e-Book of Abstracts

2025 Global Conference

UFZ HELMHOLTZ Centre for Environmental Research

LEIBNIZ IZ:HKI

M. Pohl, L. Cristiani (eds.)

Title e-Book of Abstracts
ISMET9 – 2025 Global Conference

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Lorenzo Cristiani, Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute (Leibniz-HKI), Bio Pilot Plant

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PREFACE

Welcome to ISMET9, the 9th Global meeting of the International Society for Microbial Electrochemistry and Technology (ISMET). This event is co-organized by the Helmholtz-Centre for Environmental Research (UFZ), Leipzig, and the Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute (Leibniz-HKI), Jena, under the auspices of the *International Society for Microbial Electrochemistry and Technology* (ISMET).

ISMET9 shall be a melting pot of ideas, showcasing the latest research and innovation in our field. We hope that ISMET9 will be a forum where academics, engineers, and industry experts from around the world converge to share insights, spark debates, and foster collaborations aimed at harnessing microbial technologies for sustainable development. Since the first global conference in 2008 at PennState, our society has been growing, evolving different branches and flourishing at all, reaching targets that are way more than just electric energy from bugs. Now we strive for bioremediating polluted environments like soil, sediment and water, we detect contaminants, we deal with nutrients, synthesize organics from CO_2, generate household water from salty one and push the frontiers of biobased electronics. Especially we have now a clearer picture about how electromicrobiology works, but also recognize the vast untapped field in front.

The abstracts in this book represent the forefront of our field. Each contribution has been rigorously reviewed to ensure quality and relevance, covering a broad spectrum from fundamental science to real-world applications. More than 280 participants from over 30 countries will enjoy 9 keynote and invited talks, about 75 oral lectures and around 130 posters. Our heartfelt thanks go to everyone involved in enriching this conference's content and experience—from contributors and reviewers to the local team and you, our participants. The commitment to scientific excellence and collaboration is what makes ISMET9 truly exceptional.

We trust that ISMET9 will offer you valuable insights and opportunities, inspiring continued progress in your work. Welcome, and may your time here in Leipzig be both productive and memorable. *Let's get this festivity of science started!*

Falk Harnisch
Helmholtz-Centre
for Environmental Research

Miriam Rosenbaum
Leibniz Institute for Natural Product Research
and Infection Biology Hans-Knöll-Institute

MESSAGE BY THE PRESIDENT OF ISMET

Dear attendees of ISMET9,

It is our great honor and pleasure to welcome you to the 9th Global Conference of the International Society for Microbial Electrochemistry and Technology!

We are excited about the gathering of our community for the very first time in Germany jointly hosted in Jena and Leipzig. The local organizing committee of ISMET9, as well as we as a society, do our very best to ensure that you receive the conference you deserve with great ideas, excellent scientific exchange and fond memories to take home with you.

We are confident that the ease of exchanging ideas and discussions among researchers of various disciplines and all stages of their careers forms the foundation of ISMET. This exchange is fostered by different venues for interaction, especially including the regional and global ISMET conferences. We sincerely hope for a festivity of our science and engineering taking place at ISMET9.

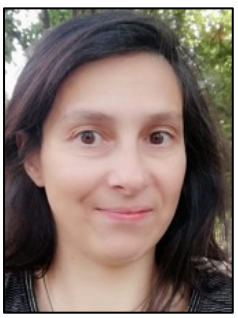
ISMET – the International Society for Microbial Electrochemistry and Technology was founded over a decade ago and has established itself as an umbrella for all activities in the truly interdisciplinary field of microbial electrochemistry & technology. Thereby ISMET is a self-renewing grass-roots-based society that can only flourish if you – the current and possible future members of ISMET – get involved. There are multiple ways of engagement. We as well as members of the board of ISMET directors and the committee chairs are more than happy to give you more information. Just approach us in the next days or afterwards!

There will be important announcements from our society's perspective at ISMET9. So stay tuned!

We are happy to have witnessed the turnover in the community, the scientific breakthroughs as well as the technology advancements since the very first global conference (MFC1 at Penn State) going now back 17 years and the first global conference under the umbrella of our (of your!) society (ISMET4 in Cairns) more than a decade ago. We are eager to get in touch with known and yet unknown great people and learn about the most recent discoveries and developments!

Can't wait to get ISMET9 started!

Jeffrey A. Gralnick
University of Minnesota, USA
Leaving ISMET President



Marianna Villano
Sapienza University, Italy
Incoming ISMET President

MESSAGE BY THE ISMET9 OMBUDSPERSON COMMITTEE

Dear ISMET9 participants,

we are the team of ombudspersons of the ISMET9. Our mission is to make the conference a safe place and space for fostering the well-being of all participants during the different activities of ISMET9.

Please be assured that there is no matter that is too "small" or too "big" for approaching one of us. We are here for you! If you experience or suspect or witness any kind of misbehavior, have any concerns or problematic issue about an incident, have lost your keys, or require advice if getting ill, contact us. We try to assist you as good as we can.

We strive that all of us can enjoy ISMET9 at any time to the max!

Catarina Paquete
Research group leader at ITQB, Lisbon, Portugal
Chair of Ombudsperson Committee
WISMET representative

Jeffrey A. Gralnick
Professor at
University of Minnesota, USA
ISMET President

Annemiek ter Hejne
Professor at Wageningen University, Netherlands
Chair Conference Committee

Sara Al-Sbei
PhD student at Leibniz-HKI, Germany
Representative of the ISMET9 Local Organizing Committee

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The SPP2440 eBiotech welcomes the ISMET community

Dear ISMET9 participants,

On behalf of the DFG-funded Priority Program 2440, eBiotech, it is my great pleasure to welcome you to the ISMET9 conference. We gather at this event to explore advancements in microbial bioelectrochemistry, a diverse field that is at the forefront of addressing global challenges in sustainability and technology.

Since 2021, the SPP2440 eBiotech program has been dedicated to fostering cutting-edge research within Germany that not only enhances our scientific understanding of bioelectrochemcial principles but also paves the way for innovative applications in biosynthesis. Our commitment to academic excellence and interdisciplinary collaboration is reflected in the diverse range of topics governed by the 11 research teams and 23 funded research scholars in this network.

ISMET9 serves as a crucial platform for bringing together leading scholars, researchers, and practitioners from around the world. It is through forums like these that we can exchange ideas, build networks, and spark the creativity necessary to advance our field.

We are particularly thrilled to support this conference, as it aligns with our mission to support sustainable biotechnological innovations. The discussions and outcomes from ISMET9 will undoubtedly contribute to shaping the future of environmental biotechnology and offer new perspectives on resource efficiency, pollution mitigation, and renewable bioprocesses.

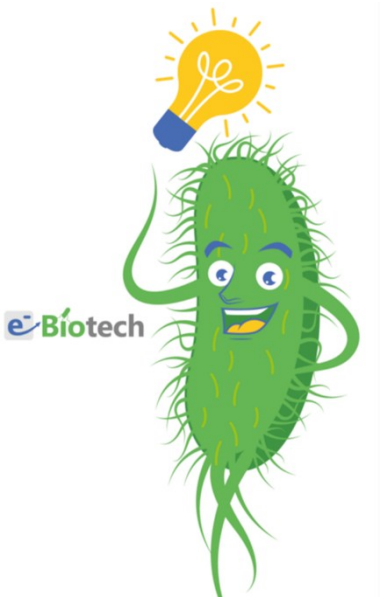
Thank you for joining us, and I look forward to the insightful exchanges and collaborations that will emerge from this gathering.

With best wishes,

Miriam Rosenbaum

www.e-biotech.de

SPP2440 eBiotech Status-Meeting, April 2025, Karlsruhe



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Helmholtz-Centre
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Miriam Rosenbaum
Leibniz Institute for Natural Product Research
and Infection Biology Hans-Knöll-Institute

UFZ HELMHOLTZ Centre for Environmental Research

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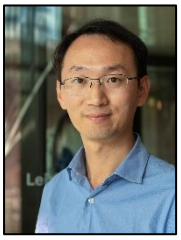


Bin Lai

Max Pohl

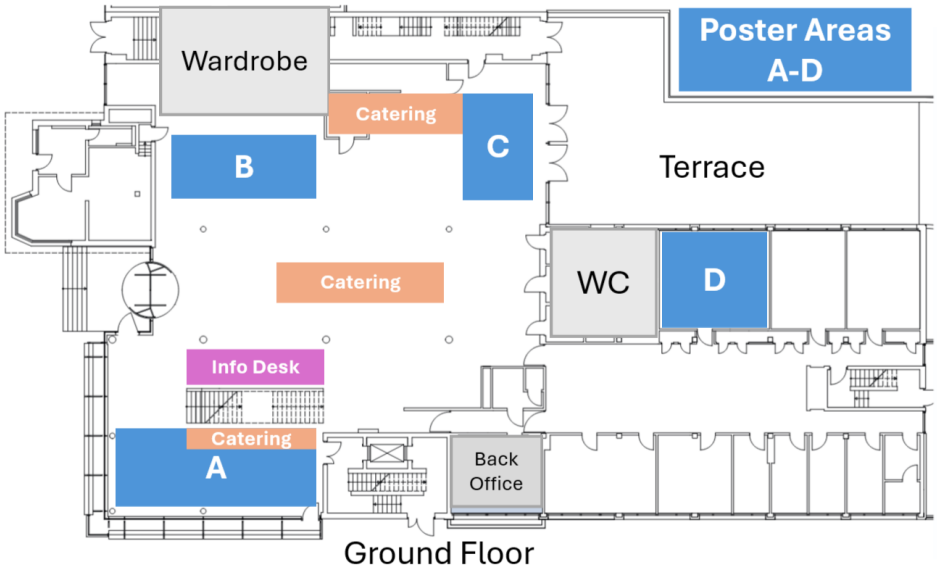
Paniz Izadi

Local Organizing Committee - Leibniz-HKI

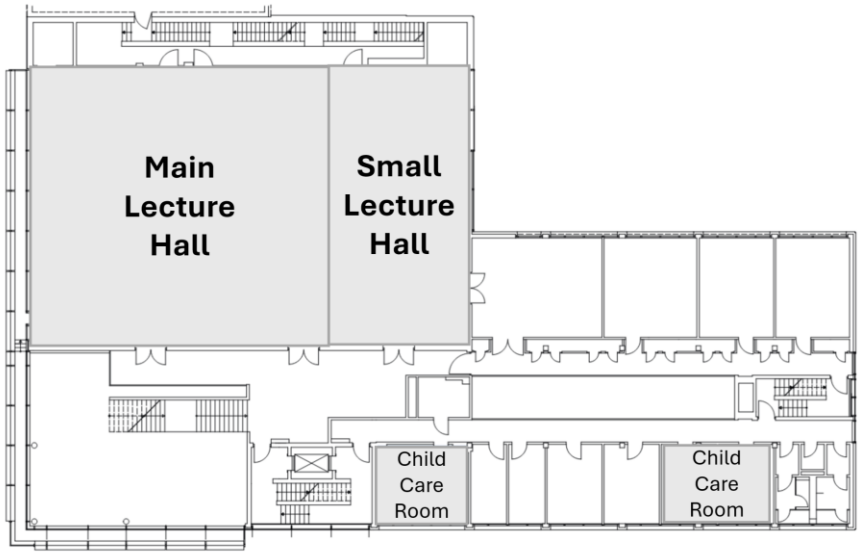
Lorenzo Cristiani

Sara Al-Sbei

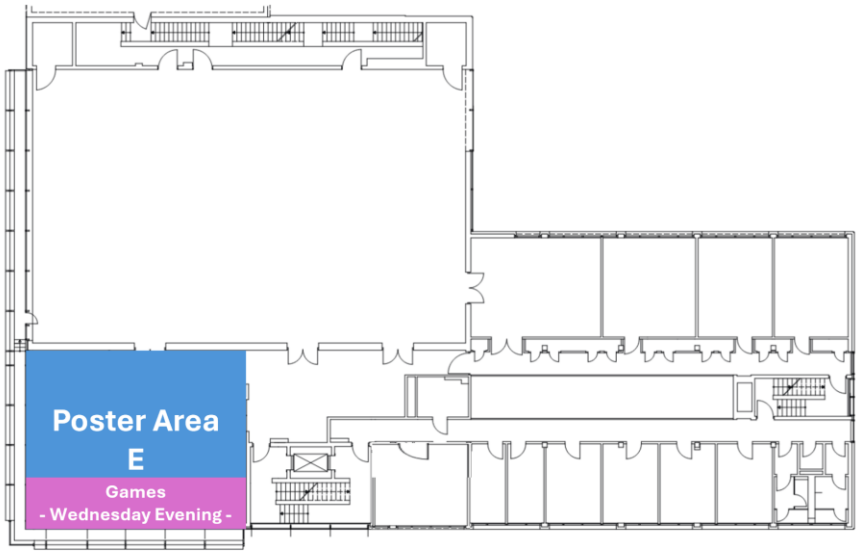
LOCATION PLAN - KUBUS



Ground Floor

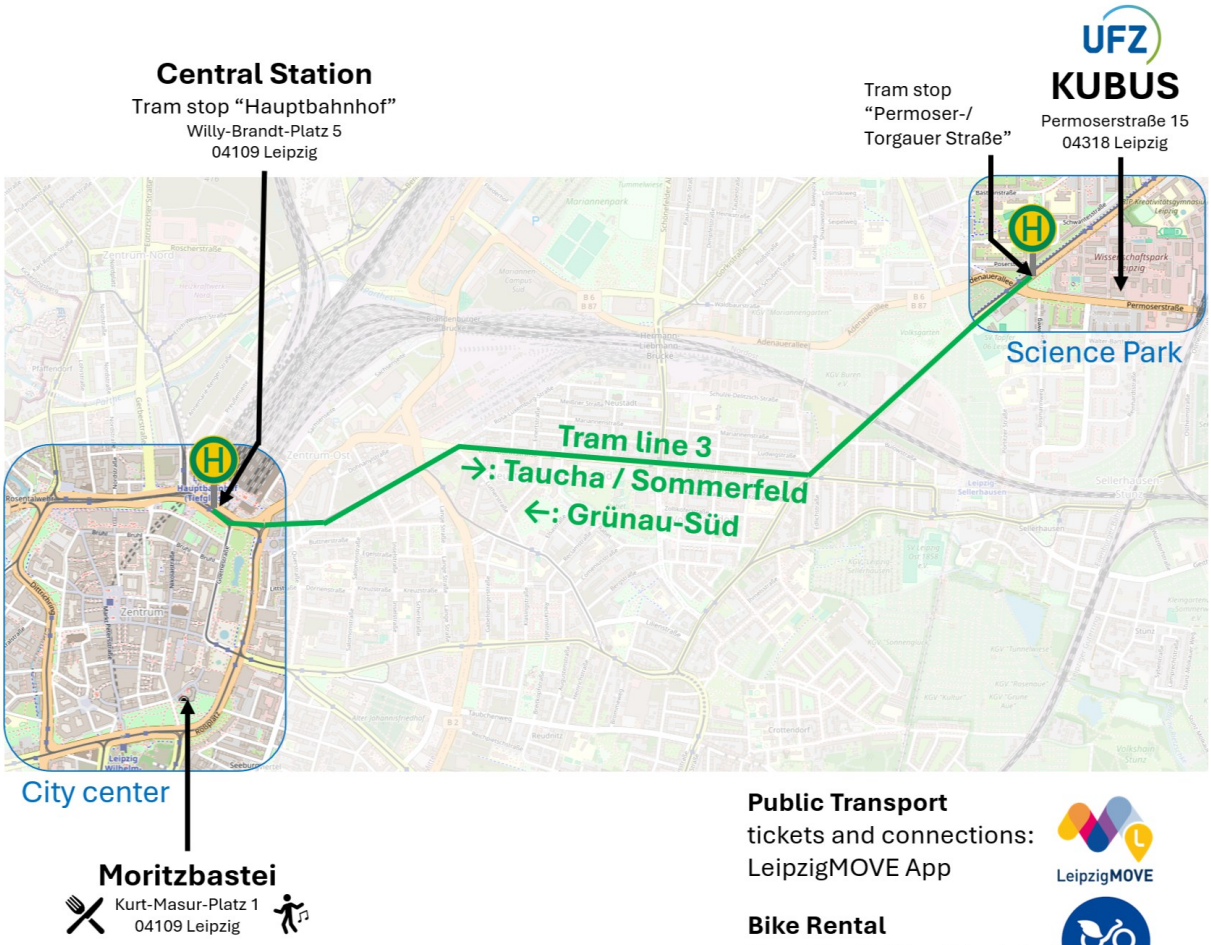


1st Floor



2nd Floor

LOCATION PLAN – LEIPZIG



How to get to the conference

The conference, ISMET 9, will take place at the Science Park in Leipzig. The event will be held at the "KUBUS", which is located at the UFZ at the following address: Permoserstraße 15, 04318 Leipzig. You can easily get there by car or tram. Maps: (Google maps)

By car

Coming from the A14 (exit: Leipzig-Ost), take Permoserstraße towards the city center until you see the sign for the KUBUS underground parking garage. Please note: Leipzig has an environmental zone that can only be entered with a green sticker.

By tram

From Leipzig Central Station, take tram line 3 (towards "Sommerfeld" or "Taucha") to the stop "Torgauer/Permoserstraße" stop, then walk to Permoserstraße. After about 300 meters, you will find KUBUS on your left. Travel time approx. 15 minutes. Tickets and further information are available at the "LeipzigMOVE" app.

PROGRAM

- Click on the session you are interested in, to jump to the abstracts of this session –

	When	Where	What	Who	Title
Tuesday, September 16th	16:00 - 18:00	Foyer	Registration		
	18:00 - 18:15	Main Lecture Hall	**Welcome Address**	**Falk Harnisch, Miriam Rosenbaum and Jeffrey Gralnick**	
	18:15 - 19:00		**Opening Keynote**	Marianna Villano	**Electrified fermentation: wiring microbial metabolism for sustainable production of valuable compounds**
	19:00 - 21:00	Foyer	**Opening Reception at Posters**		
Wednesday, September 17th		Main Lecture Hall			***Chair: Falk Harnisch***
	9:00 - 9:40		**Keynote Lecture**	Michaela TerAvest	**Inward electron transfer in *Shewanella oneidensis***
	9:45 - 12:10		***Parallel Session 1 - Microbial Physiology***		***Chair: Annette (Annie) Rowe***
	9:45 - 9:56	Main Lecture Hall	Short Talk 1-1	Matthew D. Carpenter	Investigating the extracellular electron transfer mechanism of *Vibrio natriegens* using electrodes
	9:56 - 10:07		Short Talk 1-2	Daniela Torruella-Salas	Electroactive syntrophy between *Geobacter* and *Pseudomonas* expand their role in environmental applications: A microfluidic biofilm analysis
	10:07 - 10:18		Short Talk 1-3	Conall Holohan	Electroactive Anammox: the curious reactions of an ammonium utilising bioanode
	10:18 - 10:45	Foyer	Coffee Break		
	10:45 - 10:56	Main Lecture Hall	Short Talk 1-4	Yvonne Schößow	Celebrating all the fine details in science like Neo Rauch in art: Investigation of acetate uptake kinetics of mature *Geobacter sulfurreducens* biofilm in continuous BES reveals unexpected challenges
	10:56 - 11:07		Short Talk 1-5	Anaisa Coelho	Unraveling the structural secrets of cable bacteria: From conductive nanofibers to multicellular filaments
	11:07 - 11:18		Short Talk 1-6	Han Xu	Time-resolved dynamics of extracellular polymeric substances in electroactive biofilms under multi-cycle acetate feeding: Impact on electroactivity and biofilm architecture
	11:18 - 11:29		Short Talk 1-7	Dario Shaw	Independently evolved extracellular electron transfer pathways in ecologically diverse Desulfobacterota
	11:29 - 11:40		Short Talk 1-8	Yilian Han	Extracellular electron uptake mediated by H_2O_2
	11:40 - 12:10		***Meet the Speaker Discussions***		
	9:45 - 12:10		***Parallel Session 2 - Reactor Engineering***		***Chair: Sanne de Smit***
	9:45 - 9:56	Small Lecture Hall	Short Talk 2-1	Viktorija Reinikovaite	Electroactive microorganisms and graphite granules: A deal with the devil or divine joy?
	9:56 - 10:07		Short Talk 2-2	Deepak Pant	Development and upscaling of gas diffusion electrodes for wastewater treatment and electrosynthesis of chemicals
	10:07 - 10:18		Short Talk 2-3	Zubaish Saghir	Development of a single-chamber microfluidic BES featuring a transparent gas diffusion anode for *in-situ* electrophysiological investigations of electroactive microorganisms
	10:18 - 10:45	Foyer	Coffee Break		
	10:45 - 10:56	Small Lecture Hall	Short Talk 2-4	Michael Abt	Electrochemical fluidized bed reactor for versatile and intensified electro-bio catalysis – Demonstrated by *in-situ* substrate generation
	10:56 - 11:07		Short Talk 2-5	Joshua Jack	Electro-fermentation as a path to decarbonize transportation fuels: Organic waste valorization into high-value carboxylates through fluidized bed reactors and chain elongation
	11:07 - 11:18		Short Talk 2-6	Ramineh Rad	Establishing a sustainable methanogenic carbon dioxide reduction in bioelectrochemical systems and identification of kinetic and thermodynamic constraints
	11:18 - 11:29		Short Talk 2-7	Xinyi Sun	A novel anaerobic cathodic dynamic membrane bioreactor (AnCDMBR) for efficient mitigating fouling and recovering bioenergy from municipal wastewater
	11:29 - 11:40		Short Talk 2-8	Shabnam Pouresmaeil	Leipzig's vibrancy finds its parallel in the diverse nature of biochars: Revealing the heterogeneous performance of granular biochar cathodes for abiotic hydrogen evolution reaction
	11:40 - 12:10		***Meet the Speaker Discussions***		
	12:10 - 12:20	Front of Building			**Group Picture**
	12:20 - 13:00	Foyer	Lunch break continuing into…		
	13:00 - 14:30	Foyer	**Poster Session 1, odd numbered posters**		
		Main Lecture Hall			***Chair: Angela Cabezas***
	14:30 - 15:10		**Keynote Lecture**	Paskal Saikaly	**Advancing microbial electrochemistry: Innovations in WWT and reuse with energy recovery, CO_2 valorization, and sustainable catalysis**
	15:10 - 15:35		**Invited Speaker**	Jenny Zhang	**Lighting up cyanobacterial electrochemistry**
	15:35 - 16:00	Foyer	Coffee Break		
	16:00 - 18:00	Main Lecture Hall	***Parallel Session 3 - Microbial Electrosynthesis***		***Chair: Uwe Schröder***
	16:00 - 16:11		Short Talk 3-1	Marika Zegers	Identifying key drivers of product formation in microbial electrosynthesis: A mixed linear regression analysis
	16:11 - 16:22		Short Talk 3-2	Jorge Alberto Albarracín Arias	Leveraging data from microbial electrosynthesis: Finding critical variables for ethanol production
	16:22 - 16:33		Short Talk 3-3	Louise Rigaud	Microbial electrochemical synthesis of carboxylates from organic waste: Microbial selection and functional adaptation to hypersaline conditions
	16:33 - 16:44		Short Talk 3-4	Zainab Ul Kausar	Electrochemistry meets fermentation: From CO_2 to 3-hydroxypropionic acid in a single pot approach
	16:44 - 16:55		Short Talk 3-5	Paniz Izadi	There is a sea of fog ahead: The life journey of electrochemical CO_2 reduction integrating with other technologies
	16:55 - 17:06		Short Talk 3-6	Aykut Kas	Electrochemical-microbial harmonies in high salinity: A Dittico inspired by Bach's two-part inventions transforming CO_2 into ectoine
	17:06 - 17:17		Short Talk 3-7	Dimitri van der Lee	Overlooked yet critical: Catholyte in microbial electrosynthesis
	17:17 - 17:28		Short Talk 3-8	Zhongjian Li	Iron-regulated electrochemical–microbial conversion of CO_2 to PHB
	17:28 - 18:00		***Meet the Speaker Discussions***		
	16:00 - 18:00	Small Lecture Hall	***Parallel Session 4 - Environmental BES - Nitrogen Cycling***		***Chair: Lorenzo Cristiani***
	16:00 - 16:11		Short Talk 4-1	Ugo Marzocchi	Strong electric fields within Anammox granules influence nitrate and ammonium fluxes
	16:11 - 16:22		Short Talk 4-2	Mingyi Xu	Inorganic bioelectric system for deep removal of N_2O at low temperatures
	16:22 - 16:33		Short Talk 4-3	Albert Guisasola Canudas	Bioelectrochemical ammonium recovery from high-N strength effluents
	16:33 - 16:44		Short Talk 4-4	Pratiksha Srivastava	Unveiling the unknowns: Investigating the unexplored facets of nitrite oxidation in bioelectrochemical systems
	16:44 - 16:55		Short Talk 4-5	Marco Resitano	Bioelectrocatalytic membrane reactor for nitrate reduction: Integration of *Thiobacillus denitrificans* with a CNT-coated UF membrane
	16:55 - 17:06		Short Talk 4-6	Míriam Cerrillo	Nitrogen recovery from pig slurry using four submerged MEC units
	17:06 - 17:17		Short Talk 4-7	Rahul Gautam	Ammoniacal nitrogen recovery in bioelectrochemical reactors using real reject water from biogas plant
	17:17 - 17:28		Short Talk 4-8	Gourav Dhar Bhowmick	Integration of microbial fuel cells with simultaneous partial nitrification, Anammox, and denitrification (SNAD) for energy-efficient nitrogen removal from wastewater
	17:28 - 18:00		***Meet the Speaker Discussions***		
	18:00 - end	On Site/ Outside	End of official programm and optional interactive freetime with games, food and drinks *ISMET fellows will be available for casual "Young ISMET" mentoring*		

Thursday, September 18th

When	Where	What	Who	Title
				Chair: Miriam Rosenbaum
9:00 - 9:40	Main Lecture Hall	**Keynote Lecture**	Xin Wang	**Measurement of biochemical oxygen demand using bioelectrochemical sensors**
09:40 - 10:05		**Invited Speaker**	Jeffrey Gralnick	**Emergent properties of engineered electroactive co-cultures**
10:05 - 10:30	Foyer	Coffee Break		
10:30 - 12:20		***Parallel Session 5 - Microbial Electrolysis Cells***		***Chair: Annemiek Ter Heijne***
10:30 - 10:41		Short Talk 5-1	Korneel Rabaey	Removal of organic acids for life support systems in space using a synthetic microbial community in a microbial electrolysis cell
10:41 - 10:52		Short Talk 5-2	Rouven Metz	A factorial approach to optimize biochar as an electrode material for microbial electrolysis cells
10:52 - 11:03	Main Lecture Hall	Short Talk 5-3	Chiara Capelli	Enhancing bio-electrochemical hydrogen production and organic matter removal from wastewater using *Rhodopseudomonas palustris* 42OL
11:03 - 11:14		Short Talk 5-4	Gaia Salvatori	Boosting H_2 production through electro-active anodic biofilm acclimatization in a cascade process integrating dark fermentation of cheese whey with microbial electrolysis cells
11:14 - 11:25		Short Talk 5-5	Veera Koskue	Optimising and up-scaling bioelectroconcentration for nutrient recovery from human urine
11:25 - 11:36		Short Talk 5-6	Sanne de Smit	Local hydrogen in biocathodes: Microsensors and predictive modelling
11:36 - 11:47		Short Talk 5-7	Xuemei Zhu	Biohybrid Pd catalysts harness bidirectional electron flux for enhanced C-F bond cleavage
11:47 - 12:20		***Meet the Speaker Discussions***		
10:30 - 12:20		***Parallel Session 6 - Microbial Physiology/Genetic Engineering***		***Chair: Bin Lai***
10:30 - 10:41		Short Talk 6-1	Kartik Aiyer	Unraveling extracellular electron transfer mechanisms in cable bacteria: Evidence for direct and mediated electron transfer
10:41 - 10:52		Short Talk 6-2	Joshua D. Sackett	Uncovering novel EET mechanisms and metabolic crossfeeding in a cathode-oxidizing marine sediment bacterial coculture
10:52 - 11:03	Small Lecture Hall	Short Talk 6-3	Hans Schneider	Dynamic interactions: How cellular physiology shapes and is shaped by extracellular electron transfer in biophotovoltaic systems
11:03 - 11:14		Short Talk 6-4	Ramandeep Singh	Haloalkaliphilic bacteria capable of respiring by linking sulfide oxidation to manganese(IV) oxide reduction
11:14 - 11:25		Short Talk 6-5	Annika Lenic	Investigation of mediated electron uptake in a cathodically grown biocatalyst
11:25 - 11:36		Short Talk 6-6	Annette Rowe	Towards a kinetic model of reverse electron flow in *Shewanella oneidensis*
11:36 - 11:47		Short Talk 6-7	Ahlem Filali	Thermodynamic and non-thermodynamic effects on anodic electromicrobial biofilm growth under controlled hydrodynamics
11:47 - 12:20		***Meet the Speaker Discussions***		
12:20 - 13:00	Foyer	Lunch break continuing into…		
13:00 - 14:00	Foyer	**Poster Session 2, even numbered posters**		
				Chair: Ignacio Vargas
14:00 - 14:25	Main Lecture Hall	**Invited Speaker**	Ola Gomaa	**Transformative roles of ionizing radiation in Microbial Electrochemical Bioremediation**
14:25 - 14:50		**Invited Speaker**	Benjamin Korth	**Flourishing (microbial) landscapes: The beauty of fixed-bed electrode reactors**
14:50 - 15:10	Foyer	Coffee Break		
15:10 - 17:00		***Parallel Session 7 - Electromethanogenesis***		***Chair: Lars Angenent***
15:10 - 15:21		Short Talk 7-1	Jos Steller	Designing, building and operating an up-scaled methane producing bioelectrochemical system for power-to-methane
15:21 - 15:32		Short Talk 7-2	Bruce Logan	Architecture and operational innovations for improving performance of power to gas systems generating renewable methane
15:32 - 15:43	Main Lecture Hall	Short Talk 7-3	Yeray Asensio Ramírez	Boosting biomethane production: Innovative advanced dual anerobic digesters with microbial electrochemical assistance
15:43 - 15:54		Short Talk 7-4	Silvia Bolognesi	Breathe inside the box: optimizing conditions for indoor microbial electro-methanogenesis
15:54 - 16:05		Short Talk 7-5	Pau Jimenez	BES pilot operated for 4000 h for H_2 production and biogas upgrading
16:05 - 16:16		Short Talk 7-6	Carlos Aldana	Enhancing anaerobic degradation of swine manure through electrofermentation
16:16 - 16:27		Short Talk 7-7	Annemiek Ter Heijne	High energy efficiency and long-term operation of methane-producing bioelectrochemical systems at haloalkaline conditions
16:27 - 17:00		***Meet the Speaker Discussions***		
15:10 - 17:00		***Parallel Session 8 -Electrochemistry/ Systems Engineering/Modeling***		***Chair: Paniz Izadi***
15:10 - 15:21		Short Talk 8-1	Sheila de Pablo-Casas	Analysis of electroactive biofilms for microbial electrochemical sensing
15:21 - 15:32		Short Talk 8-2	Ravineet Yadav	pyMES: An integrated Python framework for multiscale modeling and optimization of microbial electrosynthesis from CO_2
15:32 - 15:43	Small Lecture Hall	Short Talk 8-3	Tom Sleutels	Quantification of cathodic biofilm growth using optical coherence tomography
15:43 - 15:54		Short Talk 8-4	Inês Didier	Habermann and Pommer (1991) revisited: Towards a storing microbial fuel cell anode
15:54 - 16:05		Short Talk 8-5	Luis Rosa	Microbial influenced corrosion on stainless steel by Geobacter bacteria: A Butler-Volmer model
16:05 - 16:16		Short Talk 8-6	Rahul Kandpal	High-throughput screening of electroactive microbes and performance on diverse thin film electrodes in a PCB-based bioelectrochemical microreactors array
16:16 - 16:27		Short Talk 8-7	Ziyuan Wang	Understanding the tail current behavior of electroactive biofilms realizes the rapid measurement of biochemical oxygen demand
16:27 - 17:00		***Meet the Speaker Discussions***		
17:00 - 19:00	Active Surprise in the City with several options			Meeting Point: Pick up instructions at the registration desk after the last talk
19:00 - 0:00	**Conference Dinner at Moritzbastei**			Address: Kurt-Masur-Platz 1 in the City Center of Leipzig

	When	Where	What	Who	Title
Friday, September 19th		Main Lecture Hall			***Chair: Catarina Paquete***
	9:30 - 10:10		**Keynote Lecture**	Valeria Reginatto Spiller	**Exploiting the potential of *Clostridium* sp. in bioelectrochemical systems**
	10:15 - 12:25		***Parallel Session 9 - Microbial Fuel Cells***		***Chair: Sebastia Puig***
	10:15 - 10:26	Main Lecture Hall	Short Talk 9-1	Constantina Varnava	A Transcriptional and Metabolite Perspective in Microbial Fuel Cells: the case study of Pseudomonas citronellolis
	10:26 - 10:37		Short Talk 9-2	Carlos Gallardo-Bustos	Granular activated carbon tubular microbial fuel cell for decentralized greywater treatment
	10:37 - 10:48		Short Talk 9-3	Diana Yomalli Alvarez-Esquivel	A microbial electrochemical system for treating blackwater on board small-size recreational sail- or motorboats: First insights and challenges
	10:48 - 11:15	Foyer	Coffee Break		
	11:15 - 11:26	Main Lecture Hall	Short Talk 9-4	Alexandra S. Alves	*Desulfuromonas acetoxidans*: A marine bacterium to do it all, water desalination and electricity production
	11:26 - 11:37		Short Talk 9-5	Daniel Groen	Real-life data of Plant-e's SensorStick with local energy-harvesting, for remote peatland ecosystem monitoring in North Pennines National Landscape in the UK
	11:37 - 11:48		Short Talk 9-6	Rohit Kumar	Redox-tuned Ce/Fe-N-C cathode catalysts for efficient oxygen reduction reaction and high-performance microbial fuel cells
	11:48 - 12:25		***Meet the Speaker Discussions***		
	10:15 - 12:25		***Parallel Session 10 - Environmental BES - Bioremediation and Microbiology in Soils***		***Chair: Alba Ceballos-Escalera Lopez***
	10:15 - 10:26	Small Lecture Hall	Short Talk 10-1	Matteo Tucci	Magnetite nanoparticles enable self-constructed bacterial networks for long-distance electron transfer in soil
	10:26 - 10:37		Short Talk 10-2	Florian Fischer	Smart Manure: How Erich Kästner's tales of electrosorption and bioelectrochemical degradation of antibiotics and heavy metals provide safe fertilizer to our children
	10:37 - 10:48		Short Talk 10-3	Geremia Sassetto	Bioelectrochemical reactor for the remediation of trichloroethylene and chromium(VI) from contaminated groundwaters
	10:48 - 11:15	Foyer	Coffee Break		
	11:15 - 11:26	Small Lecture Hall	Short Talk 10-4	Ignacio Vargas	Enhancing bioelectrochemical perchlorate reduction by chemical electrode modification
	11:26 - 11:37		Short Talk 10-5	Christine Paul Vazhathara	Bioelectrochemical recovery of platinum group metals from spent car catalysts using *Cupriavidus metallidurans* CH34.
	11:37 - 11:48		Short Talk 10-6	Siming Chen	Microbial electrochemical reduction of vanadate by *Thiobacillus denitrificans* in groundwater
	11:48 - 11:59		Short Talk 10-7	Shunichi Ishii	Subsurface electro-biosphere: Electroactive microbes inhabiting iron-rich hot springs
	11:59 - 12:25		***Meet the Speaker Discussions***		
	12:30 - 13:00	Main Lecture Hall	**Closing Ceremony and Awards**		**Robin Bonné, Falk Harnisch, Miriam Rosenbaum**
	13:00	Foyer	Light Lunch and End of Conference		
	from 14:00		**Optional City Tours**		

CITY TOURS

Provided by Leipzig Erleben

City tours had to be booked at the conference registration. Please ask for free places on short notice at the info desk by Friday. Both tours begin on Friday, Sept 19, at 2 p.m. and last between 90 and 120 minutes. The starting point is the Tourist Information, Katharinenstraße 8, 04109 Leipzig.

Overview tour of Leipzig

A walking tour through the city center with a certified guide is the best way to discover the sights of Leipzig's city center in a short time. Walking through the beautifully renovated Speck's Hof arcade, we reach St. Nicholas Church which was the focus of events in the autumn of 1989. We continue to Augustusplatz, with the Opera and Gewandhaus Concert Hall, and the modern university complex. You will also see Leipzig's most famous arcade – Mädlerpassage. The Old Town Hall, one of the most beautiful Renaissance buildings in Germany, is situated on the eastern side of the Market Square. We then pass through Barthel's Hof, a courtyard leading to our most famous pub mile – Barfußgässchen. The last highlight of our tour will be St. Thomas Church, the main church of the Thomanerchor, Leipzig's famous boys' choir.

Music City Leipzig

If you are interested in Johann Sebastian Bach, who assumed office as organist and choirmaster of the world famous boys' choir of St. Thomas Church or Georg Philipp Telemann who achieved success as a composer in Leipzig, or perhaps Robert Schumann (who fell in love with Clara Wieck here), or Felix Mendelssohn Bartholdy who worked as orchestra director of the Gewandhaus and founded a conservatory to instruct his musical protégées, this tour is definitely for you. The city was also home to Richard Wagner, Albert Lortzing, Gustav Mahler and others. The Music City Leipzig Tour presents an eclectic score of musicians on this interesting walk through the city.

KEYNOTE SPEAKERS

Opening Keynote:
Electrified fermentation: wiring microbial metabolism for sustainable production of valuable compounds

Tuesday, Sept 16th at 6.15 p.m.

Marianna Villano - Sapienza University, Italy

Marianna Villano is an Associate Professor of Chemical Engineering at the Department of Chemistry, Sapienza University of Rome, a position she has held since January 2022. She earned her M.Sc. in Industrial Chemistry (summa cum laude) in 2007 and completed her Ph.D. in Industrial Chemical Processes in 2011, with a thesis on microbially catalyzed hydrogen and methane production in bioelectrochemical systems. During her Ph.D., she also spent six months as a visiting student at Cornell University's Department of Biological and Environmental Engineering. Her research focuses on waste and wastewater valorization via biological processes for polyhydroxyalkanoate production and biofuels generation using mixed microbial cultures. She has authored around 77 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

Keynote Lecture 2:
Inward electron transfer in *Shewanella oneidensis*

Wednesday, Sept 17th at 9.00 a.m.

Michaela TerAvest - Michigan State University, USA

Michaela TerAvest is an Associate Professor of Biochemistry and Molecular Biology at Michigan State University. She earned her Ph.D. in Biological and Environmental Engineering from Cornell University in 2014. Following her Ph.D., she served as a Research Associate at UC Berkeley's California Institute for Quantitative Biosciences from 2013 to 2015. Her research focuses on engineering microbial electron transport chains and metabolic pathways to advance bioenergy production, with particular interest in organisms like *Zymomonas*. She has authored around 35 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

Keynote Lecture 3:
Advancing Microbial Electrochemistry: Innovations in WWT and Reuse with Energy Recovery, CO_2 Valorization, and Sustainable Catalysis

Wednesday, Sept 17th at 2.30 p.m.

Pascal Saikaly - King Abdullah University of Science and Technology, Saudi Arabia

Pascal Saikaly is a Professor of Environmental Science and Engineering at King Abdullah University of Science and Technology (KAUST) in Saudi Arabia. He earned his B.S. and M.S. from the American University of Beirut and completed his Ph.D. in Environmental Engineering at the University of Cincinnati in 2005. He later worked as an Assistant Professor at the American University of Beirut (2008–2010) before joining KAUST, where he began as an Associate Professor in 2010 and now holds a full professorship. His work is renowned for combining omics, microbial ecology, and bioelectrochemistry to explore wastewater treatment, membrane bioreactors, and bioelectricity generation from wastewater. His innovative research has produced significant advancements in harvesting energy and byproducts from wastewater processes. He has authored around 147 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

Keynote Lecture 4:
Measurement of Biochemical Oxygen Demand Using Bioelectrochemical Sensors

Thursday, Sept 18th at 9.00 a.m.

Xin Wang — Nankai University, China

Xin Wang is a Professor of Environmental Engineering at Nankai University in Tianjin, China, where he has also served as Department Chair since June 2019. He earned both his B.S. (2004) and Ph.D. (2010) in Environmental Engineering from Harbin Institute of Technology. Between 2010 and 2012, he worked as an Assistant Professor at Nankai, before later being promoted to Associate Professor in December 2014 and full Professor in late 2017. His research centers on bioelectrochemical processes; including energy recovery from wastewater, microbial syntrophy in electroactive biofilms, bioelectrochemical sensing, nutrient recovery, and soil remediation via bioelectrochemical systems. Wang has been recognized as a Fellow of the Royal Society of Chemistry and has received awards such as the Excellent Young Scientists Fund and the Gold Young Scientist Award from China's Society for Environmental Sciences. He has also conducted research visits at the University of Colorado Boulder and Penn State University, enhancing his global research collaborations. He has authored around 278 peer-reviewed articles, in addition to contributing to numerous conference presentations.

Keynote Lecture 5:
Exploiting the Potential of *Clostridium* sp. In Bioelectrochemical Systems

Friday, Sept 19th at 9.30 a.m.

Valeria Reginatto Spiller - University of Sao Paolo, Brasil

Valeria Reginatto Spiller is an Associate Professor (since achieving "livre docência" in 2021) in Industrial Biochemistry at the University of São Paulo (USP), Ribeirão Preto, Brazil. She specializes in industrial biotechnology, with a strong focus on biological treatment of effluents, bioenergy, and bioproduct generation. Her recent work extends to microbial electrochemistry and its technological applications. Her contributions are significant in the Brazilian and broader Latin American context, particularly in sustainable microbiological technologies. She has authored around 55 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

INVITED SPEAKERS

Invited Speaker 1:
Lighting up cyanobacterial electrochemistry

Wednesday, Sept 17th at 3.10 p.m.

Jenny Zhang - University of Cambridge, UK

Jenny Zhenqi Zhang is a Chinese-Australian chemist and BBSRC David Phillips Research Fellow in the Department of Chemistry at the University of Cambridge, and a Fellow of Corpus Christi College since 2019. She completed her B.S. and Ph.D. at the University of Sydney (Ph.D. in 2011), with research on platinum-based anti-cancer complexes. After a postdoctoral fellowship at Cambridge funded by a Marie Skłodowska-Curie Fellowship, she began her independent research group in 2018, focusing on semi-artificial photosynthesis strategies to rewire photosystem II for sustainable fuel production. In 2020, she received the RSC Felix Franks Biotechnology Medal for her work in re-wiring photosynthesis. She has authored around 27peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

Invited Speaker 2:
Emergent properties of engineered electroactive co-cultures

Thursday, Sept 18th at 9.40 a.m.

Jeffrey A. Gralnick - University of Minnesota, USA

Jeffrey A. Gralnick is a Distinguished McKnight University Professor and Associate Dean for Faculty (currently Interim Associate Dean for Research) in the Department of Plant and Microbial Biology at the University of Minnesota, and directs the Microbial and Plant Genomics Institute. He earned his Ph.D. in Bacteriology (University of Wisconsin–Madison, 2003) and did postdoctoral research at Caltech studying *Shewanella oneidensis*. His laboratory investigates how environmental bacteria respire metals and electrodes, integrating environmental microbiology and synthetic biology to advance bioremediation and bioenergy applications. The lab explores electron transfer and respiratory processes in bacteria with broad environmental implications. He has authored around 97 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations. He served as the president of ISMET for the past years.

Invited Speaker 3:
Transformative roles of ionizing radiation in Microbial Electrochemical Bioremidation

Thursday, Sept 18th at 2.00 p.m.

Ola Gomaa - Egyptian Atomic Energy Authority, Egypt

Ola Gomaa is a Professor and Head of the Radiation Microbiology Department at the National Center for Radiation Research and Technology (NCRRT), part of the Egyptian Atomic Energy Authority (EAEA) in Cairo, Egypt. She is also a Scientific Advisor for the Central Laboratory Unit at NCRRT. Her research focuses on microbial electrochemical systems, biofilm engineering for wastewater treatment, and the enhancement of electroactive bioremediation processes, including studies of cell adhesion to electrode surfaces.
Prof. Gomaa was a Fulbright Visiting Scholar at the University of South Carolina, where she worked on engineered nanoparticle bioaccumulation. She has been actively involved in international collaborations in biofiltration, environmental microbiology, and microbial electrochemistry. She has authored around 52 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

Invited Speaker 4:
Flourishing (Microbial) Landscapes: The Beauty of Fixed-Bed Electrode Reactors

Thursday, Sept 18th at 2.25 p.m.

Benjamin Korth — Helmholtz Centre for Environmental Research, Germany

Benjamin Korth leads an independent research group titled "Thermodynamics of Electroactive Microorganisms" within the Department of Microbial Biotechnology at UFZ in Leipzig, Germany. He joined UFZ in 2017 as a scientist after completing his Ph.D. in Biochemistry at the same institution (Degree awarded by Univ. of Leipzig), where he had worked in microbial bioelectrocatalysis and bioelectrotechnology. Since 2020, he has served as team leader of his independent research group, with a focus on unraveling the thermodynamic and kinetic principles governing extracellular electron transfer in electrogenic and electrotrophic microbes. His group employs bioelectrocalorimetry, modeling, and electrochemical techniques to quantify energy fluxes and improve the efficiency of microbial electrochemical technologies. Korth has also expanded his collaboration and expertise through research stays at the University of Girona and Delft University of Technology. He has authored around 25 peer-reviewed articles and book chapters, in addition to contributing to numerous conference presentations.

ORAL PRESENTATIONS

Session 1 – Microbial Physiology

Access to the Live-Q&A of this Session

Scan the QR-Code or visit schnaq.app and enter the following access code: 8448 7634

Talk 1-1: Investigating the Extracellular Electron Transfer Mechanism of Vibrio natriegens Using Electrodes

Matthew D. Carpenter[1,2], Wen-Chia Chen[1,2], Caroline M. Ajo-Franklin[2]
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An extracellular electron transfer (EET) pathway utilizing multi-heme cytochromes was recently found to enable iron reduction in the marine bacterium *Vibrio natriegens* [1]. This organism has multiple properties, including rapid growth, easy genetic modification, robust expression of heterologous proteins, and ubiquity in diverse marine environments, that make it an optimal chassis for marine biotechnology [2]. Applications in biosensing and bioproduction could be facilitated through engineered control over electron flux between *V. natriegens* cells and electrodes. However, the capacity of the *V. natriegens* iron reduction pathway to support electron exchange with electrodes remains untested. To control and harness electrode-driven electron fluxes in *V. natriegens* for technological applications, we must first understand the mechanism of electrode reduction.

Electrode reduction by *V. natriegens* can be hypothesized to function similarly to electrode reduction by *Shewanella oneidensis* or *Aeromonas hydrophila*, given that *V. natriegens* iron reduction depends upon homologs of the CymA inner membrane cytochrome and MtrCAB outer membrane porin:cytochrome complex of *S. oneidensis* and the PdsA periplasmic cytochrome of *A. hydrophila* [1]. To test this hypothesis, we generated single knock-out and complementation mutants for each of *cymA*, *pdsA*, *mtrA*, *mtrB*, and *mtrC*. These strains were evaluated for their ability to reduce an electrode poised at +200 mV vs. Ag/AgCl in a bioelectrochemical system. Electrical current measurements revealed that for each gene, deletion significantly impaired electrode reduction and complementation restored the wild-type phenotype, indicating that all five genes are involved in EET to an electrode. Using the complementation strains, we manipulated the expression level of the complemented gene. We characterized the effect of overexpression of the complemented gene on the production of the Mtr pathway cytochromes and the electrode reduction phenotype. These experiments revealed that high expression levels of either decaheme cytochrome (MtrA or MtrC) impair the production of the other decaheme cytochrome. We also investigated whether the *V. natriegens* EET pathway could enable electrode oxidation. We found that in the presence of fumarate and a negatively polarized electrode, *V. natriegens* produced an Mtr cytochrome-dependent inward electron flux.

This study interrogated the mechanisms underlying electron flux between electrodes and *V. natriegens,* advancing fundamental understanding and helping to unlock this organism as a chassis for bioelectronic technology development. The discovery that *cymA*, *pdsA*, *mtrA*, *mtrB*, and *mtrC* are each required for electrode reduction suggests that electrode reduction utilizes the same mechanism as iron reduction, identifying target genes for developing bioelectronic sensors. Modulating cytochrome expression levels uncovered limits on decaheme cytochrome expression, elucidating clear design constraints for future engineering. Finally, the discovery of inward EET in *V. natriegens* revealed further similarity between *V. natriegens* and S*. oneidensis*, raising new prospects for metabolic engineering in this emerging bioproduction chassis. In summary, this work uncovers key properties of electrode-dependent EET in a microbe of great interest for biotechnology.

References:
[1] Conley, B. E., et al. (2020). A hybrid extracellular electron transfer pathway enhances the survival of Vibrio natriegens. Appl. Environ. Microbiol., 86(19). doi:10.1128/AEM.01253-20.
[2] Hoff, J., et al. (2020). Vibrio natriegens: an ultrafast-growing marine bacterium as emerging synthetic biology chassis. Environ. Microbiol. 22: 4394-4408. doi: 10.1111/1462-2920.15128

Talk 1-2: Electroactive Syntrophy Between *Geobacter* and *Pseudomonas* Expand Their Role in Environmental Applications: A Microfluidic Biofilm Analysis

Daniela Torruella-Salas[1,2], René Wurst[3], Edina Marlen Klein[3], Fernando Muniesa-Merino[1], Johannes Gescher[3], Abraham Esteve-Núñez[1,2]
[1]*Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Universidad de Alcalá, 28801, Alcalá de Henares, Madrid, Spain*
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[3]*Institute of Technical Microbiology, University of Technology Hamburg, Hamburg, Germany*
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Designing a synthetic microbial consortium by co-cultivating microorganisms is an approach employed in recent research for a plethora of applications [1]. This type of co-cultures are widely used in electromicrobiology mainly for a better understanding of DIET (Direct Interspecies Electron Transfer) between *Geobacter* and other bacteria [2], [3]. Creating a consortium of electroactive bacteria and hydrocarbon-degrading bacteria could open a window of applications for remediating or monitoring polluted environments. In this work, a co-culture of *G. sulfurreducens* and *P. putida* has been studied in a single-chamber MEC (Microbial Electrolysis Cell), poised at +0.4 V vs. Ag/AgCl, under micro-oxic conditions in freshwater medium. The model organic pollutant used as carbon source and electron donor was 3-methylbenzyl alcohol (3MBA), a toluene derivative. Then, independent pure cultures of *G. sulfurreducens* and *P. putida* were also studied as controls under different conditions. For one week, the results showed a high current density from the co-culture peaking at 263 μA/cm^2, corresponding to ca. 140-fold, and 190-fold higher than pure culture of *Geobacter*, and *Pseudomonas*. Cyclic voltammograms showed a higher electroactive response by the co-culture biofilm, compared to pure cultures. Moreover, biofilm development was analyzed through a microfluidic biofilm cultivation platform with OCT (Optical Coherence Tomography) imaging, revealing a significant difference between co-culture and pure culture of *Pseudomonas*. Also, FISH (Fluorescence *in situ* hybridization) analysis illustrated a clear co-culture structure with biofilm formation of a sole *Geobacter* layer in direct contact with the anodic surface and then, a sole one of *Pseudomonas* on top of *Geobacter*. These results imply that *Pseudomonas* might play a key role interacting with *Geobacter* in the co-culture, either by supplying acetate and/or through extracellular electron transfer. Both hypotheses are now subject of research; however, our results show how a stratified combination of electroactive bacteria with hydrocarbon-degrading bacteria may enhance electrobioremediation and electrochemical biosensing in hydrocarbon-polluted environments.

[1] Y. Liang, A. Ma, and G. Zhuang, "Construction of Environmental Synthetic Microbial Consortia: Based on Engineering and Ecological Principles," Front. Microbiol., vol. 13, p. 437, Feb. 2022, doi: 10.3389/FMICB.2022.829717/BIBTEX.
[2] J. A. Smith, K. P. Nevin, and D. R. Lovley, "Syntrophic growth via quinone-mediated interspecies electron transfer," Front. Microbiol., vol. 6, no. FEB, p. 121, Feb. 2015, doi: 10.3389/FMICB.2015.00121/BIBTEX.
[3] L. Semenec, I. A. Vergara, A. E. Laloo, S. Petrovski, P. L. Bond, and A. E. Franks, "Adaptive evolution of geobacter sulfurreducens in coculture with pseudomonas aeruginosa," MBio, vol. 11, no. 2, Mar. 2020, doi: 10.1128/MBIO.02875-19/SUPPL_FILE/MBIO.02875-19-SF007.TIF.

Talk 1-3: Electroactive Anammox: the curious reactions of an ammonium utilising bioanode

B. Conall Holohan[1], Phoenix Smid[1], Pablo Sanz Mendoza[1], Cornelia U. Welte[1]

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Anaerobic oxidising ammonium bacteria (anammox) harbour an enormous potential to tackle the global nitrogen crisis, however, their dependence on nitrite (NO_2^-) is a limitation for application. Recent studies demonstrate that anammox bacteria in bioelectrochemical systems (BES) can treat ammonium to N2 gas without the requirement NO2- while generating current.

Here we demonstrate a proof-of-principle study using BES anaerobically inoculated with a laboratory enrichment of anammox "Candidatus Kuenenia" and batch with up to 2mM ammonium. The system was operated at a potential of +0.6 V versus standard hydrogen electrode (SHE), with continuous current measurement via a potentiostat.

The BES bioreactors successfully converted the ammonium to N_2 gas, tracked using stable isotope probe of ammonium (N15H4+). The significant lag phase (up to c.175 h) and spike in current (up to 7 mA/m^2) (Figure 1) offer evidence of a potential storage of NO_2^- to be used up in the start-up phase before current is generated. Furthermore, the generated long-term current production serves as a proxy measurement for extracellular electron transfer (EET) and successful activity of anammox. Current production by anammox was independent of NO_2^- and cessation of electroactivity was observed with NO_2^- addition, indicating that anammox bacteria prefer the classical anammox reaction over electroactive anammox. The presence of anammox bacteria on the electrode was confirmed through fluorescent in-situ hybridisation (FISH) microscopy.

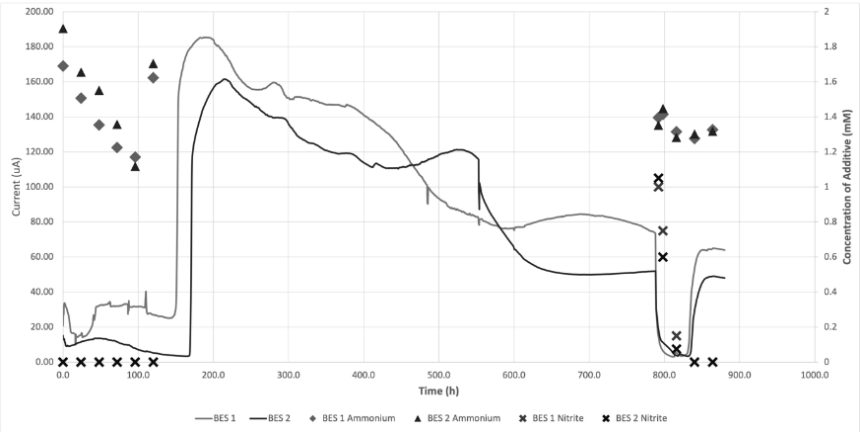

Figure 1: Outline of the current production of duplicate BES (BES 1, 2) with ammonium only medium (no NO2-). Showcasing a lag-phase in both systems of c. 175 hours. At 800 – 850 h nitrite is added leading to cessation of current production.

Here we demonstrated that anammox can treat ammonium in bioelectrochemical systems without nitrite. This study system provides both insights into the metabolism of anammox as well as potential for application through bioelectrochemical technologies to solve the nitrogen crisis.

Talk 1-4: Celebrating all the fine details in science like Neo Rauch in art: Investigation of acetate uptake kinetics of mature *Geobacter sulfurreducens* biofilm in continuous bioelectrochemical systems reveals unexpected challenges

Y. Schößow [*,1], M. Meyer [1], O. Zorc [2], P. Haus [1] and B. Korth [1]

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Microbial electrochemical technologies (MET) are promising candidates for a sustainable economy and can be applied for industrial processes, like wastewater treatment, energy production and bioremediation. In primary MET, electroactive microorganisms (EAM) exchange electrons with insoluble electron conductors like electrodes to catalyze oxidation and reduction reactions. This extracellular electron transfer (EET) extends the electron-transport chain of EAM to the exterior and bridges the electrically insulating cellular membrane. Several studies, involving cytochrome deletion studies [1], conductivity measurements [2] and surface-enhanced resonance Raman spectroscopy [3], provide a detailed picture of the EET dynamics.

While the research on EET dynamics is in full swing, far less is known about the substrate uptake kinetics. But improving the substrate uptake of EAM is as important for the optimized performance of MET as enhancing the electron transfer by the overexpression of cytochromes. A thermodynamic modelling platform [4] already demonstrated that the influence of the anode potential on the energy harvest of EAM can be only explained by kinetic effects and more data from standardized experiments needs to be generated.

On the way to more standardization, we quantified for the first time the acetate consumption of mature pure culture *Geobacter sulfurreducens* biofilms during the continuous cultivation at different acetate concentrations and used it to determine the acetate uptake parameters with the Nernst-Michaelis-Menten equation. Significant differences between this approach and the one using the anodic current as easy-to-measure proxy for the acetate uptake kinetics were shown, indicating a strong dependence on the coulombic efficiency of the used experimental set-up.

The nuanced comparison of kinetic parameters with the literature and the detailed analysis of the acetate consumption reveals the demand for highly controlled studies of *Geobacter sulfurreducens* biofilms to enable comparative analysis of single properties of *Geobacter* biofilms and expand the information pool for the modelling of MET.

REFERENCES

[1] K. Joshi, C.H. Chan, D.R. Bond, Geobacter sulfurreducens inner membrane cytochrome CbcBA controls electron transfer and growth yield near the energetic limit of respiration, Mol. Microbiol. 116 (2021) 1124–1139. DOI:10.1111/mmi.14801.
[2] Reguera, K.P. Nevin, J.S. Nicoll, S.F. Covalla, T.L. Woodard, D.R. Lovley, Biofilm and nanowire production leads to increased current in Geobacter sulfurreducens fuel cells, Appl. Environ. Microbiol. 72 (2006) 7345–7348. https://doi.org/10.1128/AEM.01444-06.
[3] L. Robuschi, J.P. Tomba, J.P. Busalmen, Proving Geobacter biofilm connectivity with confocal Raman microscopy, J. Electroanal. Chem. 793 (2017) 99–103. https://doi.org/10.1016/j.jelechem.2016.11.005.
[4] Korth, Benjamin; Rosa, Luis F. M.; Harnisch, Falk; Picioreanu, Cristian (2015): A framework for modeling electroactive microbial biofilms performing direct electron transfer. In: *Bioelectrochemistry (Amsterdam, Netherlands)* 106 (Pt A), S. 194–206. DOI: 10.1016/j.bioelechem.2015.03.010.

Talk 1-5: Unraveling the Structural Secrets of Cable Bacteria: From Conductive Nanofibers to Multicellular Filaments

Anaísa Coelho[1], Magdalene MacLean[1], Aneesh Deshmukh[1], Ravi Yadav[1], Saif Khan[1], Zenia Motiwala[1], Tingting Yang[1], Cornelius Gati[1], Mohamed Y. El-Naggar[1]
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Cable bacteria are multicellular filaments of the deltaproteobacterial family *Desulfobulbaceae*, composed of thousands of end-to-end cells, found in marine and freshwater sediments worldwide [1]. These organisms achieve remarkable centimeter-scale electron transport by coupling sulfide oxidation in deeper sediments to oxygen reduction near the sediment-water interface [1, 2]. This unique metabolic capability is facilitated by a network of periplasmic protein nanofibers that span the entire length of each cable bacteria filament [3]. Despite its significance, the structural and molecular mechanisms underlying this conductivity, along with the processes of filament growth and division, remain poorly understood.

To address these knowledge gaps, we employed a multidisciplinary approach using advanced imaging techniques, including soft X-ray tomography, cryo-electron tomography, STEM-EDX, and fluorescence microscopy. By imaging intact cable bacteria in three dimensions, we uncovered the spatial arrangement of nanofibers essential for long-range electron transport. High-resolution imaging further revealed the ultrastructural organization of these nanofibers, shedding light on their molecular composition and conductivity. Furthermore, we identified a previously uncharacterized asymmetric cell division mechanism, offering insights into how filaments elongate and maintain their metabolic integrity.

These findings uncover the intricate cellular architecture that supports the electric metabolism of cable bacteria, bridging critical gaps in our understanding of microbial conductive networks. By elucidating these processes, our work advances the study of microbial electric systems and highlights the potential of cable bacteria for applications in renewable energy and bioelectronics.

[1] Pfeffer C, et al. Nature. 2012; 491:218–221
[2] El-Naggar MY and Finkel SE. Sci. 2013; 27:38-43
[3] Cornelissen R, et al. Front Microbiol. 2018; 9:3044

Talk 1-6: Time-Resolved Dynamics of Extracellular Polymeric Substances in Electroactive Biofilms under Multi-cycle Acetate Feeding: Impact on Electroactivity and Biofilm architecture

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Toulouse, France
han.xu@toulouse-inp.fr

The architecture of electroactive biofilms (EABs) is strongly influenced by the composition and abundance of extracellular polymeric substances (EPS) [1]. Yet, the dynamic structural and functional responses of EABs to substrate feeding strategies remain poorly understood, particularly with regard to the interaction between EPS evolution and electroactivity dynamics. While acetate is a model substrate for EABs cultivation, it is unclear how acetate feeding strategy dynamically regulate EPS biosynthesis and extracellular electron transfer (EET). In this study, we conducted time-resolved acetate-fed batch EABs cultivation to explore the relationship between current production, biofilm thickness, microbial community shifts, and EPS content and distribution, focusing on polysaccharides (PS) and proteins (PN). A novel confocal laser scanning microscopy (CLSM) image analysis method, supported by conventional chemical analysis, was used to qualitatively and quantitatively assess EABs thickness and PN and PS content in EABs.

During 50 days of operation at a potential of -0.1 V vs. SCE under 6 cycles of acetate addition (AA), the cycle duration decreased and the maximum current density gradually increased throughout the first three cycles. During the following three cycles, the production of EPS kept increasing, particularly the PN fraction, reaching a 2.1-fold higher than the 3rd AA. Our results show that PN fraction exhibited a strong positive correlation with EET and, consequently, current production ($\gamma = 0.93$, $p < 0.001$). Additionally, microbial community dynamics revealed a progressive dominance of *Geobacter*, with its relative abundance increasing from 59% to 90% between the 3rd and 6th cycles. These findings highlight the progressive maturation and evolution of EABs under acetate-fed conditions. They also suggest that the PN fraction in EPS plays a key role in facilitating EET and could be leveraged to enhance the performance of EABs. Following the third AA, a seven-day period of acetate deprivation resulted in structural disintegration of the biofilm, marked by a 20.4% reduction in thickness and a 2.7-fold decrease in the PS content of the EPS. These findings underscore the operational fragility of EABs under acetate-deficient conditions and highlight the potential role of EPS—particularly PS—as energy reserves supporting EAB metabolism. To optimize EAB performance, we are currently exploring a strategy that combines controlled acetate feeding with electrode potential modulation. Specifically, we are examining the development of anodic EABs under gradually increasing acetate concentration in conjunction with gradual electrode potential adjustments. This approach aims to identify optimal conditions for promoting PN enrichment in EPS while simultaneously maximizing current generation and ensuring long-term bioanode stability.

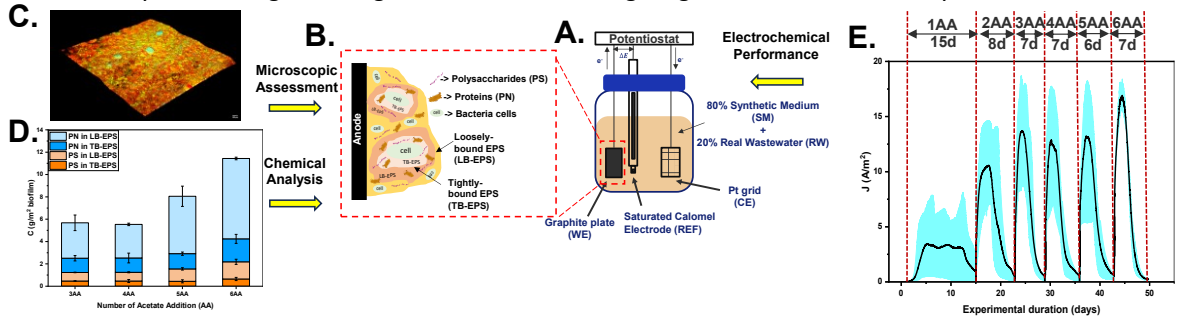

Fig. (A) Bioelectrochemical system reactor design; (B) EAB structural components; (C) 3D EAB Imaging by CLSM: total microbial cells (blue), PS (red), PN (green); (D) Relative PN and PS contents in EPS; (E) Current generation over 6 successive cycles of 20 mM acetate addition (AA)

References:

1. Martinez-Ostormujof Lucila., et al. (2023). Systemic Analysis of the Spatiotemporal Changes in Multi-Species Electroactive Biofilms to Clarify the Gradual Decline of Current Generation in Microbial Anodes. ChemElectroChem, 10(9), e202201135.

Talk 1-7: Independently evolved extracellular electron transfer pathways in ecologically diverse *Desulfobacterota*

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Extracellular electron transfer (EET) plays a crucial role in the biogeochemical cycling of carbon, metals, sulfur, and nitrogen and has wide-ranging biotechnological applications. The most extensively studied and prevalent EET pathways are the metal-reducing (Mtr) pathway (typically found in *Shewanella* spp.), the outer-membrane cytochrome (Omc) pathway (typically found in *Geobacter* spp.), and the porin-cytochrome (Pcc) pathway. Although these pathways utilize trans-outer membrane cytochrome complexes and perform similar functions, they are phylogenetically unrelated, indicating independent evolutionary origins. To date, no bacterium has been reported to encode or utilize these pathways simultaneously. In this study, we report a novel EET mechanism in which the high-current-producing bacterium *Desulfuromonas acetexigens* differentially co-expresses, at transcript and protein levels, the Pcc, Omc, and Mtr pathways, along with high-molecular-weight cytochromes containing a large number of hemes (as high as 86 heme-binding motifs) under EET growth conditions (i.e., electrode under set potential or naturally occurring iron oxide minerals as the electron acceptor). To reach these findings, we initially studied the electroactive bacterium *D. acetexigens* because it is prevalent in a wide range of natural and engineered environments and is known for displaying one of the highest current densities among pure cultures of EET-capable microorganisms. We employed a range of techniques, including bioelectrochemistry, genomics, stimulus-induced differential transcriptomics, differential proteomics, and phylogenetic analyses, to gain insight into the metabolic processes that enable this bacterium to thrive in diverse environments and produce high current densities. The study of *D. acetexigens* led to the discovery of a novel EET mechanism involving the differential co-expression, at transcript and protein levels, of phylogenetically distant EET pathways (i.e., Pcc, Omc, and Mtr) under EET growth conditions, such as an electrode poised at a set potential or iron oxide minerals serving as electron acceptors. The discovery of a conserved Mtr pathway in *Desulfuromonas* was unexpected, as the presence of *mtrCAB* genes had not been previously reported or detected in the *Desulfobacterota* phylum (formerly classified as *Deltaproteobacteria*). Moreover, no electroactive organism had been shown to express these phylogenetically distant pathways simultaneously. Consequently, we conducted phylogenetic analyses to establish the distribution and ecological context of the multiple Mtr-Omc-Pcc EET metabolisms across the tree of life. Our analyses revealed over 40 *Desulfobacterota* species from diverse ecological environments that encode both Omc and Mtr pathways, with the majority also expressing the Pcc pathway. The newly identified Mtr proteins in *Desulfobacterota* form a major divergent lineage within this phylum, spanning diverse environments and ecological contexts alongside the presence of Omc and Pcc pathways. The identification of Mtr proteins in the *Desulfobacterota* phylum suggests a greater prevalence of the Mtr mechanism and significantly expands the known phylogenetic diversity of these proteins. The co-occurrence of phylogenetically distant pathways is unprecedented in the known biology of EET-capable microorganisms and highlights the existence of unrecognized electron transfer metabolic pathways, even within relatively well-studied microbial taxa. These findings have significant ecological implications, challenging the belief that certain EET pathways are exclusive to specific taxa and suggesting that these pathways are more widespread than previously thought. Furthermore, this discovery reveals a previously unrecognized versatility in microbial electron transfer mechanisms that can be leveraged for biotechnological applications.

Talk 1-8: Extracellular Electron Uptake Mediated by H_2O_2

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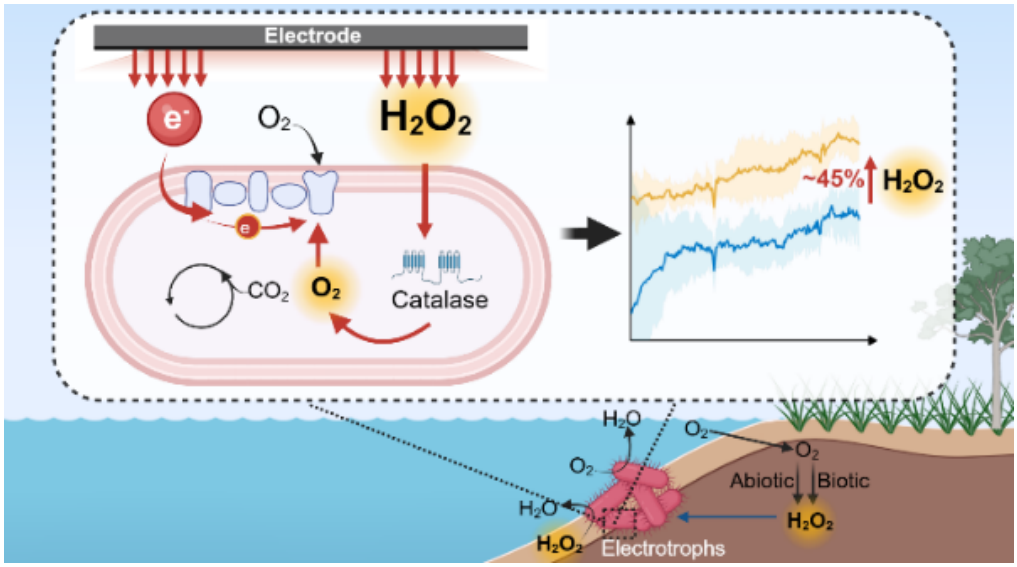
Harvesting electricity from microbial electron transfer is believed as a promising way of renewable energy generation. However, a major challenge lies in the still-unknown mechanisms of extracellular electron transfer, especially how microbes consume electrons from the cathode to catalyze oxygen reduction. The oxygen reduction reaction (ORR) is conventionally understood as a four-electron process. However, on polarized graphite electrodes, high overpotentials can shift the pathway toward a two-electron mechanism, resulting in the production of hydrogen peroxide (H_2O_2). Interestingly, many bacteria have evolved to utilize H_2O_2-driven aerobic respiration; for example, gut microbiota decompose host-derived H_2O_2 to generate oxygen, thereby gaining a competitive growth advantage. This phenomenon suggests that H_2O_2 produced via the two-electron ORR may serve as a novel pathway for aerobic survival and energy acquisition in electrotrophic microbes.

In this study, we employed MWCNTs@Ppy-modified electrodes to enhance H_2O_2 production and utilized the electroactive bacterium *Acinetobacter venetianus* RAG-1, isolated from a microbial electrochemical system acclimated over 230 days, as a model microbe to investigate the relationship between H_2O_2 generation and cathodic bioelectrical current. We report a previously undescribed yet significant extracellular electron uptake pathway mediated by inevitably produced H_2O_2, contributing up to 45% of the total biocurrent. This new H_2O_2-based bioelectrochemical respiration depend on the continuous supply of electrons from the electrode and the presence of the catalase *katG*. Selective enhancement of two-electron oxygen reduction on cathode results in a 2.4-fold increase in biocurrent, and both autotrophic biosynthesis and energy production pathways are upregulated to sustain the H_2O_2-based respiration. Our results highlight the importance of two-electron oxygen reduction in bioelectron uptake at the cathode and provide a basis for the design of bioelectricity production systems.



Graphical abstract

ORAL PRESENTATIONS

Session 2 – Reactor Engineering

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Talk 2-1: Electroactive microorganisms and graphite granules: A deal with the devil or divine joy?

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Fixed bed electrode reactors represent a promising development in microbial electrochemical technologies (MET), characterized by their compact arrangement of graphite granules (GGs) and a high electrode surface area-to-reactor volume ratio[1]. These reactors hold considerable promise in different MET applications, but especially for removing contaminants like nitrate and sulfate from groundwater and wastewater[1]. But *all theory is gray*, because single granule voltammetry revealed that the full potential of fixed bed electrode reactors is not yet exploited[2]. This could be caused by a heterogenous potential distribution within the bed electrode[3] or by limited mass transfer leading to inactive or only partially active zones that reduce the overall efficiency. For instance, it is unclear whether GGs are subject to planar or radial diffusion which would improve mass transfer.
But enough words have been exchanged – we performed single-granule voltammetry using the electroactive model organism *Geobacter sulfurreducens* oxidizing acetate to better understand mass transfer processes at granule surfaces. *G. sulfurreducens* biofilms were cultivated at single GGs to four different biofilm thicknesses. The different maturity stages and, hence, biofilm thickness at the GG were defined according to the produced charge and after the experiments validated with qPCR. Subsequently, turnover and non-turnover cyclic voltammetry with three experimental media conditions were performed: i) a low substrate concentration of 1 mM acetate, ii) a weak buffering strength of 10%, and iii) full acetate availability and high buffer capacity as control. The same set of experiments were also performed with *G. sulfurreducens* biofilms at graphite plates (GPs) at which Fick's laws of diffusion are fully applicable. The whole set of data was analyzed with different approaches, including the limiting current concept, mass transfer coefficient, and Randles–Ševčík equation.
First results indicate that a different mass transfer regime determines the performance of *G. sulfurreducens* biofilms at GGs compared to GPs. This was observed especially for weak buffer conditions and was independent of the biofilm maturity. Thus, radial diffusion is *the crux of the matter* and apparently plays a role at GGs although their size of 3-5 mm suggests only linear diffusion processes. The occurrence of radial diffusion impacts the dimensions of the diffusion boundary layer, which is important for assessing the performance and identifying limitations of fixed bed electrode reactors.
Exploring these interactions is a promising strategy to provide further insights into mass transfer limitations at graphite granules, ultimately leading to fixed bed design recommendations for improving their performance.

References
(1) Rodrigo Quejigo, J.; Tejedor-Sanz, S.; Esteve-Núñez, A.; Harnisch, F. Bed Electrodes in Microbial Electrochemistry: Setup, Operation and Characterization. *ChemTexts* 2019, *5* (1), 4.
(2) Ceballos-Escalera, A.; Pous, N.; Korth, B.; Harnisch, F.; Balaguer, M. D.; Puig, S. Ex-Situ Electrochemical Characterisation of Fixed-Bed Denitrification Biocathodes: A Promising Strategy to Improve Bioelectrochemical Denitrification. *Chemosphere* 2024, *347*, 140699.
(3) Quejigo, J. R.; Korth, B.; Kuchenbuch, A.; Harnisch, F. Redox Potential Heterogeneity in Fixed-Bed Electrodes Leads to Microbial Stratification and Inhomogeneous Performance. *ChemSusChem* 2021, *14* (4), 1155–1165.

Talk 2-2: Development and upscaling of gas diffusion electrodes for wastewater treatment and electrosynthesis of chemicals

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Technologies related to gas diffusion electrodes (GDEs) offer solutions for gaseous reagents taking part in electrochemical reactions. (Bio)electrochemical processes suffer from challenges like high costs of platinized electrodes[1]; rapid catalyst degradation and low performance due to non-uniform electrode quality[2]; difficulties in upscaling. Scaling up microbial fuel cells (MFCs) requires use of large electrodes which are often difficult to fabricate without loss in quality. VITO has developed GDEs tailored for systems with aqueous electrolytes and a gas-water interface, which are characterized by controllable pore diameters in the polymer-bound active layer, mechanical robustness and low water permeability[2]. These cold-rolled (VITOCORE®) and phase-inversion based (VITO CASE®) electrodes enable reproducible quality in sizes from 10 cm² to 1 m². Large-scale VITOCORE® air cathodes were recently developed and tested in 85 L and 255 L MFCs to evaluate the impact of the cathode size on MFC performance[4,5]. For CO_2 electroreduction, GDEs based on Sn, Cu and Pd were developed and evaluated for production of formic acid and oxalic acid.

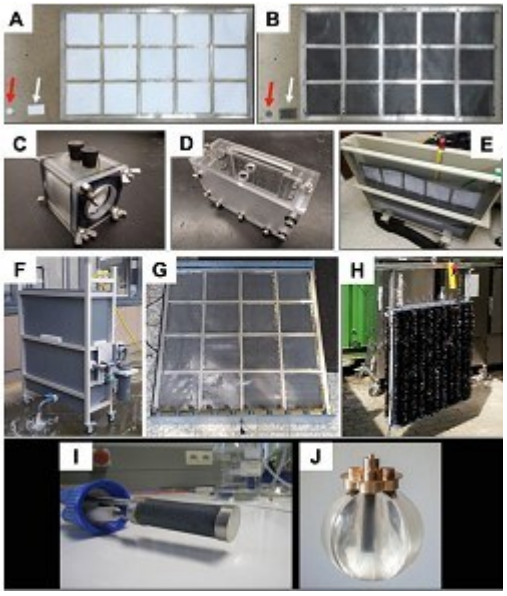

Figure 1. GDE electrode configurations in different sizes and geometries. **(A)** Gas diffusion side of an upscaled GDE **(B)** Electrolyte exposed side of an upscaled GDE. **(C, D)** Lab scale air-cathode MFCs of 10 cm² and 100 cm². **(E)** An 85 L MFC for testing GDE shown in (A) and (B). **(F)** A 255 L MFC reactor **(G)** An upscaled GDE for testing in (F). **(H)** Graphite fibre brush anode used in (F) and tested in combination with (G). **(I)** A tubular GDE. **(J)** A 'spark of life' set up employing a GDE shown in **(I)**.

Acknowledgements: The financial support by H2020 project VIVALDI contract No. 101000441 is greatly appreciated.

References:

[1] Electrode material properties for designing effective microbial electrosynthesis systems. **J. Materials Chemistry A**, 7(42), 24420, 2019.
[2] Alvarez Gallego et al., Development of gas diffusion electrodes for cogeneration of chemicals and electricity **Electrochim. Acta**, 82, 415, 2012.
[4] Rossi et al., Evaluating a multi-panel air cathode through electrochemical and biotic tests. **Wat. Res.** 148, 51, 2019.
[5] Hiegemann et al., Performance and inorganic fouling of a submergible 255 L prototype microbial fuel cell module during continuous long-term operation with real municipal wastewater under practical conditions. **Biores. Technol**., 294, 122227, 2019.

Talk 2-3: Development of a Single-Chamber Microfluidic BES Featuring a Transparent Gas Diffusion Anode for *In Situ* Electrophysiological Investigations of Electroactive Microorganisms

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Oxygen plays a crucial role in the operation of bioelectrochemical systems (BESs) (Rosenbaum, Cotta et al. 2010). Not all microorganisms and bioprocesses thrive in oxygen-rich environments; however, it is crucial for many to support their growth and metabolism. Oxygen also competes with an anode as an electron acceptor in certain systems (Lu, Chan et al. 2017). In conventional BES setups, spatial segregation of planktonic and biofilm forming communities occur when oxygen is supplied via headspace or sparging, complicating the physiological investigations. Moreover, visualizing electroactive biofilms in BES *in vivo* is challenging, as microbial characterization typically occurs post-experiment.
This study aimed at development of a microfluidic BES offering the unique advantage of real time biofilm growth visualization, even while dynamically switching between oxygen concentration (composition ranging from 0% to 21% v/v) and an anode as electron acceptors. This was achieved by a channel-type microfluidic gas diffusion layer, separated from the BES anode by a transparent Polydimethylsiloxane (PDMS) membrane, enabling controlled aeration (or anaerobic) conditions at the BES anode. To verify the accurate gas dosing function of the PDMS membrane and validate the overall infrastructure in the Micro-BES, we investigated the aerobic and anodic metabolism of the model electroactive microorganism *Shewanella oneidensis* MR-1 in the Micro-BES. An electrochemical, secondary metabolite and mediator analysis was performed. Additionally, plasmid-based constitutive GFP expression in *S. oneidensis* was used in this study to record the growth of electroactive biofilm *in-situ* under varying oxygen concentrations. Real-time oxygen quantification was performed by ratiometric fluorescence analysis of oxygen-quenching microsensor particles.
A 0.3 ml microfluidic BES was successfully constructed, allowing for online imaging under diverse operational conditions, including variations in oxygen concentration, under fed-batch, and continuous substrate and mediator availability. Under an anaerobic, fed-batch configuration, a higher max current output was observed, likely due to the higher cell retention time and use of riboflavin for mediated electron transfer. However, the continuous flow of media resulted in a more stable and long-term current generation. A comprehensive approach of simultaneously exposing *S. oneidensis* to the competing electron acceptors, i.e., electrode and oxygen at the same spatial location at varying strengths, is being used to facilitate a thorough functional characterization of the system and to generate new options for physiological understanding of electroactive microorganisms.

Lu, M., et al. (2017). "Effect of oxygen on the per-cell extracellular electron transfer rate of *Shewanella oneidensis* MR-1 explored in bioelectrochemical systems." Biotechnology and bioengineering **114**(1): 96-105.

Rosenbaum, M., et al. (2010). "Aerated *Shewanella oneidensis* in continuously fed bioelectrochemical systems for power and hydrogen production." Biotechnology and bioengineering **105**(5): 880-888.

Talk 2-4: Electrochemical Fluidized Bed Reactor for Versatile and Intensified Electro-Bio Catalysis – Demonstrated by *in-situ* Substrate Generation

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While electro-bio catalysis can offer a sustainable approach to chemical production utilizing renewable electricity, electro-enzymatic and electro-microbial syntheses still commonly rely on reactors with limited scalability (e.g. undivided reactors, H-cells or electrode stacks) typically operated in batch or recirculation modes. Achieving industrial relevance, however, demands scalable reactor designs enabling continuous operation and high space-time yields for intensified and competitive electro-bio catalysis.
We present a novel fluidized bed reactor with a 3-dimensional electrode. The electrode consists of conductive graphite particles. This reactor design enables straightforward scalability through simple particle addition and ensures intensified process conditions through excellent mixing and mass transfer characteristics inherent to fluidized beds. The electrode particles form a fluidized working electrode driven by either gas or electrolyte flow-through, supporting versatile batch-wise or continuous operation. The reactor operates effectively under neutral pH conditions and low electrolyte concentrations, benefiting from its high electrode surface-to-volume ratio and membrane-divided reactor chamber.
Demonstrating its electro-bio performance, we benchmarked the reactor through electrochemical *in-situ* generation of H_2O_2 via oxygen reduction, utilized as co-substrate by the unspecific peroxygenase *Aae*UPO for hydroxylation of ethylbenzoic acid. Tag-based immobilization of the biocatalyst facilitated robust continuous operation, and intensified gas delivery using pure oxygen significantly enhanced reactor performance. Consequently, the reactor achieved total turnover numbers up to 740,000 mol/mol and space-time yields up to 218 g/(L·d), while maintaining high current efficiencies up to 72%.
This versatile and scalable reactor concept is broadly applicable for various electro-microbial and electro-enzymatic applications, providing a promising solution for sustainable industrial electro-bio catalysis.

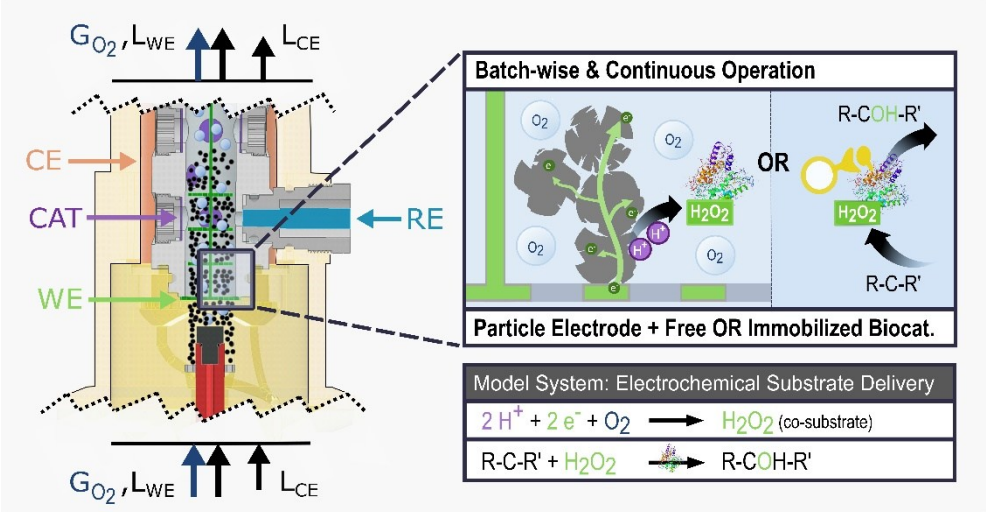

Fig. 1: Schematic of the fluidized bed reactor for electro-bio catalysis (electro-enzymatic and -microbial). Conductive graphite particles form the fluidized working electrode (WE), separated from the counter electrode (CE) by a cation-exchange membrane (CAT). In the 3-electrode setup with reference electrode (RE), gas-driven fluidization (G_{O_2}) and electrolyte flow-through (L_{WE}, L_{CE}) ensure efficient mass transport and mixing. Reactor operation is illustrated for batch-wise (free biocatalyst) and continuous operation (tag-based immobilized biocatalyst). In a model reaction, the reactor system is demonstrated by peroxygenase-driven hydroxylation, utilizing electrochemically *in-situ* generated H_2O_2 as a co-substrate.

Talk 2-5: Electro-fermentation as a path to decarbonize transportation fuels: Organic waste valorization into high-value carboxylates through fluidized bed reactors and chain elongation

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Rapid decarbonization is imperative to alleviate global climate change but the transportation sector will be difficult to fully electrify, demanding alternatives to fossil fuels that can leverage renewable electrons. Food waste presents a cheap and abundant source of renewable carbon that can be used to generate sustainable fuels. However, food waste is often underutilized and contributes additional GHG emissions to the atmosphere through landfilling or incineration.

Electro-fermentation offers a promising strategy to upgrade organic molecules from food waste into sustainable fuels that can help decarbonize the global transportation sector. Despite its potential for decarbonization, the electro-fermentation of food waste into transportation fuel precursors such as medium chain carboxylates (MCCs) is underexplored and requires improvements in rates and selectivity.

This study aims to boost the rates and selectivity of food waste electro-fermentation into MCCs using a fluidized bed reactor containing powdered activated carbon (PAC) coupled with rumen microbes enriched for microbial chain elongation. Two electrochemical H-cell reactors—R1 (control) and R2 (with PAC)—were tested under varying cathodic and anodic potentials. All reactors had a 250 mL working volume, operated via a potentiostat, with a graphite rod as the working electrode and a ruthenium-iridium coated titanium counter electrode. Each voltage condition was tested over two 10-day batch cycles using synthetic food waste (10 g COD/L) as the substrate.

R2 significantly outperformed R1, particularly at -0.8 V vs Ag/AgCl, achieving a caproic acid concentration of 6.14±0.69 g COD/L—more than double that of R1 (2.54±0.57 g COD/L). Enhanced electrochemical activity and reaction kinetics in R2 were attributed to PAC's role in expanding biofilm surface area and facilitating electron transfer. Genomic microbial community analysis revealed distinct spatial distributions across the system, especially on electrodes and PAC particles. Advanced characterization of the extra-cellular polymeric substrates (EPS) showed significant increases in EPS production and the formation of redox-active compounds in the presence of PAC. Overall, these findings highlight the ability of fluidized bed reactors with chain-elongating organisms to improve the rates and selectivity of MCC production from food waste during electro-fermentation. This technology offers a promising pathway for organic waste valorization into sustainable transportation fuels, supporting a circular carbon economy. Efforts towards testing new reactor operation modes, expanding the portfolio of products, and implementing new types of waste streams are ongoing.

Talk 2-6: Establishing a sustainable methanogenic carbon dioxide reduction in bioelectrochemical systems and identification of kinetic and thermodynamic constraints

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The conversion of wastewater treatment plants (WWTPs) into bio-refineries offers a promising route for sustainable carbon reuse and energy recovery. WWTPs emit CO_2-rich flue gases that can be biologically reduced into value-added products such as methane (CH_4) and acetate. Bioelectrochemical systems (BES) provide an efficient platform for this conversion, enabling the direct use of surplus renewable electricity via either direct electron transfer or in situ hydrogen (H_2) generation at high conversion efficiencies[1–3] .
Here, we present recent advancements in a hybrid BES designed for CH_4 production by coupling a zero-gap electrolyzer (ZGE) with a methanogenic bioreactor in a closed-loop system using real wastewater as catholyte. Building on our previous design[4], we implemented step-wise modifications, including the integration of pentlandite-based catalysts[5], which remained stable under wastewater conditions for over 120 days. This enabled long-term operation at high current densities (up to 60 mA cm^{-2}), achieving peak CH_4 production rates of 1074 $L_N\, m^{-2}\, d^{-1}$ (weekly average) - a 61% improvement over our initial system - while keeping with the previous maximal energy efficiency of 42%.
In this system with sequential electrochemical-biological processes, hydrogen acts as an electron shuttle. Thereby, the in-situ H_2 supply, i.e. directly in the growth medium, helps to circumvent challenges arising from low H_2 solubility. Nonetheless, H_2 mass-transfer rates still limit high volumetric productivity. To further optimize system performance, we developed a mechanistic model to estimate biologically available H_2 based on stable carbon isotope ($\delta^{13}C$) measurements. The model incorporates thermodynamic estimations from $\delta^{13}C$ data, along with electrochemical and biological processes[6], gas–liquid transfer, and ion migration at the anode–cathode interface.[6]
Simulations using a multi-compartment framework provide an integrated analysis of key conversion processes and mass transport phenomena. Combined with experimental investigations, we highlight the impact of catalyst stability, anode material selection, and bioreactor architecture on CH_4 productivity. These findings provide critical insights for advancing this scalable solution for decentralized renewable gas generation within WWTPs.

1 Y. Jiang, H. D. May, L. Lu, P. Liang, X. Huang and Z. J. Ren, *Water Res.*, 2019, **149**, 42–55.
2 J. S. Deutzmann, F. Kracke and A. M. Spormann, *Cell Reports Phys. Sci.*, DOI:10.1016/j.xcrp.2023.101515.
3 H. M. Fruehauf, F. Enzmann, F. Harnisch, R. Ulber and D. Holtmann, *Biotechnol. J.*, 2020, **15**, 1–11.
4 R. Rad, T. Gehring, K. Pellumbi, D. Siegmund, E. Nettmann, M. Wichern and U. P. Apfel, *Cell Reports Phys. Sci.*, 2023, **4**, 101526.
5 M. Smialkowski, D. Siegmund, K. Pellumbi, L. Hensgen, H. Antoni, M. Muhler and U.-P. Apfel, *Chem. Commun.*, 2019, **55**, 8792–8795.
6 H. Penning, C. M. Plugge, P. E. Galand and R. Conrad, *Glob. Chang. Biol.*, 2005, **11**, 2103–2113.

Talk 2-7: A novel Anaerobic Cathodic Dynamic Membrane Bioreactor (AnCDMBR) for efficient mitigating fouling and recovering bioenergy from municipal wastewater

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Concerns regarding membrane fouling and suboptimal bioenergy recovery have constrained the implementation of anaerobic membrane bioreactor (AnMBR) for treating low-strength municipal wastewater. This study presents a novel anaerobic cathodic dynamic membrane bioreactor (AnCDMBR) designed to address these challenges. A self-formed cathodic dynamic membrane (CDM) on inexpensive carbon cloth was developed to function as both a membrane and biocathode to achieve dual-function effects of mitigating membrane fouling and accelerating organics conversion. Compared with common dynamic membrane (1.52 kPa/d) and commercial membranes (7.52 kPa/d), the developed CDM presented a significantly reduced fouling rate (1.02 kPa/d), exhibiting the potential as a substitute for high-cost conductive membranes. Furthermore, efficient and stable biomethanation occurred in AnCDMBR with a superior methane yield rate of 0.26 L-CH4/g-COD (CH4 content > 95%), which was 1.42 times higher than the control, linked to the higher activities of microbial metabolism and methanogenic-related key enzymes. Further analysis revealed that electrostimulation-induced niche differentiation of microbiota regulated interspecies interactions between electroactive microorganisms and complex anaerobic digestion microbiomes, facilitating organic matter conversion to methane and leading to superior bioenergy recovery. This study offered a new strategy for effectively mitigating fouling and recovering bioenergy from low-strength wastewater, potentially expanding the application of AnMBRs.

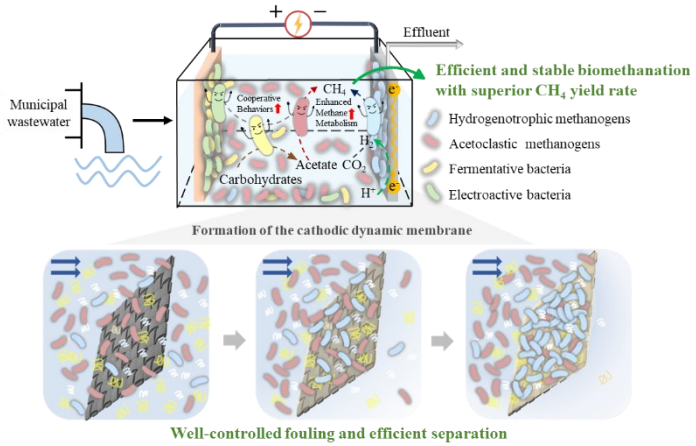

Fig 1. AnCDMBR for treating municipal wastewater

References

[1] Cheng, P., Wang, C., Zhao, X., Liu, J., Xu, X., Zhu, L., 2023. Electroactive biocake layer-driven advanced removal of dissolved organic matter at membrane interface of anaerobic electrochemical membrane bioreactor. *Water Res.* **245, 120618.**

Talk 2-8: Leipzig's vibrancy finds its parallel in the diverse nature of biochars: Revealing the heterogeneous performance of granular biochar cathodes for abiotic hydrogen evolution reaction

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Biochar is emerging as a sustainable and cost-effective cathode material for CO_2-reducing bioelectrochemical systems (BES) [1]. Biocatalysts, such as methanogenic archaea, utilize cathodic electrons, primarily in the form of hydrogen, to convert CO_2 to either mono- or multi-carbon compounds [2]. Therefore, lowering the overpotential (η) for hydrogen evolution reaction (HER) at neutral pH is crucial to develop an energy-efficient BES platform [3]. Furthermore, granular bed-cathodes provide high surface area [4], enhancing the interactions between the biocatalyst and the cathode. This study investigates η for HER at cathodes made of industrial granular biochar (GB) from beechwood and birchwood. Beechwood-based GB pyrolyzed at 740 °C exhibited the lowest η = 223.6 ± 30.0 mV, significantly lower than birchwood-based GB with η = 503.5 ± 4.9 mV and granular graphite with η = 608.3 ± 19.5 mV. The superior performance of beechwood-based GB is attributed to higher electrical conductivity, higher degree of carbonization (Thermogravimetric analysis), favorable H/C ratios, higher structural disorder (Helium ion microscopy & Raman micro-spectroscopy), and suitable porosity (N_2 adsorption-desorption). However, its bulk heterogeneity (Figure 1) suggests uneven temperature distribution during the industrial pyrolysis process. These findings highlight that the heterogeneous performance of GB cathodes for abiotic hydrogen evolution is mirroring Leipzig's dynamic diversity. Alike social and cultural aspects, the wood type and properties influence the performance calling for systematic characterization to achieve optimized production for improved bioelectrochemical system reliability.

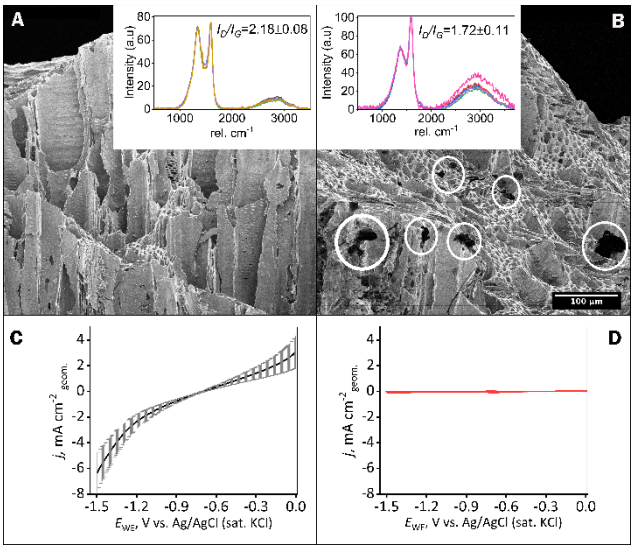

Figure 1. Characterization of granular biochar from beechwood (BEW740). **A** helium ion microscopy (HIM) on BEW740-class1 (inset: Raman micro-spectroscopy); **B** HIM on BEW740-class3; **C** geometric activity for BEW740-class1 cathodes; **D** geometric activity for BEW740-class3 cathodes.

References

[1] Patwardhan SB, Pandit S, Kumar Gupta P, Kumar Jha N, Rawat J, Joshi HC et al. Recent advances in the application of biochar in microbial electrochemical cells. Fuel 2022;311(14):122501. https://doi.org/10.1016/j.fuel.2021.122501.
[2] Kracke F, Deutzmann JS, Gu W, Spormann AM. In situ electrochemical H 2 production for efficient and stable power-to-gas electromethanogenesis. Green Chem. 2020;22(18):6194–203. https://doi.org/10.1039/D0GC01894E.
[3] Jayathilake BS, Chandrasekaran S, Freyman MC, Deutzmann JS, Kracke F, Spormann AM et al. Developing reactors for electrifying bio-methanation: A perspective from bio-electrochemistry. Sustainable Energy Fuels 2022;6(5):1249–63. https://doi.org/10.1039/D1SE02041B.
[4] Rodrigo Quejigo J, Rosa LF, Harnisch F. Electrochemical characterization of bed electrodes using voltammetry of single granules. Electrochemistry Communications 2018;90:78–82. https://doi.org/10.1016/j.elecom.2018.04.009.

ORAL PRESENTATIONS

Session 3 – Microbial Electrosynthesis

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Talk 3-1: Identifying Key Drivers of Product Formation in Microbial Electrosynthesis: A Mixed Linear Regression Analysis

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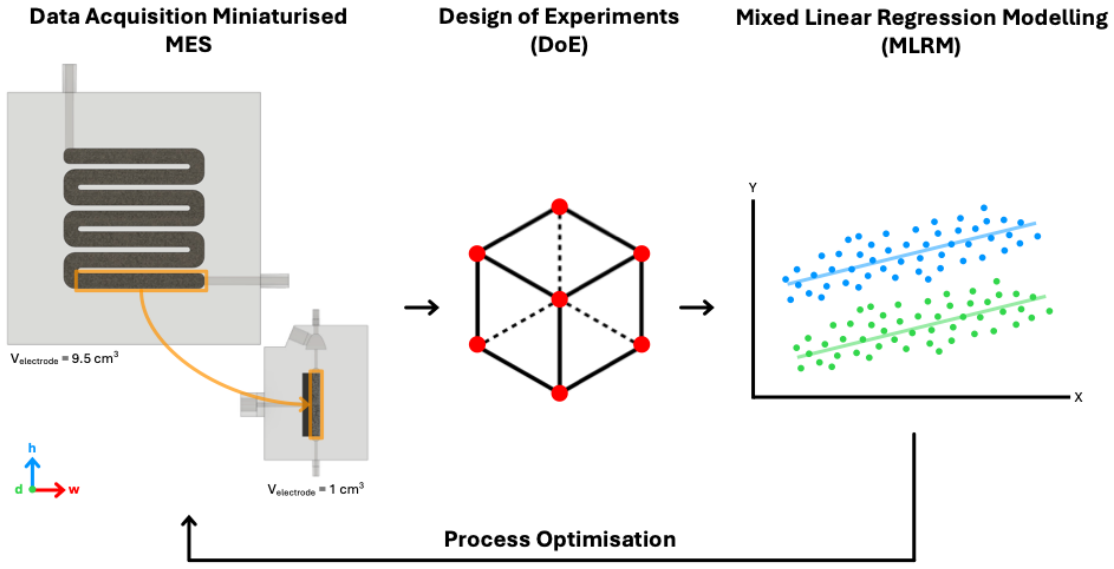
Carbon capture and utilisation technologies are crucial for reducing fossil fuel dependence and transforming the chemical and energy sectors. Microbial electrosynthesis (MES) is a promising technology where electrotrophic microorganisms convert CO_2 into valuable biochemicals using electricity. Given suitable conditions, a biofilm forms on the electrode surface, facilitating electron uptake, cell retention, and chain elongation to a mixture of acetate, butyrate, and n-caproate. Although the effects of individual parameters, such as cathode potential, pH, inorganic carbon source, nutrient availability, H_2 partial pressure, and temperature, have been widely studied, identifying the most influential factors in MES systems and their interactions remains challenging. A deeper understanding of these interplays is required to ultimately push the technology towards industrial implementation. Current models based on MES systems provide valuable insights but lack direct evidence on how microbial communities adapt to changing conditions. This study applies design of experiments (DoE) and mixed linear regression modelling (MLRM) to examine the influence of (1) pH, CO_2, and H_2 partial pressures, (2) pH, CO_2, and acetic acid supplementation, and (3) the addition of tungsten (W) and selenium (Se) on the production spectrum in biofilm-driven miniaturised directed-flow bioelectrochemical reactors. Statistical analysis revealed that pH significantly influenced the production of acetate, butyrate, and caproate, while CO_2 showed a significant impact only on acetate production. Neither hydrogen nor any of the tested parameters had a statistically significant effect on CH_4 production. The addition of acetic acid to the catholyte had a significant impact on butyrate and caproate production, highlighting its role in chain elongation. Lastly, Addition of W and Se in the trace metal solution significantly improves carbon fixation and chain elongation, leading to greater selectivity towards C4 and C6 carboxylic acids. The DoE-MLRMs approach not only reveals critical parameter interactions and optimal operating conditions, but also offers a flexible and adaptable framework for optimising MES and other bioelectrochemical systems.



Talk 3-2: Leveraging Data from Microbial Electrosynthesis: Finding Critical Variables for Ethanol Production

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Microbial electrosynthesis (MES) is a promising approach to utilise CO_2 for the production of value-added compounds such as acetic acid (HA) and ethanol (EtOH). EtOH gained interest due to the energy policies pushing for sustainable fuels. However, its production depends on a multi-stage process, including hydrogen evolution reaction, acetogenesis and solventogenesis, governed by diverse operational variables that traditional methods cannot easily optimise experimental set-ups [1]. This study integrates digitalisation into MES by implementing a real-time control and monitoring system (RTCS) to track and adjust key operational variables (i.e., dissolved CO_2 [dCO_2], pH, pressure, conductivity, temperature and cell potential). This data-driven approach enhances process efficiency, enabling the identification of optimal conditions for selective EtOH production and advancing CO_2-to-ethanol conversion towards commercial viability.
Two multivariate approaches of process data analysis were executed based on experimental results obtained in MES cells; i) a productivity-based patterns discovery, and ii) an operational-based unsupervised clustering using a sequence constrained fuzzy c-means (SCFCM) algorithm and principal component analysis to identify operational phases associated with selective acetic acid (HA), ethanol (EtOH) or concurrent production from CO_2.

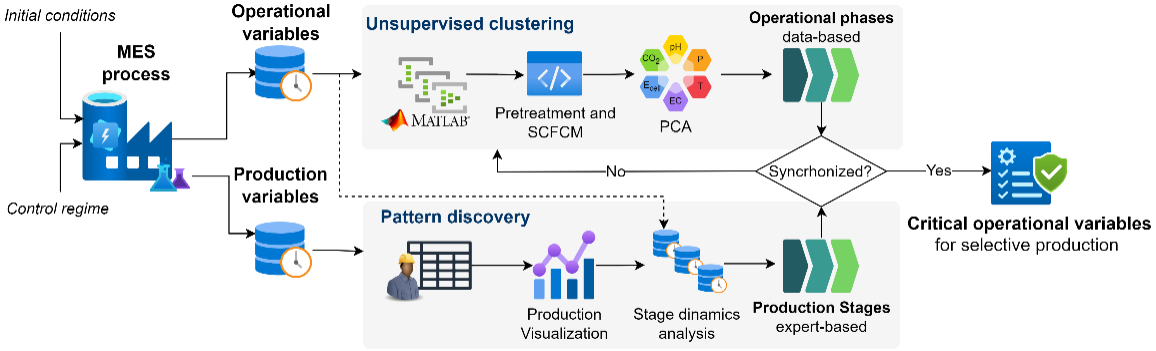

Figure 1. Methodology implemented to mine MES process data.

The RTCS enabled the identification of optimal operational conditions for each production stage, resulting in an ethanol production rate of 14.8 g $m^{-2}d^{-1}$. Process data analysis revealed operation variables presented different correlation and dynamics when EtOH, or HA were produced. The unsupervised clustering based solely on operational data, successfully identified process phases synchronised with the production stages. HA selectivity was mainly characterized by pH variability while EtOH selectivity was mostly represented by both pH and dCO_2 variations. Further control strategies should focus on critical variables for each operational phase by applying statistical monitoring charts and/or soft sensors for EtOH concentration. Overall, mining real-time operational data contributes to MES scalability by predicting production stages and reducing decision-making delays, essential for this technology optimization.

[1] Blasco-Gómez, R., Ramió-Pujol, S., Bañeras, L., Colprim, J., Balaguer, M. D., & Puig, S. (2019). Unravelling the factors that influence the bio-electrorecycling of carbon dioxide towards biofuels. Green Chemistry, 21(3), 684–691. https://doi.org/10.1039/C8GC03417F

Acknowledgements: This research was conducted within the "PANGEA" (PID2021-126240OB-I00) funded by the Spanish Ministry of Innovation and Science. J.A-A received predoctoral support from the Spanish Ministry of Science (PRE2022-101241). S.P. is a Serra Hunter Fellow (UdG AG-575) and acknowledges funding from the ICREA Academia award. LEQUiA [2021-SGR-01352] is recognised as a consolidated research group by the Catalan Government.

Talk 3-3: Microbial Electrochemical Synthesis of Carboxylates from Organic Waste: Microbial Selection and Functional Adaptation to hypersaline conditions

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Introduction
Microbial electrochemical technologies (METs) represent a promising approach to valorizing organic waste by separating oxidation at the bioanode from bio-based chemical synthesis at the biocathode. The taxonomic composition of microbial communities directly influences the spectrum of multicarbon compounds synthesized. Electrolyte salinity is a key performance parameter, reducing internal resistance and optimizing energetic yields. Hypersaline conditions might also constitue a lever for steering microbial selection and metabolic functionality in METs.

This research aims to evaluate the selection, adaptation, and functional characterization of electrosynthetic microbial communities under hypersaline conditions. It focuses on the impact of contrasted electrolyte salinity on microbial assembly and metabolic specialization, targeting the efficient bioconversion of CO2 into carboxylates.

Materials and methods
A microbial electrochemical system was designed with three compartments: (i) a bioanode for organic matter oxidation, (ii) a biocathode for electrotrophic CO2 reduction, and (iii) an intermediate compartment for selective extraction and concentration of carboxylates via electrodialysis. Two inoculation strategies were compared in triplicated labscale reactors: hypersaline communities from salt marsh sediments and industrial digester sludge as a reference non-saline inoculum. The cathode inoculum was heat-treated to enrich homoacetogenic *Clostridia* while eliminating methanogenic archaea. The anode was configured as a flat carbon cloth to maximize electrocatalytic activity, while the cathode consisted of carbon granules to enhance microbial adhesion and electron transfer.

Findings
Experiments demonstrated that the nature of salts in the electrolyte significantly influences catalytic performance. NaCl-based saline conditions enhanced electrochemical efficiency; achieving current densities up to 8 $A.m^{-2}$ as daily averages with real biowaste hydrolysates as substrate and promoting carboxylate synthesis at the biocathode. 16S rDNA sequencing confirmed taxonomic shifts between conditions with *Alkalibaculm* dominated cathodic communities versus *Sporomusa* dominated ones, under saline and non-saline conditions, respectively. Our findings reveal how selective pressures exerted by ionic environment finaly translated into in contrasted microbial community and carboxylate electrosynthesis patterns. Shotgun metagenomics and metabolic modeling from metagenome-assembled genomes (MAGs) are underway to further resolve underlying metabolic processes and community interactions.

Conclusion
This study provides insights into microbial selection in bioelectrochemical systems, demonstrating that salinity-driven adaptation can be used to steer microbial community structure and associated metabolic pathways. Metabolic models of engineered hypersaline electrosynthesis communities are being designed to further optimize the efficiency of CO_2-to-carboxylate conversion.

Talk 3-4: Electrochemistry meets fermentation: from CO_2 to 3-hydroxypropionic acid in a single pot approach

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Carbon capture and utilization strategies seek to transform CO_2 into valuable products, providing a sustainable alternative to fossil-based production. Microbial electrochemical technologies (METs) offer a promising approach by integrating electrochemical CO_2 reduction (eCO_2RR), with microbial bioconversion. In this study, CO_2 is electrochemically reduced to formate, which serves as an energy source for *Komagataella phaffii* to produce 3-hydroxypropionic acid (3-HP), a valuable platform chemical used in biodegradable plastics and specialty chemicals. A key challenge in this integration is to ensure that the medium supports both electrochemical activity and microbial growth. To enhance sustainability, NH_4^+, an essential fermentation nutrient, is recovered from wastewater using a bioelectrochemical system (BES) and incorporated into the fermentation process. This proof of concept demonstrates the potential of METs in developing circular bioprocesses.

The eCO_2RR was carried out in a 1L single-chamber system with an indium-deposited graphite cathode and a platinode anode. NH_4^+ recovery was carried out in a 1L three-chamber BES with N-rich synthetic wastewater in the anode chamber[2]. Electron flow facilitated NH_4^+ transport across a cation exchange membrane, where alkaline conditions in the cathode chamber partially converted NH_4^+ to NH_3. NH_3 was then recovered in a separate chamber using a gas diffusion electrode. Fermentation was conducted in 1L bioreactors with a genetically modified *K. phaffii* strain, utilizing methanol as a carbon source.

To establish a baseline for process integration, a 3-step setup was tested, running each process separately under optimal conditions. Four 1L bioreactors were operated under identical conditions: (1) Control (commercial NH_4^+, no formate), (2) BES-recovered NH_4^+, (3) eCO_2RR-recovered formate, and (4) both BES-recovered NH_4^+ and eCO_2RR-recovered formate. The control reached 6.84 g/L of 3-HP, while BES-recovered NH_4^+ increased titers by 6.4% (7.28 g/L). Formate from eCO_2RR had a stronger effect, boosting production by 16.4% (7.96 g/L). The fully integrated system (BES-recovered NH_4^+ + eCO_2RR-recovered formate) achieved 8.16 g/L, demonstrating the potential of MET-derived resources for fermentation. This trend was also reflected in 3-HP yield per methanol, with the fully integrated system reaching 0.26 g/g, an 18% increase over the control (0.22 g/g). To further optimize integration, a 2-step approach was explored, where eCO_2RR and fermentation were performed sequentially in the same reactor. Extensive medium optimization was required to balance electrochemical activity with microbial growth. The full presentation will also cover results from this strategy, including sequential and continuous operation, where eCO_2RR and fermentation occurred simultaneously. By directly linking eCO_2RR with microbial fermentation and incorporating BES-recovered NH_4^+, this approach enhances resource efficiency, advancing METs as a sustainable bioproduction platform.

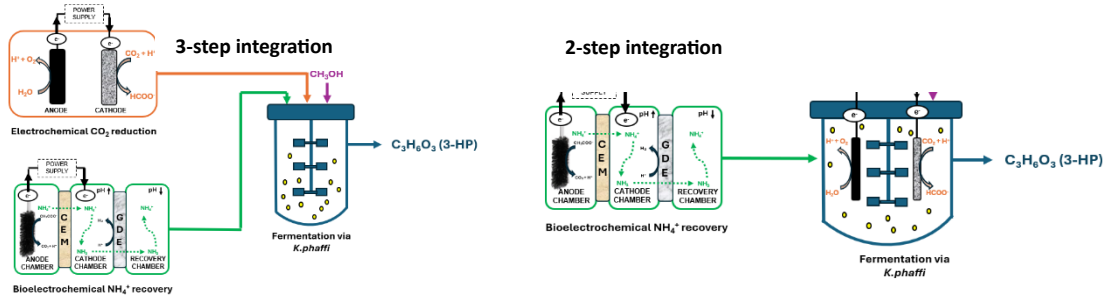

Figure 1: Schematic diagram for process integration of eCO_2RR, BES, fermentation.
[1] Langie et al., Nat. Commun.13,7482 (2022); [2] Galeano et al., Chem. Eng. J. 472, 144855 (2023).

Talk 3-5: There is a Sea of Fog Ahead: The Life Journey of Electrochemical CO_2 Reduction Integrating with Other Technologies

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Observing the life's path in "Wanderer Above the Sea of Fog" by Caspar David Friedrich, 1817.

Electrochemical CO_2 reduction reaction (eCO_2RR) can become a key technology not only for reducing CO_2 but also for producing valuable products with various applications. However, current eCO_2RR products are mainly restricted to C_1-compounds, such as formate and CO, which have limited commercial applications. Integrating electrochemical and biological processes presents a promising approach to converting CO_2 into more valuable chemical products with broader industrial uses. This is due to the diversity in the products through biological pathways. Previously, we discussed that a single reactor configuration can be one of the methods to combine eCO_2RR and biological processes [1].
However, the way to achieve this integration is uncertain and we stand in front of a Sea of fog that is covering the path to wander.
In this study, eCO_2RR to formate in 1 L electrobioreactor was integrated with formate and CO_2 conversion to itaconic acid, a known building block for a large number of compounds such as plastics, using engineered *Komagataella phaffii* and the performance of this integrated technology was evaluated. The reactors were designed in a single chamber configuration in order to take advantage of the oxygen generated at the counter electrode for the aerobic activities of the strains. Over the first 48 h of the experiment, itaconic acid concentration has increased as the formate generated through eCO_2RR, reaching *ca.* 120 mg L^{-1} at hour 48. The rate of itaconic acid production during this time was much higher compared to the sole fermentation processes of *K. phaffii* when formate and CO_2 were manually provided, being *ca.* 25 mg L^{-1}. However, maintaining optimal conditions for both processes was challenging, as the oxidative nature of the counter electrode was detrimental to both product accumulation and the viability of *K. phaffii* cells. Nevertheless, this study demonstrated the potential of integrating these two technologies for CO_2 conversion and the production of highly valuable products. Various strategies used to overcome current challenges and further scale up this technology will be discussed. *In other words, we went ahead and the fog started clearing up.*

Reference

[1] P. Izadi, F. Harnisch, Microbial| electrochemical CO_2 reduction: To integrate or not to integrate?, Joule, 6 (2022) 935-940.

Talk 3-6: Electrochemical-Microbial Harmonies in High Salinity: A *Dittico* Inspired by Bach's Two-Part Inventions Transforming CO_2 into Ectoine

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The transition towards a circular carbon economy requires efficient CO_2 valorization strategies. The electrochemical CO_2 reduction reaction (eCO_2RR) offers a promising route to convert CO_2 into platform chemicals such as formate, which can serve as intermediates for microbial synthesis of value-added products. We show the integration of eCO_2RR with microbial synthesis to produce ectoine, a high-value osmoprotectant used for its protective properties in pharmaceuticals, cosmetics, and biotechnology.

A process for eCO_2RR to formate at halophilic conditions was established using Sn-based gas diffusion electrodes (GDEs) operated in saline electrolyte solutions containing 3%, 10%, and 17% (w/v) NaCl, reflecting natural saline environments. Experiments were conducted in a custom-built reactor [1], operated in flow-through mode with electrolyte solution recirculation and continuous gas and liquid phase monitoring [2], enabling to establish a near-complete electron balance throughout the process. Current density of 50 mA cm^{-2} yielded stable formate production rates of 1.30 ± 0.13 mmol L^{-1} h^{-1} cm^{-2} across all salinities. High salinity poses challenges for maintaining reaction selectivity and long-term stability [3] that is potentially also due to reduced CO_2 solubility. However, the use of GDEs effectively mitigated CO_2 supply limitations, ensuring stable delivery of formate across all tested salinities. Energy efficiency was enhanced with increasing salinity by reducing ohmic losses, along with a likely contribution from partial replacement of the oxygen evolution reaction (OER) with the chlorine evolution reaction (CER) at the anode. When using 10% NaCl, a 52.1% decrease in cell voltage and a 178% increase in energy efficiency were observed when compared to sodium phosphate buffer.

To assess the potential of formate as a C1 substrate for ectoine biosynthesis, cultivations were carried out using the halophilic bacterium *Methyloligella halotolerans* in minimal media supplemented with formate, methanol, or electrochemically generated formate (e-formate) at 20 mmol L^{-1} concentration. While formate-based growth presented energetic limitations compared to methanol, ectoine production was observed under all conditions, with concentrations reaching 2.57 ± 0.81, 1.10 ± 0.04 and 0.62 ± 0.20 µg mL^{-1} for methanol, formate and e-formate, respectively. These results establish a proof-of-concept pathway for the biotechnological production of fine chemicals from CO_2 via electrochemically produced intermediates, using an integrated approach allowing to use saline wastewater streams. This two-step synthesis, an electrochemical-microbial *dittico*, brings together distinct but harmonized processes for the transformation of CO_2 into ectoine.

REFERENCES

[1] Stöckl, M.; Harms, S.; Dinges, I.; Dimitrova, S.; Holtmann, D. From CO_2 to Bioplastic – Coupling the Electrochemical CO_2 Reduction with a Microbial Product Generation by Drop-in Electrolysis. *ChemSusChem* 2020, *13* (16), 4086–4093.
[2] Izadi, P.; Song, J.; Singh, C.; Pant, D.; Harnisch, F. Assessing the Electrochemical CO_2 Reduction Reaction Performance Requires More Than Reporting Coulombic Efficiency. *Adv. Energy Sustain. Res.* 2024, *5* (6), 2400031.
[3] Kas, A.; Izadi, P.; Harnisch, F. High Salt Electrolyte Solutions Challenge the Electrochemical CO_2 Reduction Reaction to Formate at Indium and Tin Cathodes. *ChemElectroChem* 2023, *10* (23), e202300311.

Talk 3-7: Overlooked Yet Critical: Catholyte in Microbial Electrosynthesis

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Biofilm-driven Microbial Electrosynthesis (MES) reactors have shown to be able to produce a variety of products, including methane, alcohols, and medium chain carboxylic acids (MCCAs) solely from CO2 and electricity on a minimal medium. In MES, this medium supplies all nutrients, yet its composition has been adopted from other biotechnologies, thus overlooking specific needs of MES. For this reason, we examined how catholyte design impacts MES performance at microbial, electrochemical, and process levels. We highlight mismatches in metal availability, electrode interactions, and medium origins from many reported MES experiments. One of those mismatches was found to be the specific metals supplied for the production of methane, alcohols, or MCCAs. Even though the metabolic pathways are comparable, the types and amount of metal-dependent enzymes were found to be varying between the pathways, with methane production utilizing the least amount of metalloenzymes, and alcohol production requiring the most. Taking this and several process design attributes into account, we propose a flowchart for qualitative medium design, which should serve as a starting point for critically evaluating existing medium design. This analysis highlights the need for more generalized methods for medium design evaluation, thereby matching medium composition with process-specific requirements.

Talk 3-8: Iron-Regulated Electrochemical–Microbial Conversion of CO_2 to PHB

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The electrochemical and thermochemical conversion of CO_2 are widely studied for carbon utilization, but are often limited by low selectivity and short carbon-chain products. In contrast, inorganic–biological hybrid systems offer a promising route for the selective production of multi-carbon compounds. In this study, we developed a hybrid platform coupling water electrolysis with *Cupriavidus necator* H16 to convert CO_2 into poly-β-hydroxybutyrate (PHB), using electrolytically generated H_2 as the energy and reducing source for microbial carbon fixation. To enhance the efficiency of this system, we first investigated the role of iron ions in the electrolyte. By tuning Fe ion concentration via electrochemical regulation, we reduced interfacial electron transfer resistance and suppressed reactive oxygen species (ROS) generation. Iron ions were also found to facilitate hydrogen mass transfer and modulate key enzymes involved in hydrogen and electron transfer within *C. necator* H16. Building on these findings, we further engineered the microbial interface using nitrogen-doped carbon-supported iron single-atom catalysts (Fe-ISA). Surface modification of *C. necator* H16 cells enhanced local hydrogen availability and promoted H_2 dissociation into protons and electrons, improving ATP generation. Meanwhile, ISA catalyzed ROS decomposition, protecting cells from oxidative stress.By integrating electrochemical tuning and microbial interface engineering, our hybrid system achieved a record PHB production of 1.06 g L^{-1} from CO_2, representing a significant advancement in artificial photosynthesis and carbon-neutral biomanufacturing.

ORAL PRESENTATIONS

Session 4 – Environmental BES - Nitrogen Cycling

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Talk 4-1: Strong electric fields within Anammox granules influence nitrate and ammonium fluxes

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In this study, we present the first evidence of strong electric fields developing naturally within Anammox granules. Granules were collected from two wastewater treatment plants in Aarhus, Denmark, and ranged in diameter from 0.3 to 3 mm. Electric fields were measured as an increase in electric potential with depth using microsensors. In freshly collected granules (1-3 days old) incubated at 32°C, electric fields ranged from 145 to 319 V/m (n. 10). Mathematical modeling suggests that negative charges associated with bacterial cell walls and extracellular polymeric substances inside the granules act as weak ion exchangers for soluble cations, thereby generating diffusion potentials. The electric fields stimulate the movement of NH_4^+ towards the core of the granule while slowing the flux of negatively charged NO_2^-. Under electric fields of 120 V/m, the contribution of ionic migration to the total ion flux matched in magnitude the contribution of molecular diffusion. Our data indicate that neglecting ionic migration could result in significant errors in estimating mass transport and thereby in identifying limiting reactants and reaction rates within Anammox granules, and potentially in a broader range of natural and artificial biofilms.

Talk 4-2: Inorganic Bioelectric System for Deep Removal of N_2O at low temperatures

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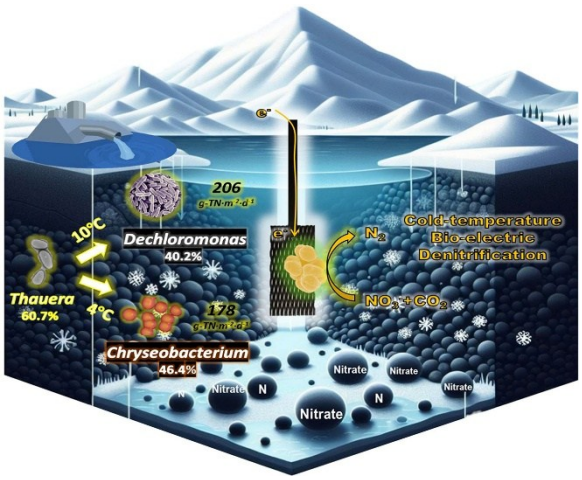
Nitrate contamination in groundwater poses significant ecological and public health challenges, particularly under low-temperature conditions and limited organic carbon availability. This study presents an inorganic bioelectric remediation (BER) system designed for efficient hydrogenotrophic denitrification at temperatures between 4–10°C, while minimizing nitrous oxide (N_2O) emissions, providing a sustainable in-situ groundwater treatment solution.

The BER comprised a stainless steel mesh cathode and an IrO_2-coated titanium anode, operating in both batch and continuous modes with synthetic and actual nitrate-contaminated groundwater. Microbial communities were enriched from a Danish drinking water treatment plant and acclimated to lower temperatures. Performance metrics included nitrogen removal efficiency, N_2O emissions, and system resilience to operational disruptions. Microbial composition and functional gene abundance (e.g., *narG*, *nirS*, *nosZ*) were analyzed using 16S rRNA sequencing and qPCR to understand temperature-driven adaptations.

The BER achieved total nitrogen (TN) removal efficiencies of 95.4% at 10°C and 90.9% at 4°C with a 2-hour hydraulic retention time (HRT). Remarkably, under a high nitrate load (240 g $N \cdot m^{-3} \cdot d^{-1}$) at 1-hour HRT, TN removal rates reached 206.0 ± 6.3 g $N \cdot m^{-3} \cdot d^{-1}$ at 10°C and 178.3 ± 9.4 g $N \cdot m^{-3} \cdot d^{-1}$ at 4°C, while N_2O emissions remained <2% of TN removed due to balanced production and reduction rates, confirmed by *nosZ* abundance. The system demonstrated resilience, recovering within 3 days after a power outage (10°C) and 8 days after a flow interruption (4°C). Coulombic efficiencies reached 82% at 10°C and 71% at 4°C with a 1-hour HRT, surpassing previous bioelectrochemical systems.

Gene analysis indicated minimal dissimilatory nitrate reduction to ammonium (DNRA), favoring complete denitrification. Microbial analysis indicated distinct dominant genera: *Dechloromonas* (40.2%) at 10°C and *Chryseobacterium* (46.4%) at 4°C, highlighting adaptation to cold environments. Functional genes (*narG*, *nirS*) were enriched in biofilms, whereas *norB* and *nosZ* were more prevalent in suspended biomass, suggesting spatial stratification of denitrification processes.

This research advances bioelectrochemical denitrification at low temperatures, achieving high efficiency without chemical additives. It emphasizes the potential for tailored microbial consortia and electrode designs in cold environments, presenting a scalable, energy-efficient approach to tackle nitrate pollution in groundwater. Future work will focus on optimizing operational parameters and field-scale implementation to enhance practical sustainability.



Graphical Abstract

Talk 4-3: Bioelectrochemical ammonium recovery from high-N strength effluents

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Ammonium (NH_4^+) recovery is crucial for creating a more sustainable and resilient future by ensuring food security, protecting the environment, offering an alternative to the energy-demanding Haber-Bosch process and promoting resource efficiency. Traditional methods like air stripping or reverse osmosis are energy and resource-intensive, impacting environmental sustainability. Microbial electrochemistry leverages an electric gradient between anode and cathode to recover NH_4^+. Although NH_4^+transfer rates may be lower compared to electrochemical methods, MET allow for milder operating conditions that significantly enhance energy efficiency, particularly when waste organics are employed as a substrate.

The full presentation will comprehensively summarize the experimental results of 3 years of work under a three-chamber MET configuration with multiple replicates under batch and continuous conditions (Figure 1d). In short, organic matter is oxidized at the anode side and electrons flow from the anode to the cathode since a potential is applied in the cell. The charge balance due to the electron transport is balanced by cation transport over the cation exchange membrane (CEM) to maintain electroneutrality. Therefore, NH_4^+ and other cations are concentrated in the cathode compartment. NH_4^+ cations, once transported into the catholyte, are converted into NH_3 molecules because H_2 production increases the pH of the catholyte. NH_3 can pass through the pores of the hydrophobic membrane and is absorbed by a strong acid on the other side to produce an ammonium salt. The hydrophobic membrane prevents the permeation of other ions, enhancing ammonia purity in the recovery solution.

The full presentation will critically discuss the results under batch and continuous mode to better understand the effect of key parameters on ammonium recovery performance: applied voltage, hydraulic retention time, pH and influent conditions. The results obtained (Figure 1 a) are among the best reported on the literature with high NH_4^+ recovery rates (>150 g $N/m^2/d$) or NH_4^+ recovery efficiency (>80%) at high current densities (around 10 A/m^2). Moreover, we could obtain custom-made effluents at a desired pH (Figure 1 b and c) with different recovery acids: sulfuric, nitric and phosphoric. We will compare the results from those obtained under pure electrochemical conditions and will provide perspectives of the application of this novel technology in a real environment.

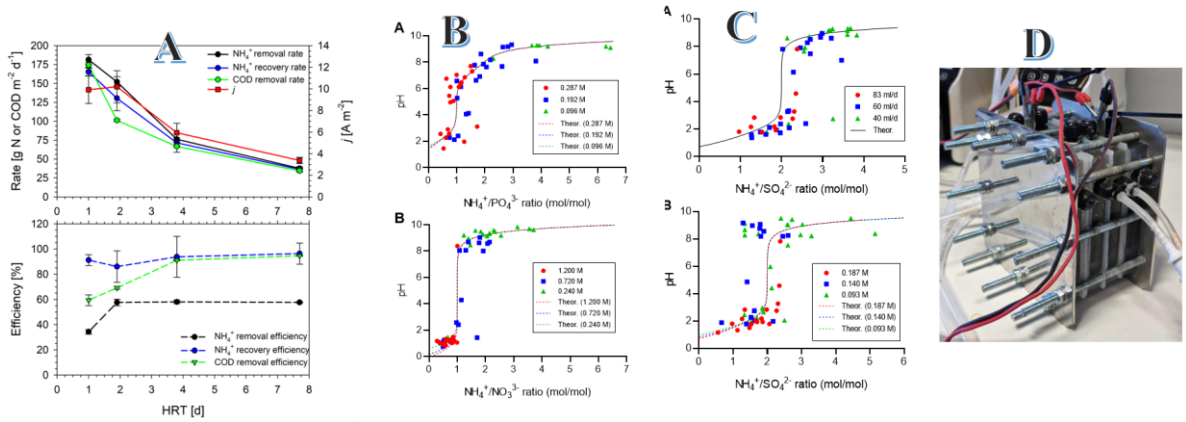

Figure 1 a) NH_4^+ recovery rate and efficiency at different HRTs, b-c) effluent pH at different influent NH_4^+/anion ratios obtained by changing the acid solution concentration or flowrate and d) image of one of the MET set-ups.

Talk 4-4: Unveiling the Unknowns: Investigating the Unexplored Facets of Nitrite Oxidation in Bio electrochemical Systems

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Nitrite oxidation is essential for maintaining nitrogen balance in ecosystems. The accumulation of nitrites contributes to nutrient pollution, leading to eutrophication, which results in excessive algal growth and oxygen depletion in water bodies[1]. Traditionally, nitrite oxidation relies on aerobic nitrification, a process that requires oxygen supplied through aeration, consuming significant amounts of energy (approximately 2 kWh/kg O_2)[2]. To minimize oxygen requirements, electrochemical oxidation of NO_2^- to NO_3^- is a promising option. Typically, the electrochemical oxidation of NO_2^- to NO_3^- thermodynamically requires applied potential of 0.34 V vs. SHE at pH 7[2]. However, in practical applications, the required applied potential is usually higher than the thermodynamic value due to overpotentials (activation losses), mass transfer or biokinetic barriers[1]. Therefore, the typical applied potential in neutral and alkaline conditions ranges from 1 to 1.8 V vs. SHE[3], which requires a significant amount of energy for oxidation. In this study we unveil an interesting phenomenon, whereby electroactive bacteria use electrodes as electron acceptors for the oxidation of NO_2^- to NO_3^- at low overpotential barriers[4].

Materials & methods: For this purpose, laboratory-scale 300 mL microbial electrochemical cells (MECs) were assembled. MECs were equipped with a graphite electrode at the anode, a platinum wire electrode as the cathode, and an Ag/AgCl reference electrode. The reactors were inoculated with mixed microbial culture of activated sludge from an activated sludge plant in Melbourne, Australia. The initial pH of the synthetic wastewater was maintained at 7.0, and the dissolved oxygen (DO) was removed by purging high-purity N_2 gas continuously at a flow rate of 0.3 mL/min. The applied potential provided was 0.8 V vs. SHE.

Results: The results demonstrated that 12 mM of NO_2^- was completely oxidized to NO_3^- within two weeks in the microbial electrolysis cell (MEC) (Fig. 1a). The process exhibited a high coulombic efficiency (~80%), indicating efficient electron utilization. The complete oxidation of NO_2^- to NO_3^- was facilitated by microbial catalysis, wherein electrogenic microorganisms acted as biocatalysts to drive the oxidation reaction, as demonstrated by turnover and non-turnover cyclic voltammetry (CV). A noticeable turnover sigmoidal oxidation wave matched the non-turnover CV peak at 0.8 V (Fig. 1b), suggesting that microbial electrochemical activity was responsible for nitrite oxidation. No appreciable nitrite oxidation was observed in an abiotic control at the same applied potential. Proteomic analysis revealed significant variations in protein intensities under applied and non-applied (control) potential conditions, signifying metabolic adaptations of the microbial community in response to the applied potential. A Student's t-test for differentially expressed proteins confirmed statistically significant differences between applied and non-applied potential conditions ($p < 0.05$), suggesting that specific proteins were upregulated under applied potential conditions. Overall, this study provides compelling evidence for microbial anaerobic NO_2^- oxidation at a low overpotential barriers, suggesting the existence of an alternative biochemical pathway for nitrite oxidation in natural environmental settings. This finding has profound implications for bioelectrochemical nitrogen cycling.

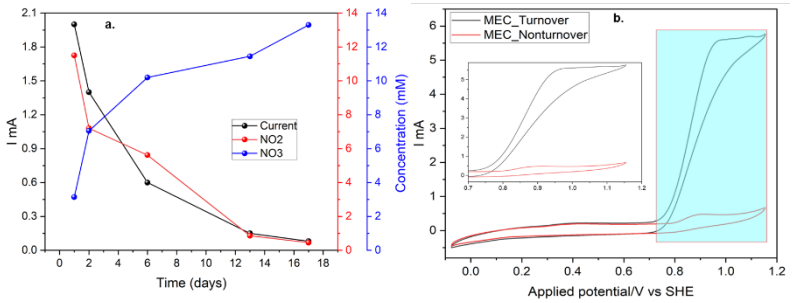

Fig 1. a) Concentration and current, b) Cyclic voltametry of turnover and non-turnover.

[1]Wu, L., et al (2022). Nitrite oxidation in oxygen-deficient conditions during landfill leachate treatment. *Environmental Research*, *214*. https://doi.org/10.1016/j.envres.2022.114090

[2]Sun, J. et al (2016). Reducing aeration energy consumption in a large-scale membrane bioreactor: Process simulation and engineering application. Water Research, 93, 205–213. https://doi.org/10.1016/j.watres.2016.02.026

[3] Wargon J.A., & Arvía A.J. (1972). Kinetics and mechanism of the electrochemical oxidation of nitrite ion dissolved as sodium nitrite in dimethylsulphoxide. Electrochemical Acta

[4] Wang, Y., Laborda, E., & Compton, R. G. (2012). Electrochemical oxidation of nitrite: Kinetic, mechanistic and analytical study by square wave voltammetry. *Journal of Electroanalytical Chemistry*, *670*, 56–61. https://doi.org/10.1016/j.jelechem.2012.02.016

Talk 4-5: Bioelectrocatalytic Membrane Reactor for Nitrate Reduction: Integration of *Thiobacillus denitrificans* with a CNT-Coated UF Membrane

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Background information: Electrocatalytic membrane reactors (ECMRs), merging electrochemical technologies with membrane separation hold remarkable potential to selectively remove specific pollutants from contaminated water while simultaneously producing ultra-filtrated effluents [1]. Despite being a promising technology, ECMRs still face numerous challenges such as limited electrode stability and sluggish and unstable catalytic activity, particularly when treating complex water. Integrating ECMRs with living, self-regenerating microbial biocatalysts presents a transformative approach, offering enhanced catalytic efficiency, improved long-term stability, and a more cost-effective, sustainable water treatment solution. This study aims to develop a bioelectrocatalytic membrane reactor that integrates a carbon nanotube (CNT)-coated ultrafiltration (UF) membrane as a cathodic platform, enabling the biologically driven reduction of nitrate to nitrogen gas by *Thiobacillus denitrificans*.
Experimental setup: The ECMR consisted of a single-chamber reactor equipped with a carbon cloth anode and a CNT-coated conductive membrane cathode. The system was operated in galvanostatic mode (2 mA). The reactor configuration included an inlet, a retentate line and an outlet line for permeate collection. The system was operated under both batch and continuous flow conditions. Both synthetic solutions and real groundwater (Navata, Spain) were tested to evaluate the process performance.
Main results: Preliminary abiotic batch tests using a synthetic nitrate solution (95 mgN L^{-1}) achieved a maximum nitrate removal rate of 72 mgN m^{-2} d^{-1}. Remarkably, inoculation with *Thiobacillus denitrificans* led to a 12-fold enhancement, reaching an average nitrate reduction rate of 866 ± 129 mgN m^{-2} d^{-1} without the accumulation of intermediates such as NO_2^-, N_2O or NH_4^+. To evaluate the process robustness, the ECMR was operated in continuous-flow mode with real nitrate-contaminated groundwater, sustaining an average nitrate removal rate of 1415 ± 94 mgN m^{-2} d^{-1} (HRT 0.2 d) for over 40 days at a permeate flow rate of 26 L m^{-2} h^{-1}. Electrochemical impedance spectroscopy (EIS) measurements revealed a decrease in charge transfer resistance due to due to bacteria accumulation on the electrode, enhancing stability and activity without affecting membrane permeability.
Conclusions: This study demonstrates the feasibility of integrating electrocatalytic ultrafiltration technologies and electro-bioremediation for nitrate removal. The combined system exploits the dual benefits of advanced filtration and microbial denitrification, achieving high nitrate removal rates and producing high-quality water effluents.

References:

[1] A.O. Rashed, C. Huynh, A. Merenda, S. Qin, M. Maghe, L. Kong, T. Kondo, J.M. Razal, L.F. Dumée, Electrocatalytic ultrafiltration membrane reactors designed from dry-spun self-standing carbon nanotube sheets, Chem. Eng. J. 458 (2023). https://doi.org/10.1016/j.cej.2023.141517.
[2] A. Ceballos-Escalera, N. Pous, P. Chiluiza-Ramos, B. Korth, F. Harnisch, L. Bañeras, M.D. Balaguer, S. Puig, Electro-bioremediation of nitrate and arsenite polluted groundwater, Water Res. 190 (2021) 116748. https://doi.org/10.1016/j.watres.2020.116748.

Talk 4-6: Nitrogen recovery from pig slurry using four submerged MEC units

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The management of slurry in pig farms presents a significant environmental challenge due to ammonia (NH_3) and greenhouse gas (GHG) emissions. These emissions contribute to air pollution, climate change and water pollution [1]. Simultaneously, nitrogen in slurries, if recovered, represents a resource for producing organic fertilizers, reducing the reliance on synthetic fertilizers. Bioelectrochemical systems (BES) are a promising biotechnological approach that allows the recovery of ammonia from livestock manure while potentially mitigating emissions [2]. This study focuses on the design, construction, and operation of a new submerged Microbial Electrolysis Cell (MEC) system aimed at ammonium (NH_4^+) recovery and emission reduction from pig slurry.

The system comprises four flat-plate single-compartment MEC modules (60x60 cm each) arranged vertically on a support structure submerged in a 1 m^3 swine slurry storage tank. Each module consists of a stainless-steel mesh supporting a carbon felt layer (anode) on both external sides of the reactor where microbial biofilm develops in contact with the pig slurry. The cathode, also made of stainless-steel mesh, is located within the module and is in contact with a saline solution (NaCl 0.1 g/L) to recover ammonium. A cation-exchange membrane (CEM) separates both anodes from the cathode compartment, facilitating selective ammonium migration. The modules are connected in parallel to a DC power supply applying an initial current density of 15 mA/cm^2.

A preliminary pilot-scale experiment, using a different design, based on four-unit tubular MEC, was conducted in two 1 m^3 slurry storage tanks, one equipped with a MEC and the other serving as a control, operating for 117 days. The MEC system recovered 4.25% of the total nitrogen in the slurry. The MEC tank demonstrated a reduction in methane emissions, with a lower emission rate (0.09 $mg/m^2/s$) compared to the control tank (0.15 $mg/m^2/s$).
This new flat-plate prototype will be submerged in the tank for 2-3 months to evaluate the recovery rate of the system, monitoring the concentration of ammonia in the slurry and in the recovery solution. The pH will be monitored to avoid the conversion of ammonium (NH_4^+) to ammonia (NH_3), which could volatilize and lead to nitrogen losses. The ammonia and GHG emissions will be monitored with laser and photoacoustic technologies.

ACKNOWLEDGEMENTS:
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REFERENCES:
[1] Philippe, F. X., & Nicks, B. (2015). Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. *Agriculture, Ecosystems & Environment*, *199*, 10-25.
[2] Cerrillo, M., Riau, V., & Bonmatí, A. (2023). Recent advances in bioelectrochemical systems for nitrogen and phosphorus recovery using membranes. *Membranes*, *13*(2), 186.

Talk 4-7: Ammoniacal Nitrogen Recovery in Bioelectrochemical Reactors using Real Reject Water from Biogas Plant

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The ammoniacal nitrogen (NH_4-N) in wastewaters can lead to eutrophication depleting dissolved oxygen from water bodies[1]. Therefore, recovery of ammoniacal nitrogen is necessary but challenging in terms of energy inputs. Bioelectrochemical systems are capable of NH_4-N removal and have proven energy efficient at laboratory scale[2].

The present study aims at NH_4-N removal in 2-chamber bio-electrochemical systems. Initially, removal efficacy was studied in small scale reactors (SSR) with anodic working volume of 38 mL, which was scaled up to 1 L working volume reactors (1-LR) stable performance of NH_4-N removal. The pretreated graphite granules were used as anode and stainless steel or titanium mesh as cathode, separated by cation exchange membrane. These reactors were first fed with synthetic wastewater containing 1.5 g/l of NH_4-N and later fed with real wastewater with NH_4-N concentration of 2.5 g/l and COD of 180 mg/L.

The SSR resulted in peak current density of 2.44 A/m^2 using synthetic wastewater. In terms of the NH_4-N recovery, SSR resulted in 79 to 81% NH_4-N removal with hydraulic retention time (HRT) of 1.56 hrs (continuous mode and 13.4 hrs in fed batch), while 1-LR had improved NH4-N removal efficiency of 82-84% with HRT of 8.2 hours and current density of 1.02 A/m^2 The reactor performances were further investigated by using real reject water from biogas plant treating sewage sludge (Nokia, Finland) resulted in around 58% % NH4-N removal in a fed batch mode of the reactor operations with current density of 1.43 A/m^2.

When comparing with other methods of NH_4-N removal from reject waters, e.g bioelectroconcentration, the NH_4-N removal in BES at laboratory scale resulted in higher removal efficiency (84%) in comparison to bioelectroconcentration (75.5%) [3]. This study also aims to scale up the results obtained in the 1 L BES reactors to 75 L pilot scale BES reactors, which has a sandwiched cathode between the two anodes, and further compare the NH4-N removal efficiencies upon scaling-up.

Keywords: Ammoniacal nitrogen, Bioelectrochemical system, Biogas reject water, Sandwiched cathode, Pilot scale BES

References

[1] E. E. Licon Bernal, C. Maya, C. Valderrama, and J. L. Cortina, "Valorization of ammonia concentrates from treated urban wastewater using liquid–liquid membrane contactors," *Chem. Eng. J.*, vol. 302, pp. 641–649, Oct. 2016, doi: 10.1016/j.cej.2016.05.094.

[2] M. B. Galeano, M. Sulonen, Z. Ul, M. Baeza, J. A. Baeza, and A. Guisasola, "Bioelectrochemical ammonium recovery from wastewater: A review," *Chem. Eng. J.*, vol. 472, p. 144855, Sep. 2023, doi: 10.1016/j.cej.2023.144855.

[3] V. Koskue, S. Freguia, P. Ledezma, and M. Kokko, "Efficient nitrogen removal and recovery from real digested sewage sludge reject water through electroconcentration," *J. Environ. Chem. Eng.*, vol. 9, no. 5, p. 106286, Oct. 2021, doi: 10.1016/j.jece.2021.106286.

Talk 4-8: Integration of Microbial Fuel Cells with Simultaneous Partial Nitrification, Anammox, and Denitrification (SNAD) for Energy-Efficient Nitrogen Removal from Wastewater

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The anammox process, while effective for nitrogen removal in reject wastewater, faces challenges in mainstream applications due to high C/N ratios and residual nitrate in effluent. The Anammox process gets inhibited over high C/N ratios (Singh et al., 2022), and requires external nitrite as an electron acceptor. In this study, all these limitations were addressed by the integration of microbial fuel cells (MFCs) with an advanced anammox-based process - SNAD (Simultaneous Partial Nitrification, Anammox, and Denitrification) for sustainable and energy-positive wastewater treatment. A dual-chamber H-shaped MFC (250 mL per chamber) with 2 mm thick carbon felt electrodes sieved with titanium wires as current collectors was integrated with a 24-hour cycle SNAD reactor of the same volume. Dissolved Oxygen (0.2 - 0.7 mg/L), pH, and temperature were strictly regulated using the IKS aquastar system for SNAD startup. The reactors were individually optimized and then integrated to treat high C/N ratio(3.5 to 4.75) wastewater. A control SNAD reactor, fed directly without MFC pretreatment, was used for comparison. The system was tested with synthetic feed and validated with mainstream municipal wastewater (C/N = 3.5) from the Kleinsteinbach Wastewater Treatment Plant. The integrated MFC-SNAD system fed with mainstream municipal wastewater achieved an ammonium nitrogen removal efficiency of 99.8 ± 0.1%, total nitrogen removal efficiency of 93.3 ± 0.4%, and COD removal efficiency of 91.9 ± 0.3%. Stoichiometric model-based evaluation of the SNAD system revealed that 56.9% of nitrogen transformation was through partial nitrification, 33.9% was through an anammox process, and the remaining 9.2% of ammonium nitrogen was through the denitrification process. The total internal resistance of MFC was found to be 270.1 ohms, and the maximum power density of 301.2 mW/m^2 was obtained from the polarization plot. Electrochemical characterization of MFC was performed, and the cyclic voltammogram curve shape indicates direct electron transfer rather than mediated electron transfer. The Nyquist plot, obtained through EIS, showed a charge transfer resistance of 58.1 ± 0.5 ohm in the MFC. The coulombic efficiency of the MFC was calculated to be 34.4 ± 0.6%, while the Normalized Energy Recovery was 0.3 ± 0.01 kWh per kg COD. Results of the relative abundance of the microbial community at the phylum level in 0th-day inoculum sludge and 80th-day SNAD reactor sludge, respectively, revealed that the phylum *Pseudomonadota* has a dominant increase from 14.30% to 35.15%, which includes denitrifiers(e.g., *Thauera*) and Partial nitrifiers(e.g., *Nitrosomonas*). The phylum *Nitrospirota* also showed proliferation from 0.06% to 0.33% which includes Nitrite Oxidizing Bacteria (NOB) like *Nitrospira*, which is undesirable in the SNAD process and to inhibit the activity of NOB, chemical inhibition was applied by using 5 mg/L Nitrogen of hydroxylamines in the synthetic feed (Zhao et al., 2022). The phylum *Planctomycetota*, which includes anammox bacteria, showed a decline from 3.25% to 2.57%, possibly due to competition with partial nitrifiers, NOB, and denitrifiers. The integrated MFC-SNAD system achieved 93.3 ± 0.4% total nitrogen removal efficiency, outperforming the control SNAD reactor's 74.7 ± 8.8% efficiency while treating municipal wastewater (C/N = 3.5). Future research will focus on upscaling and further inhibiting Nitrite Oxidizing Bacteria (NOB) through advanced chemical or operational strategies.

Singh, V., Ormeci, B., Mishra, S., & Hussain, A. (2022). Simultaneous partial Nitrification, ANAMMOX and denitrification (SNAD)–A review of critical operating parameters and reactor configurations. *Chemical Engineering Journal*, *433*, 133677.
Zhao, J., Lei, S., Cheng, G., Zhang, J., Shi, B., Xie, S., & Zhao, J. (2022). Comparison of inhibitory roles on nitrite-oxidizing bacteria by hydroxylamine and hydrazine during the establishment of partial nitrification. *Bioresource Technology*, *355*, 127271.

ORAL PRESENTATIONS

Session 5 – Microbial Electrolysis Cells

Access to the Live-Q&A of this Session

Scan the QR-Code or visit schnaq.app and enter the following access code: 8448 7634

Talk 5-1: Removal of organic acids for life support systems in space using a synthetic microbial community in a microbial electrolysis cell

Korneel Rabaey[1,2], Jolien De Paepe[1,3], Amanda K. Luther[4], Antonin Prévoteau[2,5], Jean-Romain Bautista-Angeli[2,5], Dries Demey[4]
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To enable astronauts to survive in space, a life support system is essential to maintain good air quality, provide potable water and food, and deal with wastes, be they solid, liquid or gaseous. As missions of increased duration are envisaged for the future, the life support system needs to become increasingly efficient and consider all waste as a resource to be recycled. The European Space Agency has developed the so-called MELiSSA loop to create almost full circularity. In this loop, the fecal matter, food residues and other (semi)solid wastes such as unused plant material are first fermented to liquefy the waste while also unlocking already some 15% of the carbon as CO_2. The filtrate from this stage contains some 10 g/L of unused organics, mainly organic acids.
We have developed in the past 10 years a microbial electrolysis cell (MEC) able to treat this stream by oxidizing the organics at the anode while creating needed alkalinity at the cathode. In the initial project, we observed that while removing the organic acids well (>90% overall), the microbial community fluctuated over time giving rise to, e.g., unwanted methanogenesis. Given the need for control, a second generation system (Figure 1) was designed ("breadboard") in which the MEC can be sterilized via steaming in place (SIP) and in which the fermenter effluent is brought after membrane separation to be treated by a synthetic community.

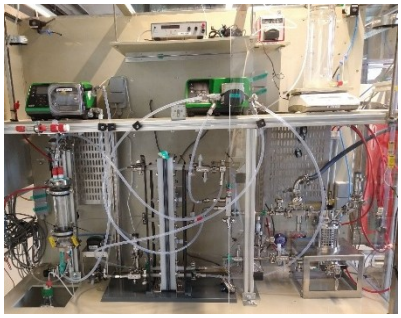

Figure 1. Photograph of the MEC breadboard including centrally the PVDF based MEC enabling steam sterilization

The synthetic community was selected based on the need to remove all organic acids from C1 to C6, and consisted of 6 species including *Geobacter* and *Shewanella* species along with a facultative aerobe (in part to scavenge oxygen) and syntrophs.
In a laboratory set-up (100 cm^2 anode) the community removed all organic acids tested albeit at different extents, from synthetic and real feeds. Subsequently, the community was used to inoculate the breadboard system which was operated with a PVDF membrane separating the two anodes from the cathode. This configuration allowed influent flow to the anode with fluid passing through the membrane towards the cathode where OH^- production was targeted along with H_2. The pH was controlled in the anode compartment by transfer of part of the basic cathode effluent.
After steam sterilization and verification, the system was initially fed with a batch of fermenter effluent. Upon the emergence of microbially-induced electrical current the system was switched to continuous mode with increasing loadings for an operation over several months. Whereas initial organic acids removal followed supply quite well, in time organic acids accumulated. Analysis of the microbial community revealed that over time the synthetic community could not be maintained and re-inoculation to boost the target community could not lead to higher performance. Interestingly, whereas the cathode was steam sterilized and separated from the anode by a PVDF membrane not allowing any microbial transfer, over time a community developed dominated by *Sporomusa sp.* (which was not part of the inoculum) which caused acetate concentrations in the cathode to decrease. This finding was not only surprising, but also shows the resilience that even such strict anaerobes can exhibit. Even though we were able to show that a synthetic community can remove all organic acids from complex waste, a main conclusion is that even with all precautions maintaining a synthetic, defined community over longer time durations is likely not realistic.
Acknowledgement: This work was funded by the European Space Agency (MECO2 project)

Talk 5-2: A Factorial Approach to Optimize Biochar as an Electrode Material for Microbial Electrolysis Cells

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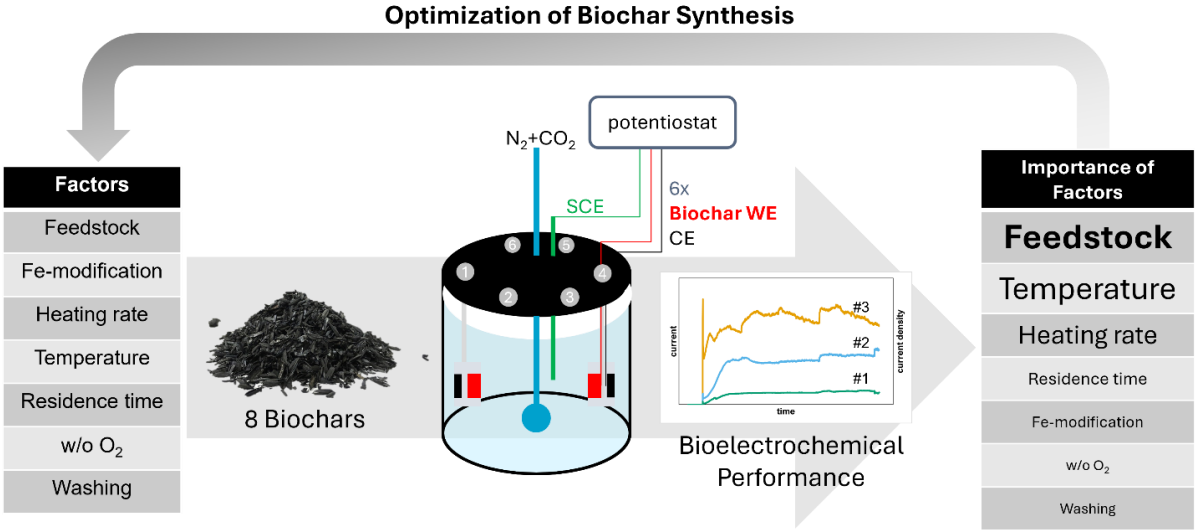
Wastewater treatment (WWT) is energy intensive and emits significant amounts of CO_2. Microbial electrolysis cells (MECs) enable energy recovery in various forms (H_2, CH_4, biopolymers) during WWT, but face challenges in upscaling a.o. due to the lack of cost-effective, suitable electrode materials. Biochar derived from organic wastes offers a low-cost, conductive, and biocompatible alternative that also serves as a C-sink and advances circular economy goals.
However, biochar is a diverse material; to investigate its suitability as MEC electrode material, and to understand the influence of feedstock and pyrolysis conditions, eight biochars were synthesized, by varying seven factors: feedstock, Fe-modification, heating rate, temperature, residence time, w/o O2 and washing. These biochars were thoroughly characterized, and tested in a MEC setup, which allowed simultaneous comparison of six biochars under identical conditions. The electroactive bacterium *Geobacter sulfurreducens* was inoculated into the reactor filled with synthetic wastewater. The biochar electrodes were polarized at -241 mV vs. SCE serving as anode; Ti-meshes as counter electrodes. The electrochemical performance was evaluated via chronoamperometry, polarization curves and CVs. Further, pH, conductivity, COD, OD600, and organic acids were monitored.
Despite the strong transport limitations, caused by the specific experimental setup, the achieved limiting current densities (j_{lim}, 0.04–0.25 mA cm^{-2}) were comparable to or exceeded those of other common materials (e.g. graphite foil, carbon paper, stainless-steel mesh) tested in a similar reactor. Further, j_{lim} varied considerably between the biochars and we could determine the relative contribution and importance of the single biochar synthesis factors on the bioelectrochemical performance. In combination with the biochar characterization, this enables a targeted optimization of the synthesis method to enhance bioelectrochemical properties.
The results demonstrate biochar as a promising electrode material in MECs and provide critical insights to improve their performance, addressing a major limitation for scaling up MECs in WWT.



Talk 5-3: Enhancing bio-electrochemical hydrogen production and organic matter removal from wastewater using *Rhodopseudomonas palustris* 42OL

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Introduction: Rising energy costs in Europe are diminishing economic competitiveness, particularly impacting sectors like food processing. Beer production is energy-intensive and generates significant amounts of brewer's waste (BW)[1]. While BW is an environmental problem due to its high chemical oxygen demand (COD), it also offers opportunities for resource recovery and sustainable energy production[1]. Bio-electrochemical systems (BESs) are a promising method for improving BW. They use bacteria to transfer electrons between electrodes. Purple non-sulfur bacteria (PNSB), such as *Rhodopseudomonas palustris*, are electroactive and can be integrated into BESs, using electrons from the bio-cathode to enhance H_2 yields while reducing total COD at the anode[2]. This strategy could offer a sustainable approach to support circular economy goals by minimizing waste and generating bioenergy.

Materials and Methods: BESs experiments were performed in triplicate in 250 mL single-chamber reactors fed with sterile BW. Each chamber was inoculated with *R. palustris* 42OL. The system was operated under anaerobic conditions with continuous illumination. Different conditions were compared to explore the effect of cathodic polarization: −0.3V, -0,6V, and -0.8V (vs. Ag/AgCl); furthermore, a control without electrodes was set up. A graphite rod was used as the working electrode, with reference to an Ag/AgCl reference electrode, and a platinized titanium mesh as counter electrode. A non-polarized reactor was operated as a control. COD, gas production, total organic carbon, total nitrogen, and optical density were measured over time.

Results and discussion: H_2 production in BESs increased when *R. palustris* 42OL was cultured in the presence of an electrode as an electron donor. The maximum H_2 production was obtained with a potential of -0.6V vs Ag/AgCl (Fig. 1). The presence of the electrode also promoted higher % COD removal. At a more negative potential (-0.8V vs Ag/AgCl), the removal was about 70% (Fig. 2).

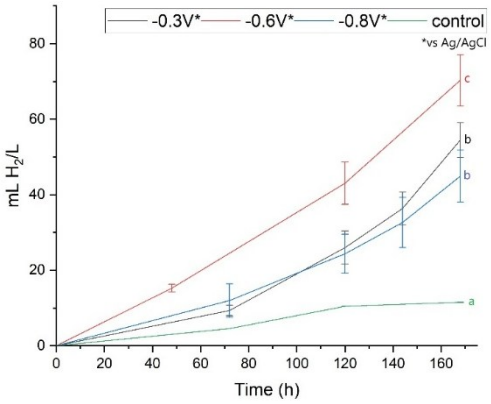

Figure 1. H_2 production (mL/L) at different cathode potentials

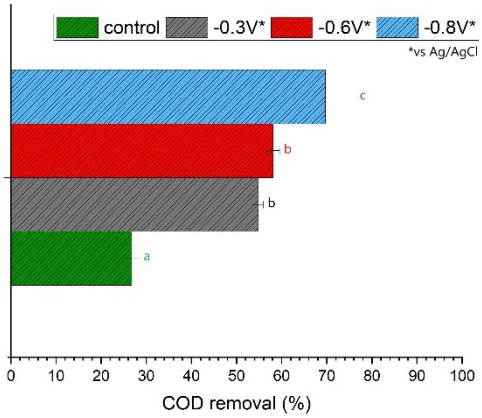

Figure 2. COD removal (%) after 1 week of polarization

Acknowledgements: The financial support by Italian Ministry of University and Research during the research program PRIN2022 PNRR project WHISPER contract No. P2022W4MNM is greatly appreciated.

References:

1. Brewers Association. (2020). "Sustainability Benchmarking Report: Brewery Waste and Water Use."
2. Grattieri, M. (2020). Purple bacteria photo-bioelectrochemistry: enthralling challenges and opportunities. Photochemical & Photobiological Sciences, 19, 424-435.

Talk 5-4: Boosting H_2 production through Electro-Active anodic biofilm acclimatization in a cascade process integrating Dark Fermentation of cheese Whey with Microbial Electrolysis Cells

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Hydrogen gas production was investigated in an integrated process exploiting Dark Fermentation (DF) and Microbial Electrolysis Cell (MEC) to enhance the overall H_2 recovery from Cheese Whey (CW) [1] (Figure 1). CW fermentation with indigenous microflora pointed out a hydrogen production yield of 42±8 NmL_{H2}/gVS. To optimize H_2 production, an in-depth investigation was conducted on the growth of Electro-Active Biofilms (EABf) in H-type cells (180 mL each chamber). Specifically, the cells were equipped with a carbon-felt anode and a stainless-steel cathode to facilitate the H_2 evolution reaction. Various acclimatization strategies were adopted to enhance the EABf growth, with the aim of improving the degradation of different carboxylic acids and subsequently increasing H_2 production. The acclimatization of EABf was evaluated using either acetate (as a model substrate) or a synthetic mixture of carboxylic acids (containing ethanol, lactate, acetate, and butyrate), which was ad hoc designed to simulate the composition of a real effluent deriving from a lab-scale DF reactor fed with CW. All bioelectrochemical experiments were performed in triplicate and operated with the anode poised at +0.20 *vs*. SHE (Standard Hydrogen Electrode). Once EABf were acclimatized on synthetic substrates, the real DF effluent was fed to the H-cell systems to assess the H_2 production performance. The Chemical Oxygen Demand (COD) removal efficiency for the cells acclimatized on acetate was 23±5 % compared to 58 ±6 % for the cells acclimatized on the synthetic mixture. In addition, the positive effect of the EABf acclimatization strategy pointed out a higher Cathodic Capture Efficiency (CCE). Indeed, tests acclimatized on synthetic acids mixture accounted for 98±2 (%, $meqH_2/meq_i$) with respect to 67±13 (%, $meqH_2/meq_i$) for the tests acclimatized on acetate. Furthermore, a characterization with advanced genomic and bioinformatic analysis of third-generation sequencing clearly evidenced the selection of significant diverse anodic communities for the two acclimatization conditions. According to these results, a two-chamber MEC reactor (1.5 L total volume) was set up and fed with the synthetic acids mixture to stimulate the EABf acclimatization at the anode and simultaneously boost the cathodic H_2 production and recovery. The MEC was operated under the same polarized conditions as the H-type cells. Overall, H_2 production accounted for 40 $meqH_2/d$, resulting in a high CCE value (93±4 %, $meqH_2/meq_i$), and the COD removal efficiency (63±5, %) was comparable to that obtained in H-cell cells when the EABf was acclimatized on the synthetic organic mixture. Further MEC runs are ongoing using a real feedstock (i.e., the effluent from a DF lab-scale reactor treating CW) to evaluate the performance of the integrated DF-MEC process in terms of H_2 recovery.

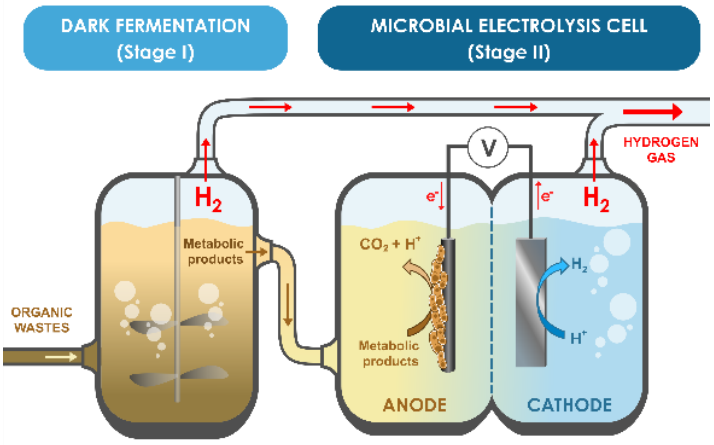

Figure 1. Scheme of the studied integrated DF-MEC process

This research was funded by the European Union – NextGeneration EU from the Italian Ministry of Environment and Energy Security POR H_2

[1] A. Marone et al., *Int. Journal of Hydrogen Energy,* 2017, 42,3,1609-1621.

Talk 5-5: Optimising and up-scaling bioelectroconcentration for nutrient recovery from human urine

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Nitrogen (N), phosphorus (P) and potassium (K) are macronutrients used as ingredients in fertilisers in agriculture. A large amount of these nutrients end up in human urine, from where they could be recovered for reuse. Human urine is a concentrated stream with up to 8.6 g_N L^{-1}, 0.5 g_P L^{-1}, and 2.4 g_K L^{-1} [1]. Hydrolysed urine also has a high organic content, buffer capacity and electric conductivity [1,2], which make it an ideal electrolyte for microbial electrochemical systems, including bioelectroconcentration cells (BECs).

BECs combine microbial electrolysis and electrodialysis. At the anode, electroactive microbes convert chemical energy in organic matter into electrical energy [3], which drives the migration of charged ionic nutrients, particularly ammonium (NH_4^+), potassium (K^+) and phosphate (HPO_4^{2-}), through cation- (CEM) and anion-exchange membranes (AEM) for recovery as a liquid fertiliser. As part of the electricity is produced in situ from the organic matter in urine, only a low external cell voltage input of 1.5–2 V is required, and the energy consumption of the recovery system remains low at 2–4 kWh per kilogram nitrogen recovered [1,4].

BECs have been demonstrated to be highly suitable for nutrient recovery from human urine [1,4], however, some technical and operational challenges have remained. First, bioanode enrichment has traditionally required several months due to the high pH and toxic ammonia concentration of hydrolysed urine [1,4]. Second, mass transfer in the BECs has not been ideal due to imperfect flow distribution and accumulation of gaseous oxidation and reduction products in the system. In this study, these operational challenges were first addressed in laboratory-scale experiments. Four parallel 0.8-litre reactors were operated continuously with both synthetic and real human urine, treating up to 2 L of urine per day. The anode chambers were filled with graphite granules (AJJA Technologies, Australia) and 200 cm^2 CEMs (CMI-7000) and AEMs (AMI-7001, Membranes International, USA) were used to separate the recovery chamber from the anode and cathode, respectively. A peristaltic pump (Watson-Marlow Fluid Technology Solutions, UK) was used to provide mixing to the anode and cathode.

Inoculating the anodes with hydrolysed urine diluted with waste activated sludge proved to be an efficient start-up strategy, where continuous feeding with undiluted hydrolysed urine could be commenced within 11 days from start-up [5]. Flow distribution in the BECs was successfully improved by increasing the number of feed channels from one to four. Gases produced at the electrodes were removed from the system by inserting simple gas-liquid separation columns into the anolyte and catholyte circulation loops.

These technical and operational advances were then implemented in the design of a pilot-scale 18-litre BEC, designed to treat 40–100 L of hydrolysed urine per day. Public field trials with the pilot-scale BEC will commence during 2025 under the Nutrients in a Circular Economy (NiCE) Research Hub in Australia.

References

[1] Monetti et al. (2021), Separ. Purif. Technol. 279: 119793, DOI: 10.1016/j.seppur.2021.119793
[2] Zamora et al. (2017), J. Power Sources 346, pp. 491–499, DOI: 10.1016/j.jpowsour.2017.02.089
[3] Rabaey et al. (2007), ISME Journal 1, pp. 9–18, DOI: 10.1038/ismej.2007.4
[4] Ledezma et al. (2017), Environ. Sci. Technol. Lett. 4(3), pp. 119–124, DOI: 10.1021/acs.estlett.7b00024
[5] Koskue & Freguia (2024), Bioelectrochemistry 158: 108706, DOI: 10.1016/j.bioelechem.2024.108706

Talk 5-6: Local Hydrogen in Biocathodes: Microsensors and Predictive Modelling

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In bio-electrochemical systems for CO_2 valorisation, microbial conversions are combined with electrochemical input of energy to create a system that converts CO_2 and electricity to multi-carbon products. Products from these systems can be used to replace current fossil-based platform chemicals. In these systems, hydrogen is a crucial energy carrier between the electrodes and the CO_2 converting microorganisms. However, often the hydrogen is not bioavailable for micro-organisms because it is not distributed property. We discovered how measuring and promoting local hydrogen production in bio-electrochemical CO_2 conversion systems can boost the productivity and shorten start-up times.

To measure local hydrogen concentrations, microsensors were used in reactors with graphite felt and granular activated carbon electrodes. Based on the obtained profiles of local hydrogen distribution over the porous electrode material, great differences were observed between different areas, ranging from 200 to 1200 µM. These insights were used to stimulate local hydrogen availability throughout the cathode surface area by modification of flow-through patterns from recirculated catholyte.

Furthermore, we developed a model based on theoretical equations that describes the hydrogen distribution throughout the electrode material under abiotic conditions. We validated the model with data from the abovementioned microsensor experiments. The validated model was also run at different parameters that represent operational settings. As a result, the porosity and tortuosity of electrode material, applied current, recirculated flow rate, and catholyte influent pH and contained phosphate buffer concentration all influence the local hydrogen and pH distribution in the modelled system (Figure 1). In the lab experiments, improving hydrogen distribution over the cathode volume showed immediate start-up of acetate or methane formation from CO_2. The acetogenesis was followed by *n*-butyrate formation after 20 days and *n*-caproate formation after 30 days (Figure 1). Based on the model output we can predict how different operation parameters can stimulate or inhibit hydrogen distribution. In our work we showed that the combination of microsensor analysis to study local conditions and modelling of hydrogen distribution in microbial electrosynthesis holds great promise to boost CO_2 valorisation.

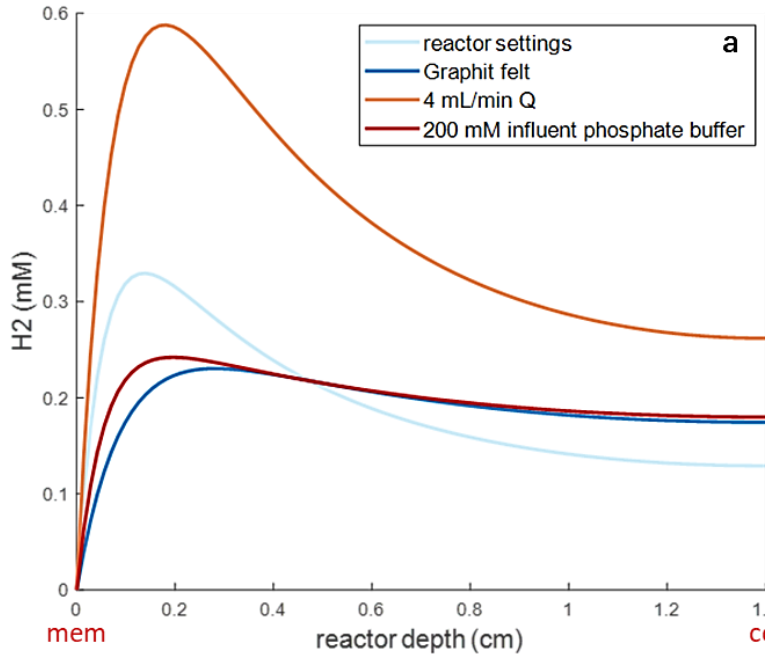

Figure 1. *Modelled hydrogen distribution in granular activated 3D electrode with different operational settings.*

Talk 5-7: Biohybrid Pd Catalysts Harness Bidirectional Electron Flux for Enhanced C-F Bond Cleavage

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Abstract
Per- and polyfluoroalkyl substances (PFASs), persistent environmental contaminants due to strong C–F bonds, pose significant remediation challenges. Conventional degradation methods suffer from inefficiency, energy demands, and byproduct formation. This study introduces a biohybrid palladium (Pd)-electrochemical system that synergizes microbial biosynthesis of monodispersed Pd nanoparticles (PdNPs, 4.6 ± 1.1 nm) with hydrodefluorination (HDF) via atomic hydrogen (H*) generation. Biofilm-enabled PdNP synthesis enhanced surface coverage by 56% compared to electrodeposition, optimizing catalytic interfaces for C–F bond cleavage. System optimization (−0.5 V to −1.1 V vs. Ag/AgCl, 1.5 mM Pd(II)) achieved mass activity of 19.7 μM min^{-1} (μg $Pd)^{-1}$, with 83% phenol reduction yield and bond energy-dependent defluorination efficiency (48% for florfenicol vs. 20% for 4-fluorophenol). Synergistic adsorption-catalysis mechanisms were driven by H*-mediated reduction (ΔG=−180.38 kJ/mol) and direct electron transfer, facilitated by *Geobacter*-dominated biofilms (78%). Transcriptional upregulation of outer membrane cytochromes (*OmcE*, *OmcG*) and periplasmic cytochrome *PpcA* established direct electron conduits from microbial respiratory chains to Pd catalytic sites. These cytochromes mediated transmembrane electron transfer, reducing Pd(II) precursors to Pd(0) nanoparticles. Concurrently, Hox-type hydrogenases catalyzed H_2 dissociation into reactive atomic hydrogen (H*) at Pd surfaces, amplifying reductive defluorination pathways. While anionic PFASs faced electrostatic repulsion, the biohybrid platform demonstrated scalable defluorination via interfacial electron flux optimization. Future integration with advanced oxidation or membrane separation is proposed to address mass transfer limitations. This work advances mechanistic insights into bio-abiotic synergy for fluorinated pollutant remediation under ambient conditions.

Keywords
PFASs; C-F bond; reductive defluorination; hydrodefluorination; biohybrid

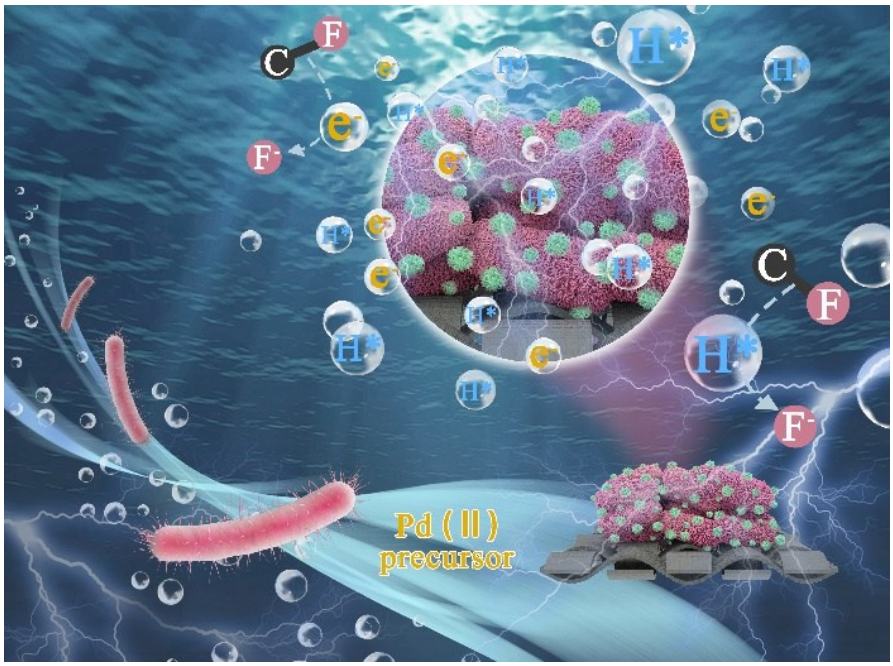

Figure 1. Cleavage mechanism of bio-palladium hybrid catalysts for C-F bond.

ORAL PRESENTATIONS

Session 6 – Microbial Physiology/Genetic Engineering

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Talk 6-1: Unraveling Extracellular Electron Transfer Mechanisms in Cable Bacteria: Evidence for Direct and Mediated Electron Transfer

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Extracellular electron transfer (EET) is a key metabolic function of electroactive bacteria, enabling interactions with external electron donors or acceptors and supporting diverse applications. Cable bacteria, a unique class of sulfide-oxidizing filamentous microbes capable of long-distance electron transport in marine and freshwater sediments, have been observed to interact with electrodes in bioelectrochemical systems (Bonné et al., 2024). However, the mechanisms underlying their electrode interactions remain unclear. In this study, we investigate these mechanisms through electrochemical and biochemical analyses, with results supporting both direct and mediated electron transfer. Differential pulse voltammetry of the freshwater cable bacterium *Electronema aureum* GS revealed redox peaks at +0.25V and +0.6V (vs Ag/AgCl), suggesting the presence of redox-active components involved in EET. The +0.25V peak is reminiscent of cytochrome-associated electron transfer seen in other electroactive bacteria, indicating a possible role of multiheme cytochromes or similar redox-active proteins in *E.aureum* GS. This similarity suggests a conserved mechanism, potentially allowing *E. aureum* GS to interact with external electron acceptors in a manner analogous to well-characterized *Geobacter* or *Shewanella* species. Notably, the +0.6V peak appears to represent a distinct high-potential redox-active moiety, possibly linked to the periplasmic conductive fiber, and unlike other cytochromes observed in electroactive bacteria. Proteinase K treatment and heating altered both peaks, confirming the peaks to be outer membrane proteins in *E.aureum* GS. Electrochemical cells poised at +0.25V exhibited higher current outputs in cable bacteria-containing sediment compared to controls, while at +0.6V, only cable bacteria-containing systems generated a progressively increasing current. These findings suggest the involvement of two sets of redox-active proteins facilitating electron transfer at different potentials. Additionally, we observed significantly elevated riboflavin concentrations in electrochemical cells containing cable bacteria compared to controls, suggesting flavin-mediated electron transfer. These insights shed light on the metabolic abilities of cable bacteria, with the potential to use extracellular electron acceptors in the absence of oxygen. Figure 1 depicts *E. aureum* GS stretching towards poised electrodes.

Figure 1: Cable bacteria stretching from sulfidic sediment and aggregating on poised electrodes.

Reference: Bonné, R., Marshall, I. P. G., Bjerg, J. J., Marzocchi, U., Manca, J., Nielsen, L. P., & Aiyer, K. (2024). Interaction of living cable bacteria with carbon electrodes in bioelectrochemical systems. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.00795-24

Talk 6-2: Uncovering Novel EET Mechanisms and Metabolic Crossfeeding in a Cathode-Oxidizing Marine Sediment Bacterial Coculture

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Extracellular electron transfer (EET) is the process by which some microorganisms transfer electrons across membrane(s) to/from solid-phase surfaces, such as minerals or electrodes. Significant advances in our understanding of EET mechanisms have been gleaned from physiologic investigations of iron-oxidizing and iron-reducing bacteria; however, considerably less is known about the mechanisms and ecological implications of EET in mineral-oxidizing organisms that do not interface with iron. To better elucidate the mechanisms and ecological implications of EET in oxidative processes, we are investigating the genetic basis of oxidative EET in pure cultures, and the nature of interspecies interactions during EET in cocultures, of two electrode-oxidizing bacteria: *Thioclava electrotropha* ElOx9^T and *Idiomarina* sp. strain FeN1. These two organisms, isolated from the same marine sediment, are physiologically distinct and genetically tractable. Genomic and physiologic investigations into these organisms have shown that *Thioclava* is metabolically versatile and capable of chemoorganoheterotrophic growth or chemolithautotrophic growth with hydrogen or reduced sulfur species as electron donors. Conversely, *Idiomarina* is an obligate aerobic heterotroph and is auxotrophic for eleven amino acids. Both organisms engage in cathode oxidation (-278 mV vs. SHE) – a proxy for mineral oxidation – yet lack homologs to canonical genes implicated in EET, such as the multi-heme outer membrane cytochromes responsible for iron respiration in *Shewanella* and *Geobacter* species.

To gain insight into the genetic basis of oxidative EET in these organisms, we conducted a suite of high-throughput whole-genome mutagenesis screens, transcriptome analysis, and construction and testing of gene deletion mutants and complementation strains. Whole-genome mutagenesis screens in *Thioclava* identified over 50 genes essential for cathode oxidation, including several hypothetical proteins and poorly characterized oxidoreductases that are predicted to localize to the cellular envelope. Transcriptome sequencing and comparative analysis of pure and cocultures has illuminated shifts in gene expression profiles and interspecies interactions under oxidative EET conditions that suggests metabolite sharing may be important component of coculture EET. Through untargeted extracellular metabolome profiling, we are gaining insight into metabolic crossfeeding interactions that occur during coculture EET. These experiments provide insight into novel mechanisms of extracellular electron uptake and have expanded our knowledge of interspecies interactions in a polymicrobial electrode-oxidizing bacterial consortium. In this presentation, I will discuss the results of these high-throughput screens, report on our progress to elucidate mechanisms of oxidative EET in these organisms, and share insights into metabolic crossfeeding interactions during coculture EET.

Talk 6-3: Dynamic Interactions: How Cellular Physiology Shapes and is Shaped by Extracellular Electron Transfer in Biophotovoltaic Systems

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Introduction
The study of extracellular electron transfer (EET) in biophotovoltaic systems is crucial for understanding how cyanobacteria, such as the model organism *Synechocystis* sp. PCC 6803, contribute to an establishment of this technique for real-world application and thus sustainable energy production. The development of this process depends not only on the improvement of current production by optimization of the EET but also on understanding its implications for the cellular physiology that governs this process. This work investigates the influence of different cellular physiologies on the current production in a biophotovoltaic system in interaction with varying availability of terminal electron donors and acceptors, and the application of specific electron transfer inhibitors (*1, 2*). On the other hand, the impact of the extracellular electron transfer on cellular physiology directly correlates with the number of electrons harvested at the electrode (i.e. biophotovoltaic productivity). Therefore, this work also investigates the influence of current production on the cellular physiology of the biocatalyst in a biophotovoltaic system.

Methods
To elucidate the influence of cellular physiology on current production and the responsible pathways, a combination of statistical analysis of electrochemical experiments under different conditions, advanced spectrophotometry, and application of specific electron transfer inhibitors was employed. *Synechocystis* cultures were grown under varying conditions to obtain different cellular physiologies, particularly with regard to their intracellular glycogen storage pools. The ability of cells to sustain a current in the absence of light was quantified in relation to their glycogen storage. Furthermore, the progression of light-induced currents was investigated under different availabilities of electron donors and acceptors. Additionally, cellular parameters such as cell growth, size, pigment content, and media pH were examined under current-producing conditions and compared with multiple control conditions. Metabolomic and proteomic analyses were applied to gain molecular-level insights into the adaptation of cyanobacteria to biophotovoltaic performance.

Results
The findings indicate that cellular physiology plays a significant role in determining the responsible electron pathways, and thus, the efficiency of EET in biophotovoltaic systems. Distinct physiological states, induced through varied cultivation protocols, influence the availability of respiratory electrons which played a crucial role for current production under dark conditions, but also under illumination, when electron donors (i.e. PSII) and acceptors (i.e. O_2) were restricted. A systematic evaluation of electrochemical data reveals that the fluidity of electron pathways is responsive to these variables. Furthermore, key parameters for cellular physiology, like cell growth and size as well as media pH, showed distinct influences from EET during biophotovoltaic current production compared to control conditions. Metabolomic and proteomic analyses show molecular-level implications of how cyanobacteria adapt to biophotovoltaic performance, offering insights into cellular mechanisms and pathways that could be of interest for further investigation of extracellular electron transfer. These results offer new perspectives on the interplay between cellular physiology and bioelectric performance.

1. H. Schneider, B. Lai, J. O. Kromer, "Understanding the electron pathway fluidity of Synechocystis in biophotovoltaics". *Plant Journal* **121**, e17225 (2025). Doi: 10.1111/tpj.17225
2. H. Schneider, B. Lai, J. O. Kromer, "Interference of electron transfer chain inhibitors in bioelectrochemical systems". *Electrochemistry Communications* **152**, (2023). Doi: 10.1016/j.elecom.2023.107527

Talk 6-4: Haloalkaliphilic bacteria capable of respiring by linking sulfide oxidation to manganese(IV) oxide reduction

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Manganese and sulfur are vital elements that support microbial respiration under anoxic environments. MnO_2, a prevalent mineral in aquatic sediments, acts as an electron acceptor for microorganisms, while sulfide serves as an electron donor. The redox interaction among these two leads to increased mobility and bioavailability of elements, establishing a redox gradient that enables electron flow and energy transfer across different microbial groups[1]. Microbially mediated sulfide oxidation linked to MnO_2 reduction has been hypothesized for years but was confirmed recently with the isolation of *Sulfurimonas marisnigri* from the Black Sea[2]. Microbes can use extracellular electron transfer (EET) to achieve respiration with insoluble electron acceptors like MnO_2. Although studying the roles of EET-capable microbes in biogeochemical element cycles is gaining attention, research in extreme environments remains limited. This study presents evidence of sulfide oxidation linked to MnO_2 reduction by haloalkaliphilic bacteria enriched from subsurface sediments of Lonar Lake (India), a haloalkaline crater lake formed by a meteorite impact. This chemolithotrophic process was examined under autotrophic conditions in serum bottles containing a minimal growth medium (pH 9.5 & 20 g/L NaCl). Sulfide (2 mM), MnO_2 (10 mM), and $NaHCO_3$ (20 mM) served as the sole electron donor, electron acceptor, and carbon source, respectively. With microbial culture, rapid sulfide oxidation and MnO_2 reduction were observed with sulfate production (0.128 ±0.005 mMS^{2-}/day, 0.187 ±0.015 $mMMn^{2+}$/day & 0.062 ±0.009 $mMSO_4^{2-}$/day). In abiotic controls, sulfide-linked MnO_2 reduction occurred much slower (0.095 ±0.013 mMS^{2-}/day & 0.097 ±0.015 $mMMn^{2+}$/day), and without sulfate production. Enhanced redox reaction rates with sulfate production confirm the microbial role in this particular respiratory process. Whole genome metagenome sequencing revealed *Desulfurivibrio alkaliphilus* and *Desulfurispirillum indicum* as the dominant taxa in the enriched microbial community with a relative sequence abundance of 23.28% and 11.25%, respectively. *D. alkaliphilus* is reported for autotrophic sulfide oxidation-linked nitrate reduction[3] and as the dominant taxa in sulfide-oxidizing anodic biofilms[4], suggesting its EET capability. *D. indicum* is neither reported for autotrophy nor for sulfide oxidation[5]. These bacteria have not been reported for MnO_2 reduction-based respiration yet. Further work on the pure culture strains of these microbes would confirm their role in this EET-driven MnO_2 reduction-linked sulfide oxidation process. This study provides evidence for the microbial role in driving manganese and sulfur cycling under haloalkaline conditions, emphasizing their importance in biogeochemical processes under extreme habitats.

References:

1. Henkel J v., Schulz-Vogt HN, Dellwig O, et al. Biological manganese-dependent sulfide oxidation impacts elemental gradients in redox-stratified systems: indications from the Black Sea water column. ISME Journal. 2022;16(6):1523-1533. doi:10.1038/s41396-022-01200-3
2. Henkel J v., Dellwig O, Pollehne F, Herlemann DPR, Leipe T, Schulz-Vogt HN. A bacterial isolate from the Black Sea oxidizes sulfide with manganese(IV) oxide. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(25):12153-12155. doi:10.1073/pnas.1906000116
3. Thorup C, Schramm A, Findlay AJ, Finster KW, Schreiber L. Disguised as a sulfate reducer: Growth of the deltaproteobacterium Desulfurivibrio alkaliphilus by Sulfide Oxidation with Nitrate. mBio. 2017;8(4). doi:10.1128/mBio.00671-17
4. de Rink R, B. Lavender M, Liu D, et al. Continuous electron shuttling by sulfide oxidizing bacteria as a novel strategy to produce electric current. Journal of Hazardous Materials. 2022;424. doi:10.1016/j.jhazmat.2021.127358
5. Rauschenbach I, Narasingarao P, Häggblom MM. Desulfurispirillum indicum sp. nov., a selenate- and selenite-respiring bacterium isolated from an estuarine canal. International Journal of Systematic and Evolutionary Microbiology. 2011;61(3):654-658. doi:10.1099/ijs.0.022392-0

Talk 6-5: Investigation of mediated electron uptake in a cathodically grown biocatalyst

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Until now, biosynthetic processes on the cathodic side of bioelectrochemical systems have been severely limited by the dependency of biocatalysts on hydrogen as an electron mediator into cell metabolism or on the fairly rare trait of direct electron uptake. Thereby, typical biotech workhorses are excluded from BES reactions as they possess no native electro-activity.
Direct electron transfer requires intense investigation of biofilm formation, substrate, and nutrient availability. Further, the dependency on the electrode surface leads to complex reactor designs, especially considering future reactor upscale. Hydrogen utilizing biocatalysts pose restrictions towards the acetogenic product spectrum, and in the current BES reactor design, unutilized hydrogen may be lost in off-gas streams, leading to decreased overall efficiency.
Therefore, in our studies, we focus on finding and evaluating a natural redox mediator for cathodic electron uptake that can be self-produced and utilized by a natively non-electroactive biocatalyst host strain.

To evaluate the function and electro-activity of a possible deazaflavin target mediator, we used a self-constructed electro-cuvette system. We monitored the absorbance spectrum of the target molecule during exposure to different cathodically applied potentials to conclude on the reduction state. A deeper evaluation focused on the conditions for optimized combined mediator activity and strain cultivation conditions.
A one-enzyme-dependent electron transfer reaction from the reduced deazaflavin mediator towards NADPH was heterologously expressed in an *Escherichia coli* host. However, as the strain grows on glucose in anaerobic conditions, it was suspected that the NADPH pool of the host was already saturated and additional NADP reduction through the electron mediator was not required by the host. Consequently, to avoid saturation of NADPH and enhance the electron drive into the metabolism, we are currently implementing a metabolic electron sink involving an easy-to-follow NADPH-depleting enzyme-based reaction that enables the monitoring of the rate of metabolic electron uptake.

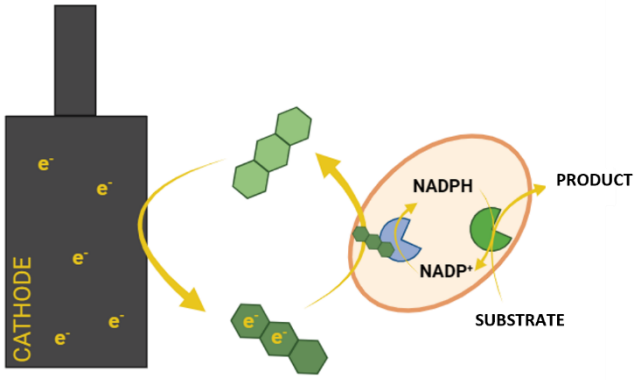

Figure 1: Electron transfer from the cathode into the host cell metabolism and towards NADPH via the reduced mediator molecule. Subsequent NADPH utilization via an enzymatic substrate conversion to a designated product.

The results of this strategy are evaluated and will further deepen the understanding of the feasibility of inward electron transfer of the targeted mediator molecule.

Talk 6-6: Towards a Kinetic Model of Reverse Electron Flow in *Shewanella oneidensis.*

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Shewanella oneidensis MR-1 is an important model system for understanding bi-directional extracellular electron uptake, however much less is known about the mechanism and/or physiologic relevance of electron uptake from redox active surfaces compared with the transfer to these susbtrates. On cathodes, it has been shown that many of the proteins involved overlap across the organism's previously characterized extracellular electron transport machinery. However, there are still many questions surrounding the mechanics and physiologic relevance of electron uptake in *Shewanella* biofilms, stemming from large abiotic currents in cathodic systems that confound results, as well as the challenge of quantifying biomass on electrode surfaces. This has limited our understanding of the physiologic and kinetic constraints of electron uptake, as well as our ability to make meaningful comparisons across systems. To investigate the relationship between cathodic activity and biomass, we used a previously described *Shewanella oneidensis* strain genetically modified with cell aggregation protein CdrAB behind a blue light-controlled promotor. Using blue light exposure to control cell deposition, we then investigated the relationship between cathodic activity and biomass. Electrochemical impedance spectroscopy (EIS) confirmed a decrease in biofilm impedance over a range of blue light exposures (i.e., 2 to 8 h). Consistent with previous results, after this timepoint, a drop-in electrochemical activity was observed, and impedances increased. For biofilms within the 2-8 h light exposure range, we observed a trend towards increased biological current consumption by quantifying the difference between pre and post kill currents. Using an equivalent circuit model to extrapolate specific biofilm parameters we quantified the charger transfer resistance within the biofilm that corresponds to varying biofilm thicknesses and matches previously observed activities. On an individual reactor basis, we correlate this biofilm charge transfer resistance with biologic cathodic current. We observed a linear trend with a correlation score of 0.87 ($r^2 = 0.773$). To the best of our knowledge, this is the first investigation of biofilm physiology on *Shewanella* cathodes using EIS. Continued efforts in this direction will further our understanding of biofilm-electrode interface during extracellular electron uptake with the goal of enhancing applications to bioelectrochemical systems.

Talk 6-7: Thermodynamic and Non-thermodynamic Effects on Anodic Electromicrobial Biofilm Growth under Controlled Hydrodynamics

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Microbial electrochemical systems (MES) utilize electroactive biofilms to convert organic substrates into valuable molecules. Optimizing the performance of these biofilms requires a comprehensive understanding of how electrode potential influences their growth and electroactivity. Moreover, hydrodynamic conditions in MES are challenging to monitor and control, yet they can significantly impact experimental outcomes. To address these challenges, we cultivated anodic biofilms under precisely controlled hydrodynamic conditions using an innovative electrochemical Taylor-Couette reactor (eCTR, Fig.1, right), designed to ensure homogeneous shear stress and mass transfer across electrodes. Biofilms were cultivated on graphite electrodes in triplicate at three applied potentials (92, -59, and -209 mV/SHE) over 45 days, using synthetic electrolyte with acetate as substrate. Biofilm development and activity were assessed via chronoamperometry, cyclic voltammetry, chemical oxygen demand (COD) measurements, and microscopic analyses, while microbial composition was characterized using 16S rDNA sequencing.

Chronoamperometric monitoring revealed successful biofilm formation at 92 and -59 mV/SHE. Notably, the intermediate potential (-59 mV/SHE) yielded biofilms with greater long-term electroactivity (2.54 ± 0.25 A/m²) compared to those at 92 mV/SHE (1.60 ± 0.19 A/m²) during the second feeding cycle (Fig.1, left). Electrodes poised at -209 mV/SHE showed negligible biofilm development, consistent with limited thermodynamic energy availability. The sequenced electrodes and bulk samples all displayed *Geobacter anodireducens* as the sole known electroactive species, which was predominant at -59 mV/SHE but outnumbered by *Alcaligenes* at the higher potential (92 mV/SHE).

Short-term shifts to the lower potential (-209 mV/SHE) showed that pre-established biofilms maintained partial electroactivity, with a linear response between current density and applied potential, indicating a short-term activity governed by thermodynamic constraints. In contrast, enhanced biofilm performance at the intermediate potential during long-term cultivation highlights the importance of non-thermodynamic factors, potentially involving microbial community adaptation, biofilm structuration, or differential energy allocation strategies. Exceptionally high reproducibility among replicates was achieved due to the homogeneous hydrodynamic conditions established by the eCTR, with coefficients of variation consistently below 10% across replicates.

This study thus emphasizes the complex interplay of thermodynamic and non-thermodynamic factors governing anodic biofilm activity. Furthermore, it demonstrates that precise control of hydrodynamic conditions improves data reproducibility. These insights are critical for developing robust predictive models and optimizing electromicrobial systems for practical applications.

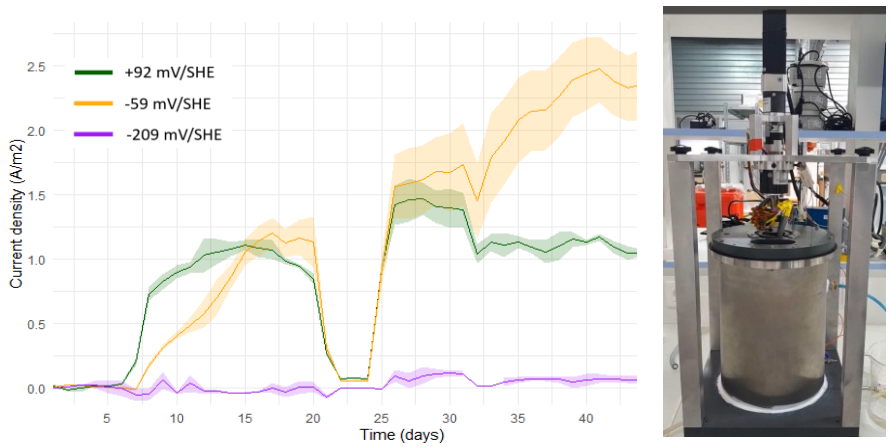

Figure 1.

Chronoamperometric monitoring of anodic biofilms at +92, -59, and -209 mV/SHE during two batch-fed cycles. Shaded areas represent the standard deviation for each triplicate (left). Photograph of the electrochemical Taylor-Couette reactor (right)

ORAL PRESENTATIONS

Session 7 – Electromethanogenesis

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Talk 7-1: Designing, building and operating an up-scaled methane producing bioelectrochemical system for power-to-methane

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Power-to-methane in a bioelectrochemical system (BES) is an innovative technology that provides a sustainable and efficient method to convert carbon dioxide and electric energy into a chemical carrier (methane). The vast majority of BES studies have been performed in lab-scale reactors (~100 mL) as proof of concept, and, so far, only few scale-up attempts have been made. To scale up BES, it is crucial to limit energy losses and address diffusion limitations to increase the volumetric conversion rate. We designed a scalable 17 L methane-producing BES and operated the system long term to analyze its performance over time. The following design considerations were implemented: (1) the use of granular activated carbon (GAC) as the cathode material, (2) the integration of commercially available tubular membranes, and (3) the adoption of a hexagonal prism structure that can be easily scaled up in modules. The methane-producing BES was operated for 470 days with stepwise increases in applied current density, starting at −6 A m^{-3}_{BES}. The highest methane production rate of 280 NL m^{-3} BES d^{-1} was achieved at a current density of -125 A m^{-3}_{BES} . For the majority of the experimental period the faradaic efficiency ranged between 80% and 100% and energy efficiency was around 40%. At each increase in current density, the distribution of voltage losses was assessed. To minimize these losses, the anode surface area and electrolyte composition (i.e. alkalinity) were adjusted throughout the experiment. Besides the BES, the line-up included a bubble column for CO_2 absorption, an oxygen stripping unit and an electrolyte interchange vessel. We didn't test the limiting rates; however, larger methane production rates could likely have been achieved based on the following observations: (1) the faradaic efficiency remained consistently high throughout the operation, and (2) no hydrogen (H_2) was detected in the outlet gas. This work represents a step towards commercial applications of a methane-producing BES and design opportunities and challenges when upscaling BES.

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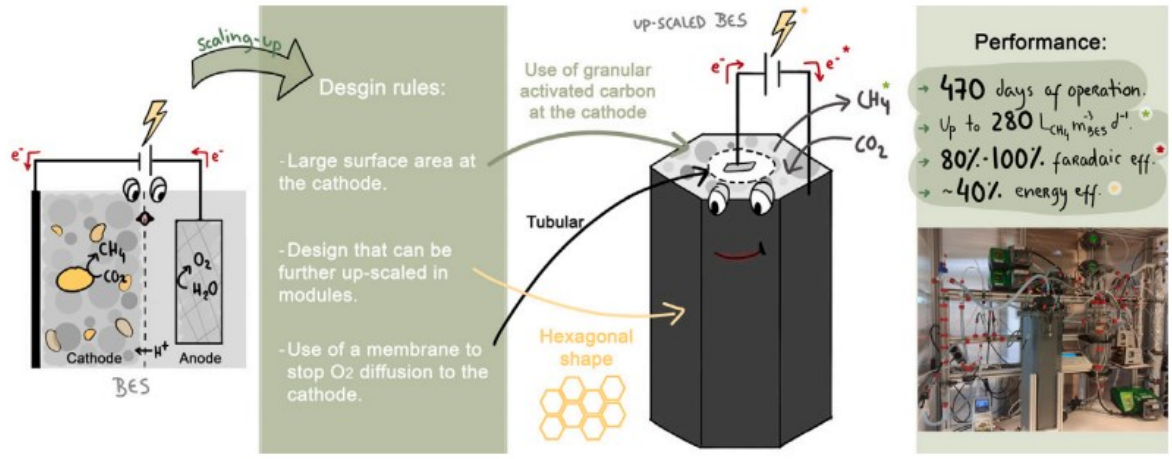

Talk 7-2: Architecture and operational innovations for improving performance of power to gas systems generating renewable methane

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Microbial methanogenesis cells (MMCs) produce renewable methane from captured CO_2 using methanogens on a cathode with water splitting on the anode using renewable electricity. The key challenge for MMCs is increasing methane production rates while maintaining high energy efficiencies. Several modifications of a zero-gap reactor are being investigated to improve rates and efficiencies with the aim of accomplishing greater conversion of hydrogen gas as methane (i.e. minimizing H_2 gas in the effluent).

A dual-layer cathode design was developed to substantially improve biomass retention and thus net methane production rates. Conductive 3D materials (reticulated vitreous carbon, RVC, or multiple layers of carbon nanoparticle-coated stainless-steel mesh, CN-SSM), were placed on top of a thin carbon cloth to form a biocathode. The catalyst was placed on the carbon cloth that was adjacent to a cation exchange membrane and used in a zero-gap design with a vapor-fed anode. Electrolyte flow directed through the biocathode generated 9-20 L/L-d of methane over multiple cycles, averaging 12 ± 3 L/L-d for RVC (50 ± 5 A/m^2) and 16 ± 3 L/L-d for CN-SSM (54 ± 5 A/m^2), at an applied voltage of 2.8 V. These rates were achieved with energy conversion efficiencies (electricity to methane) of 20 ± 4% (RVC) and 23 ± 4% (CN-SSM). Current densities of up to 148 A/m^2 were temporarily obtained by spiking the anode feed daily with water, indicating that better control of water transport in the vapor-anode system could lead to greatly increased performance. The biocathode archaeal community was dominated by hydrogenotrophic methanogens of the genus *Methanobacterium*. Based on a machine learning model cathodic methane recovery was found to be the most important operational component.

To improve the conversion of H_2 into CH_4 we further focused on increasing hydrogen gas retention and improving hydraulic flow distribution by using a new rectangular configuration. Multiphase flow modeling showed that this new design substantially reduced flow dead zones and nearly tripled hydrogen retention time compared to previous circular cells. At –1 V vs Ag/AgCl, increasing catholyte flow rate from 0.8 to 2.5 mL/min raised current densities from 19 to 24 A/m^2 (30 °C), reaching a peak cathodic efficiency (CE) of 82% for methane production (7.0 L/L-d). Further increasing catholyte flow rate to 7.5 ml/min or temperature to 37 °C slightly improved methane production but reduced hydrogen retention, lowering CE and energy efficiencies due to unreacted hydrogen. Matching cathode potential to flow rates and operating temperatures could balance H_2 production and retention, significantly improving CE to 96% and achieving a high energy efficiency of 36% with 7.5 L/L-d of methane (–0.95 V vs Ag/AgCl, 37 °C).

These findings demonstrate the importance of making architecture changes that can enable better retention of biomass in biofilms that more effectively consume the H_2 gas generated and convert it to CH_4. Improving flow distribution by avoiding dead zones in the flow system and increasing hydrogen retention using zero-gap cells successfully enhanced both energy and cathodic efficiencies. Current work is underway on multiple-port systems (Figure 1) and multiple-chamber systems. These new reactors are being constructed based on the above architectural and operational improvements.

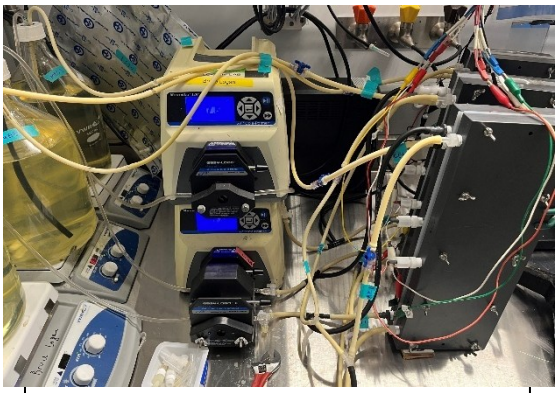

Figure 1: New flow reactors for improved renewable methane production (gray blocks on right)

Talk 7-3: Boosting biomethane production: Innovative advanced dual anerobic digesters with microbial electrochemical assistance.

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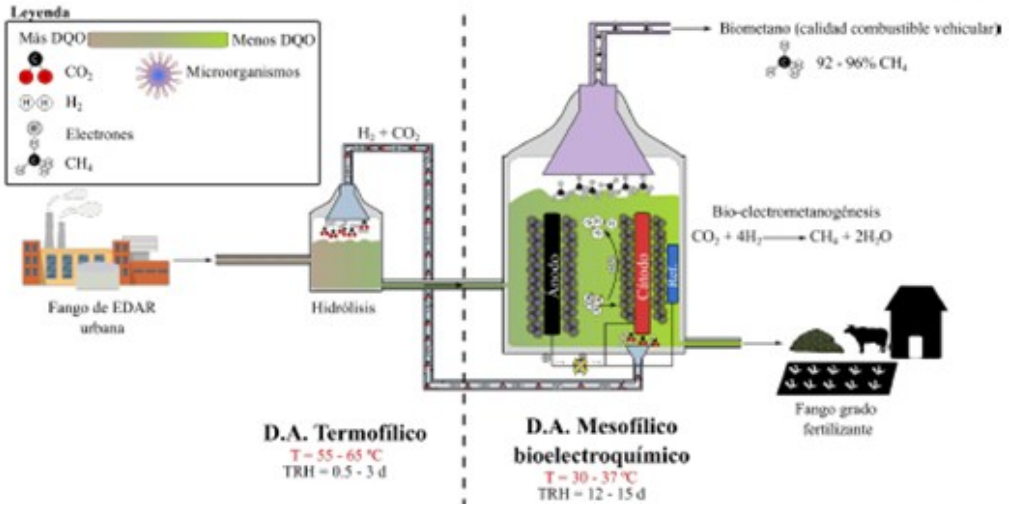

Anaerobic digestion (AD) is a crucial wastewater treatment process that offers significant environmental benefits, including reduced greenhouse gas emissions and renewable energy production (biogas). However, challenges such as low and variable methane yields and the need for energy-intensive digestate treatment limit its widespread application. To address the main problems of AD, the scientific community has been intensively working on developing dual anaerobic digestion, presenting a promising alternative with separate thermophilic and mesophilic stages enhancing biogas production and facilitating by-product recovery. While this approach improves methane content, achieving biomethane quality suitable for direct utilization within WWTPs (e.g., as vehicle fuel) remains a significant hurdle.

This work has developed a highly efficient anaerobic technological train that integrates thermophilic anaerobic reactors to favor the hydrolysis of the organic matter contained in urban sewage sludge (T=55°C, HRT=2-3 days), enhancing the volatile fatty acids (VFAs) of the organic stream that is further fed to a microbial electrochemical mesophilic reactor (T=32°C, HRT=16 days), where the organic matter oxidation is promoted over the surface of the electrodes. During this study, the technical solutions were fed with urban sewage sludge with an organic load of 14.2 kgVS/m³d for the thermophilic AD, and 2.4 kgVS/m³d for the microbial electrochemical mesophilic AD. In addition, the pH of the thermophilic AD was maintained at 5 to boost the production of biological H_2, which was recirculated to the mesophilic bioelectrochemical reactor to maximize the bio-methanization of the CO_2 produced during the AD process. The cathode was polarized under the following values: -0.35V, -0.55V, and -0.75V (vs. Ag/AgCl reference electrode), obtaining an enriched biogas with a methane content of 85%, 93%, and 87%, respectively. Additionally, an average COD removal of 52% was achieved during the semi-continuous operation of the whole train.

This research demonstrates the feasibility of producing high-purity biomethane (93%) by integrating thermophilic anaerobic digestion with microbial electrochemical technologies. Machine learning models are currently being developed to optimize reactor operation, maximizing and predicting biomethane production from the initial sewage sludge composition while minimizing costs and environmental impact to enhance this proposed technology's technical and commercial potential.

Talk 7-4: Breathe inside the box: optimizing conditions for indoor microbial electro-methanogenesis

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Human metabolism and activities within indoor environments with low or poor ventilation can cause significant increases of CO_2 concentration, eventually leading to discomfort issues. Indoor Direct Air Capture (DAC) can improve air quality in those environments, although, while being thermodynamically advantageous compared to atmospheric DAC, it remains underutilized due to challenges in concentrating available CO_2 (< 1500 ppm) to highly concentrated CO_2 streams. Among many biological processes for CO_2 conversion, bioelectrochemical systems (BES) have potential for bio-electro CO_2 recycling into carbon-neutral biofuels. One such process, microbial electro-methanogenesis (EM), converts CO_2 into methane (CH_4). This study introduces an innovative approach by developing a portable, hybrid unit, integrating indoor CO_2 DAC (using a micro-concentrator module, CO_2-MCM) with an EM-BES. The DAC system efficiently concentrated CO_2 up to 20%, with variable oxygen presence (2-7%). A commercial low-gap electrolyzer (ElectroCell, Denmark) was used as test reactor (cathode surface 0.001 m^2). The EM-BES was operated at room temperature (25 °C) and optimized to be synchronized with the adsorption-desorption cycles of the DAC unit by applying intermittent CO_2 feeding (200 mL d^{-1}). 8-12h power cuts were introduced to simulate solar energy surplus as electricity supply, at different current applied values (10-40 A m^{-2}) according to the CO_2 content. The presence of oxygen did not significantly affect the methane production rates at 10 A m^{-2}, ranging from 6.9 to 23.9 L m^{-2} d^{-1} (T=25 °C and p=1 atm) and varying consistently with the CO_2 content. The control reactor fed with 100% CO_2 showed similar production rates (17.5 ± 9.3 L m^{-2} d^{-1}), although the energy consumption with indoor carbon increased by 20% due to the presence of oxygen competing with CO_2 as electron acceptor compared to the control (30.1 ± 8.8 kWh Nm^{-3}). The EM-BES showed high tolerance to oxygen intrusion, process flexibility and reliability. Electric power interruptions did not affect CH_4 productivity (108.2 ± 21.2 L m^{-2} d^{-1}) and reduced energy consumption by 50% (21.7 ± 4.3 kWh Nm^{-3}) at the higher current rates tested (40 A m^{-2}). This study provided the framework for the validation of the portable unit in real indoor environments.

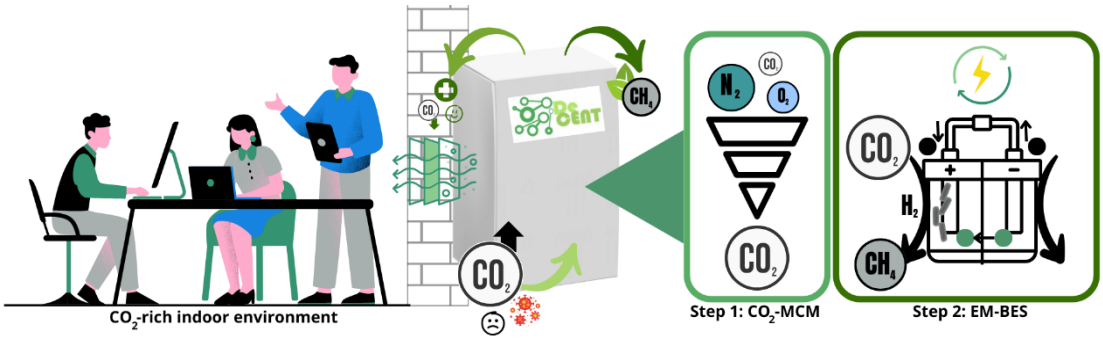

Figure 1. De-Cent project concept.

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Talk 7-5: BES pilot operated for 4000 h for H_2 production and biogas upgrading

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This study presents a novel pilot-scale bioelectrochemical system (BES) integrated with a biogas upgrading process to produce renewable gases (H2 and CH4) and valorize wastewater and CO2. The 3-chamber BES reactor generates H_2 and NaOH in the cathodic chamber. Simultaneously, in the anodic chamber, electroactive bacteria oxidize organic matter from wastewater, providing electrons. In the saline chamber, the electric field between anode and cathode allows the migration of Na^+ to the cathodic chamber and HCO_3^- to the anode chamber, removing/desalinating the $NaHCO_3$. The NaOH-enriched electrolyte from the cathode is employed in the alkaline biogas upgrading scrubber, capturing CO_2. The $NaHCO_3$ stream produced is regenerated in the BES pilot, first it is introduced into the saline chamber to remove Na^+ and HCO_3^- and the resulting effluent is then introduced into the catholyte chamber to generate the NaOH. This innovative approach facilitates the generation of H_2 and CH_4, and enables renewable electricity storage, wastewater treatment, and chemical-free operation of scrubber due to NaOH in-situ regeneration.

The pilot system consists of a flat-plate BES reactor (based on an ElectroCell Europe cell) with a section of 0.8 m^2 and an internal volume of 24 L (8 L per chamber), with the buffer tanks a total system volume of 175 L. The anode is carbon felt with a graphite current collector, the cathode is Ni felt with a Ni plate as the current collector and FAB-PK-130 and FKB-PK-130 membranes were used. Additionally, a 35 L scrubber, integrated with a 50 L buffer tank, is incorporated into the pilot for biogas upgrading.

Before pilot construction, a 100 cm^2 lab-scale BES was used to evaluate membranes and cathode materials to optimize system performance and operational parameters. The optimal results were obtained at an applied voltage of 1 V, yielding current densities in the range of 4-6 A/m^2. The system exhibited a theoretical H_2 production rate of 2-3 $m^3H_2/m^3_{reactor}$ day, along with effective $NaHCO_3$ desalination from the saline chamber. At the pilot scale (Figure 1), the system operated for 4000 hours under real environmental conditions (temperature range: 10-20°C). During fully automated operation (cycles 13-21, exceeding 2000 hours), the BES pilot demonstrated stable performance with an average current density of 1.7 A/m^2, a H_2 production rate of 0.8-1 $m^3H_2/m^3_{reactor}$ day, and a Faradaic desalination efficiency of 75%. Notably, no catholyte or saline solution replacement was required during automated operation, highlighting the system's low OPEX potential for biogas upgrading, as NaOH used and consumed in the scrubber is regenerated in BES without additional chemical inputs.

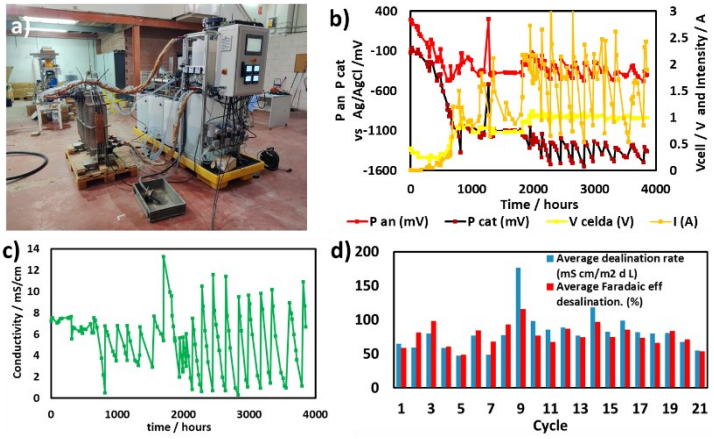

Figure 1. a) BES and scrubber pilot. b) Electrochemical parameters of BES pilot. c) Conductivity of saline chamber during operation, which shows de desalination. d) Desalination rate and desalination Faradic efficiency of pilot. Each cycle corresponds to a complete desalination process, removing both HCO_3^- and Na^+ from the saline electrolyte.

Talk 7-6: Enhancing Anaerobic Degradation of Swine Manure through Electrofermentation

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According to the Worldwatch Institute, global meat production and consumption have tripled over the last four decades [1]. Swine farming is the most widely consumed meat worldwide, generating approximately 1.7 billion tons of liquid swine manure annually and contributing to 18% of livestock greenhouse gas (GHG) emissions [2]. Conventional anaerobic digestion has the potential to recover energy from swine manure, but only a fraction of the waste can be stabilized [2]. Electrofermentation (EF) can enhance methane production, accelerate organic waste degradation, and promote H_2 generation. The EF process, based on Microbial Electrolysis Cells (MECs), can promote organic matter oxidation with the formation of electrons and H_2 that can be used by hydrogenotrophic methanogens. In this study, EF of swine manure was first evaluated at mesophilic temperatures (37±1°C) under batch conditions for 54 days. Carbon plate electrodes were installed in four 250-mL reactors, where two reactors were supplied with 1 V (EF), and two were maintained as an open circuit (AD). EF treatments showed a 57% higher, faster, and more consistent methane production than the AD controls (905±16 *vs.* 575±103 mL CH_4/g VS) (Fig. 1A). Following this study, eight 1-L CSTRs were set up in a semicontinuous feeding mode with a 20-d HRT under ambient temperatures. All the reactors were equipped with carbon felt electrodes, where four reactors were supplied with 1 V (EF) and two were maintained as an open circuit (AD).

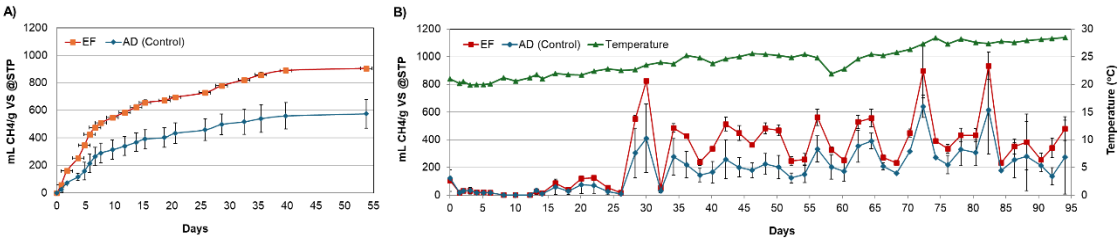

Fig. 1. Methane production of swine manure under (A) batch, (controlled) mesophilic and (B) semi-continuous, (uncontrolled) ambient temperature conditions.

During 94 days of operation temperature was maintained within the suboptimal microbial psychrophilic and mesophilic ranges, ranging between 20 and 29°C (24±3°C). After *ca.* 26 days of acclimation, methane production became somewhat more stable for both the EF and AD reactors. After this period, average methane production was 24% higher for the EF reactors, with 460±210 mL CH_4/g VS *vs.* 372±174 mL CH_4/g VS for the AD reactors. Methane production for the semi-continuous reactors was considerably lower than that observed under batch mode; however, at least three considerations should be noted that may, in part, explain these differences: 1) CSTRs HRT = 20 d *vs.* complete degradation batch time; 2) CSTRs lower, uncontrolled, and suboptimal temperatures; 3) CSTRs higher influent VS = 25.3±14.7 g/L vs. batch VS = 1.9 g/L. Overall, EF CSTRs demonstrated a higher OCP (304 vs 2.12 mV) and increased VS (48.6 *vs.* 43.8%) and COD (55.9 *vs.* 49.2%) degradation *vs.* AD CSTRs, suggesting that voltage application effectively stimulated the growth of electrochemically active microorganisms and increased the overall performance of swine manure treatment. This is an ongoing study, which upon completion, will include specific bioelectrochemical analyses and changes in the microbial community using Illumina MiSeq sequencing.

References:

[1] M. Karwowska, S. Łaba, and K. Szczepański, "Food loss and waste in meat sector—why the consumption stage generates the most losses?" 2021. doi: 10.3390/su13116227.
[2] A. Makara and Z. Kowalski, "Selection of pig manure management strategies: Case study of Polish farms," *J Clean Prod*, vol. 172, 2018, doi: 10.1016/j.jclepro.2017.10.095.

Talk 7-7: High energy efficiency and long-term operation of methane-producing bioelectrochemical systems at haloalkaline conditions

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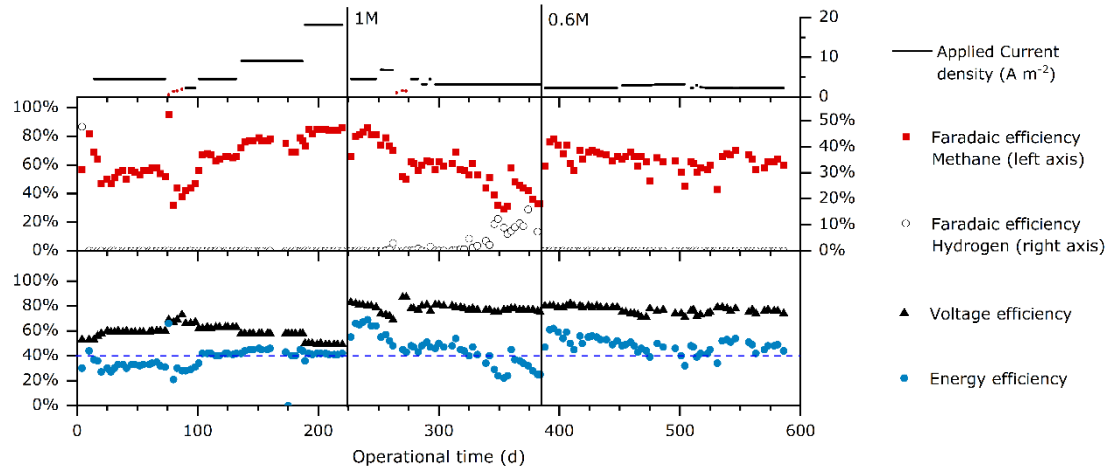
Methane-producing bioelectrochemical systems (BES) are a sustainable form of Power-to-gas technologies, converting electricity into a chemical energy carrier, methane, in a single step. Conversion of electricity into methane allows for stable and long-term energy storage and can make use of currently existing infrastructure for gas storage and transport: qualities that are relevant for shorter-term solutions to the energy transition towards circular and greener energy. The main indicators in order to fully assess performance of methane-producing BES are methane production rate, faradaic efficiency and energy efficiency. However, up to now, short operational times and low (or underreported) energy efficiency and lack of long-term stability studies stand in the way of developing methane-producing BES as an energy storage technology.

Here, we studied the operation of methane-producing BES under a haloalkaline electrolyte as a strategy to increase energy efficiency. We operated 2 BESs for 380 and 590 days, with granular activated carbon (GAC) as cathode material, through current control. The cells were first operated with standard electrolyte (50 mM phosphate buffer at pH 7) and later at haloalkaline conditions, with a concentration between 0.6 and 1.0 M bicarbonate at pH~8.5. The highest methane production rate was 40 L/m^2.d at standard electrolyte at a current density of -20 A/m^2. We observed unprecedented energy efficiency up to 70% at haloalkaline conditions, and energy efficiency was higher than 40% throughout most of the operational period (see Figure). Interestingly, the biocathodes reached very low overpotential, in line with previous work using GAC, at cathode potentials between -0.5 and -0.7V vs Ag/AgCl, while current density varied between -2 and -20 A/m^2. The higher energy efficiency at haloalkaline conditions was the result of ~20% lower total internal resistance in comparison to operation with standard electrolyte. Highest resistance was found for the anodes, followed by cathode, transport and ionic losses.

Stability at halo-alkaline conditions was a challenge: at higher bicarbonate concentration of 1.0 M, cathode overpotential increased with time, and hydrogen formation was observed. Stability was re-established by lowering the current density to -2 (BES1) and -4.5 (BES2) A/m^2 and decreasing the bicarbonate concentration to 0.6 M. Next challenges is to increase the current density (methane production rate), while maintaining high energy efficiency, and to test adaptation strategies to maintain methanogenic activity at haloalkaline conditions at these higher rates.



ORAL PRESENTATIONS

Session 8 – Electrochemistry/ Systems Engineering/ Modeling

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Talk 8-1: Analysis of electroactive biofilms for microbial electrochemical sensing

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Microbial electrochemical sensors have been proposed as real time water quality indicators in several applications. The information provided by these biosensors has been revealed as unvaluable for making decisions in biotechnological processes where microorganisms are involved. Special and relevant advances have been achieved in monitoring effluents and influents of wastewater treatment plants, with the implementation through an online IoT platform[1] of a three electrodes bioelectrochemical system and an electronical system responsible for the control, acquisition and transmission of the electrical current in real time.
However, there is still lot of work to be done in order to gain insightful knowledge about the different events and environmental conditions that influence the response of these systems. Key aspects like the availability of procedures for developing mature and reproducible biofilms in microbial and electrochemical terms, and the variation of coulombic efficiencies due to complex mixtures of different carbon source substrate present in real scenarios must be addressed in detail. Furthermore, the effect of electrode potential in these aspects deserved a thoroughly consideration.
In this work, a protocol has been developed to achieve biofilm replicates with *G.sulfurreducens* by using a bipotentiostat, an electrochemical instrumentation that allows to work with two different working electrodes in the same culture under potentiostatic control sharing the same reference and counter electrodes. These biofilms grown in identical physiological and physicochemical conditions have been studied under different electrode potentials, including OCP situation, and exposed to different carbon source substrates. Electrochemical performance was used as standard check for reproducibility by means of chronoamperometry and cyclic voltammetry. The cyclic voltammetry response of the replicates at 15 days showed a sigmoidal curve with an onset potential close to 0 V vs Ag/AgCl (3.5M), and a maximum limiting current at 0.1 V vs Ag/AgCl (3.5M), features typical of mature *Geobacter sulfurreducens* biofilms[2,3].
Once replicable and mature biofilms were obtained, a study with different biodegradable compounds was performed: i) acetate as the sole carbon source for G. sulfurreducens and ii) glucose and its fermentation products (lactate and pyruvate) , to investigate the different contribution to the recorded electrical current values by every single organic compound. The application of this results is focused on the interpretation of the concept of biodegradable organic matter in wastewater treatment plants when using microbial electrochemical sensors[4].

References:

1. Berná, A. et al. (2024). Microbial Electrochemical Sensors: A new Platform for Online and Real Time Water Quality Monitoring. XXVIII International Symposium Bioelectrochemistry and Bioenergetics organized by The Bioelectrochemical Society, Alcalá de Henares, Spain.
2. Marsili, E., Rollefson, J.B., Baron, D.B., Hozalski, R.M., Bond, D.R. (2008). Microbial Biofilm Voltammetry: Direct Electrochemical Characterization of Catalytic Electrode-Attached Biofilms. Appl Environ Microbiol 74:. https://doi.org/10.1128/AEM.00177-08.
3. Schröder, U., Harnisch, F., Angenent, L. T. (2015). Microbial electrochemistry and technology: Terminology and classification. Energy and Environmental Science, 8(2), 513–519. https://doi.org/10.1039/c4ee03359k.
4. Simoska, 0. et al., (2021). Recent trends and advances in microbial electrochemical sensing technologies: An Overview. Current Opinion in Electrochemistry. 30:100562

Talk 8-2: pyMES: An Integrated Python Framework for Multiscale Modeling and Optimization of Microbial Electrosynthesis from CO_2

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Microbial Electrosynthesis (MES) enables the bioconversion of CO_2 into value-added multi-carbon chemicals by coupling electrochemical water oxidation at the anode with CO_2 reduction by chemolithoautotrophic microbes at the cathode[1]. The MES process encompasses tightly coupled phenomena—including electrochemical water oxidation, direct and indirect microbial extracellular electron transfer, ionic migration, gas-liquid mass transfer, and microbial growth and metabolism—operating across multiple spatial (micro-, meso-, and macro-) and temporal scales[2]. Despite its potential for CO_2 utilization and a decade of research efforts, the mechanistic understanding of MES remains limited, primarily due to fragmented investigations that focus separately on electrochemical, biological, physical or operational components, without accounting for their interdependencies[4]. To bridge these gaps, a unified, multiscale modeling framework is critical for capturing the complex physicochemical and microbial interactions that govern MES performance, guiding rational experimental design and overall conceptual understanding[2,3,5]. To this end, we have developed a modular, multiscale modeling framework in Python to simulate the physicochemical and biological processes in a commonly used two-chamber MES reactor configuration. The model integrates electrochemical kinetics, proton and gas transport, CO_2 sparging, headspace pressure dynamics, biofilm and H_2-mediated production, pH buffering, reactor volume. Each sub-process is implemented as an independent, interacting module, enabling time-resolved predictions under both galvanostatic- and potentiostatic-controlled modes. All equations are unit-consistent and parameterized using experimentally relevant values derived from literature and previous experiments. This model enables detailed in-silico analysis of key parameters, such as electrochemical kinetics, mass transport, gas dynamics, proton flux, and microbial metabolism that together govern MES reactor performance. It can simulate the effect of different operational parameters, reactor scale and design (e.g., electrode area and reactor volume), electrochemical parameters, and microbial metabolism (mainly substrate uptake rates). This allows predictive evaluation of experimental setups and identification of performance-limiting bottlenecks under different operating scenarios. Python based Microbial Electrosynthesis (pyMES) is expected to facilitate systematic experiment design, standardizing protocols, and process analysis. It thus addresses a crucial gap in MES technology development, offering a comprehensive set of tools to facilitate further development of computational modeling approaches which remain underutilized thus far.

References:

1. Gadkari, S., Shemfe, M., Modestra, J.A., Mohan, S.V., Sadhukhan, J., 2019. Understanding the interdependence of operating parameters in microbial electrosynthesis: a numerical investigation. Phys. Chem. Chem. Phys. 21, 10761–10772. https://doi.org/10.1039/C9CP01288E
2. Jourdin, L., Burdyny, T., 2021. Microbial Electrosynthesis: Where Do We Go from Here? Trends in Biotechnology 39, 359–369. https://doi.org/10.1016/j.tibtech.2020.10.014
3. Kazemi, M., Biria, D., Rismani-Yazdi, H., 2015. Modelling bio-electrosynthesis in a reverse microbial fuel cell to produce acetate from CO_2 and H_2O. Phys. Chem. Chem. Phys. 17, 12561–12574. https://doi.org/10.1039/C5CP00904A
4. Prévoteau, A., Carvajal-Arroyo, J.M., Ganigué, R., Rabaey, K., 2020. Microbial electrosynthesis from CO_2: forever a promise? Current Opinion in Biotechnology 62, 48–57. https://doi.org/10.1016/j.copbio.2019.08.014
5. Salimijazi, F., Kim, J., Schmitz, A.M., Grenville, R., Bocarsly, A., Barstow, B., 2020. Constraints on the Efficiency of Engineered Electromicrobial Production. Joule 4, 2101–2130. https://doi.org/10.1016/j.joule.2020.08.010

Talk 8-3: Quantification of Cathodic biofilm growth using optical coherence tomography

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Microbial electrosynthesis (MES) technology is a promising technology to convert CO_2 into green chemicals, with microbial community development being crucial for MES performance optimization.
This study uses Optical Coherence Tomography (OCT) as an in-situ, non-invasive, monitoring technique for studying the biofilm formation of acetogenic bacteria over time on the surface of the cathode of a MES cell using different saline concentrations in the electrolyte.
The experiments used dual-chamber bio-electrochemical cells previously reported by Molenaar et al., 2018. FTO-coated glass was used as the cathode. An IVIUMnStat potentiostat was used to control the cathode potential either at -0.8 V or -0.7 V vs Ag/AgCl. These potentials were chosen as the FTO coating is not stable under more reductive conditions.
The catholyte chambers were inoculated using 15 mL of enriched mixed-culture biomass. Each chamber contained a total of 150 mL of electrolyte with a NaCl concentration of 5 or 10 g/L. A higher concentration should reduce the internal resistance and, therefore, the required energy input for VFA production.
Samples of the anolyte and catholyte were taken through sampling ports twice a week to analyze the pH using a pH sensor. The composition of the electrolyte was measured using high-performance liquid chromatography (HPLC). OCT scanning procedure and data processing were done twice a week throughout the operation of the reactors. Finally, EIS spectra were measured at the end of the operation.
Figure 1 shows the increase of the biofilm thickness observed through OCT, normalized to the initial value recorded for the FTO electrode before inoculation. The results show a faster development for the biofilm with 10 g/L of NaCl in the electrolyte, especially at a potential of -0.7 V, with growth almost immediately after inoculation, and biofilm thickness remained quite low throughout 35 days. The biomass yield on electrons was around 10%. However, for all reactors, acetic acid production stayed below the detection limit (<5 mg/L) of the HPLC for the duration of the experiment (~35 days).

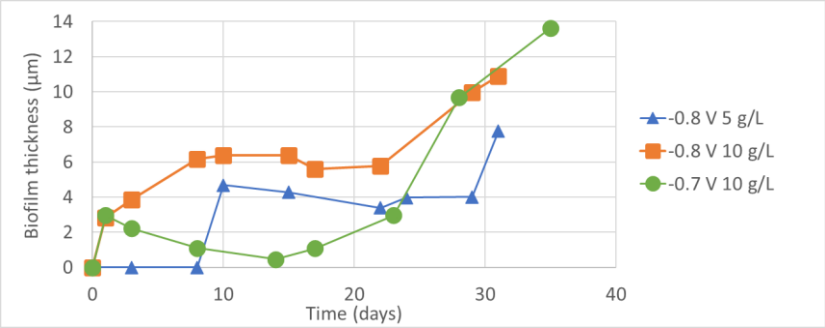

Figure 1 Biofilm development over time

1. Molenaar, S. D. *et al.* In situ Biofilm Quantification in Bioelectrochemical Systems by using Optical Coherence Tomography. *ChemSusChem* **11**, 2171–2178 (2018).

Talk 8-4: Habermann and Pommer (1991) Revisited: Towards a Sulphide Storing Microbial Fuel Cell Anode

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In 1991, Habermann and Pommer published their work on a microbial fuel cell based on sulphide (S^{2-}) mediation at cobalt hydroxide modified graphite anodes with impressive performance characteristics [1], however to this day, literature does not show a follow-up study or a successful replication of the results.
The understanding, replication and possible improvement of the original work by Habermann and Pommer would allow employing this MFC concept especially for sulphide-rich environments with the purpose of current generation and sulphide remediation.
In this study the electrochemical properties of cobalt-deposited electrodes when brought in contact with sulphide-containing solutions were investigated. Results show that cobalt acts as a catalyst accelerating the oxidation of S^{2-} to higher oxidation products ($S_xO_y^{2-}$), thereby avoiding sulphur build-up on the electrode surface. The sulphide oxidation can proceed either directly at the cobalt oxide surface (continuous mode), or via soaking and subsequent oxidation mechanism (discontinuous mode). In the latter, the cobalt layer itself is "charged" by transformation to cobalt sulphide (CoS), which is subsequently "discharged" oxidatively resulting in the production of current. These reactions are repeatable for numerous cycles, illustrating the chemical reversibility of the process. Data reveals that the overall rate of the discontinuous operation is significantly higher than that of the continuous mode – reaching 200 % overall current / charge generation when compared to the continuous mode. [2] Based on the findings of this study a schematic representation of this MFC system is proposed in Figure 1.
Ongoing work is directed into understanding the underlying functional mechanism between the cobalt layer and the sulphide in solution under physiological pH conditions in the absence and presence of bacteria, with special focus in anode stability and reaction reversibility.
The aim is to replicate the MFC by Habermann and Pommer with an exhaustive comprehension of the underlying functional mechanism of this type of MFC so that it can be utilised to its full potential.

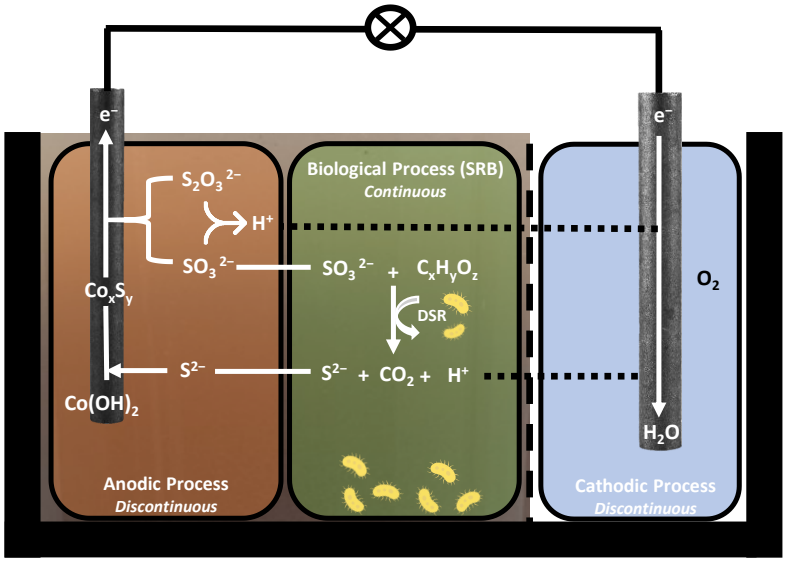

Figure 1. Schematic representation of processes in the Habermann-Pommer sulphur cycling MFC.

[1] W. Habermann and E.H. Pommer, 'Biological Fuel Cells with Sulphide Storage Capacity', *Applied Microbiology and Biotechnology*, 35.1 (1991), 128–33 <https://doi.org/10.1007/BF00180650>.
[2] Erik Lindemann, Inês Didier, and Uwe Schröder, 'Habermann and Pommer (1991) Revisited: Decoding the Mechanism of a Sulphide Storing Microbial Fuel Cell Anode', *ChemElectroChem*, 2025 <https://doi.org/10.1002/celc.202500071>.

Talk 8-5: Microbial influenced corrosion on stainless steel by Geobacter bacteria: a Butler-Volmer model

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In this work we will show theoretical results from a Butler-Volmer based finite element model, that includes anaerobic stainless-steel corrosion and Geobacter sp. bacterial cells, considered as a surface attached biofilm. The reversible electrochemical connection to the bacterial metabolism is accomplished by the expression of multiple c-type multiheme cytochromes at the outer membrane/pili, that enable anodic respiration (1) via direct electron transfer and subsequent biofilm growth.
Results will show the model calibration steps taken to obtain several parameters that are extracted from abiotic corrosion experiments and biotic cyclic voltammetry, and will demonstrate the influence of the growth of a bacterial biofilm at the metal surface with modelled potentiodynamic polarization data. The pool of mediators that the bacteria express at the outer membrane surface, as well as the relative abundance of each cytochrome are fundamental parameters for the successful interaction of the cytochromes with the corrosion electrochemical cell. Finally, we will also show how the bacterial biofilm can promote metal pitting in these highly passive metal alloys, and we will analyze the outcome of our finite element model in scenarios where changing local environmental conditions originate different corrosion potentials (Ecorr) and currents (Icorr) and compare these with experimental results and results found in literature (2,4).

(1) Nature Reviews Microbiology, volume 14, pages 651–662 (2016), doi.org/10.1038/nrmicro.2016.93
(2) mBio, volume 10 (issue 3), (2019), doi.org/10.1128/mBio.00303-19
(3) The ISME Journal, volume 15 (issue 10), pages 3084–3093 (2021), doi.org/10.1038/s41396-021-00990-2
(4) Corrosion Science, volume 52 (issue 11), pages 2596-2604 (2009), doi.org/10.1016/j.corsci.2009.06.041

Talk 8-6: High-Throughput Screening of Electroactive microbes and Performance on Diverse Thin Film Electrodes in a PCB based Bioelectrochemical microreactors array

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This study presents a novel microfluidic PCB-based bioelectrochemical microreactor array with 96 individually addressable microreactors for high-throughput screening of materials and electroactive microbes utilized in Bioelectrochemical systems. Each microreactor comprises a gold working electrode surrounded by reference and counter electrodes within a 3 mm deep well, replicating the standard microtiter plate format. This design enables parallel enrichment of electroactive biofilms from wastewater under controlled potential using a multichannel potentiostat. We demonstrate the utility of this platform by investigating the influence of twelve different thin film metal electrodes on biofilm development, power generation, dye degradation, and chemical oxygen demand (COD) removal from textile wastewater. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analysis of the biofilm-electrode interface provided insights into microbe-metal interactions and potential biofilm secretions relevant to electron transfer and dye degradation. This platform offers a powerful tool for accelerating the discovery and optimization of bioelectrochemical materials for wastewater treatment applications.

Keywords: *High-throughput screening, Bioelectrochemical system, Microfluidic reactors, Wastewater treatment, PCB-based bioelectrochemical microreactor array.*

Talk 8-7: Understanding the Tail Current Behavior of Electroactive Biofilms Realizes the Rapid Measurement of Biochemical Oxygen Demand

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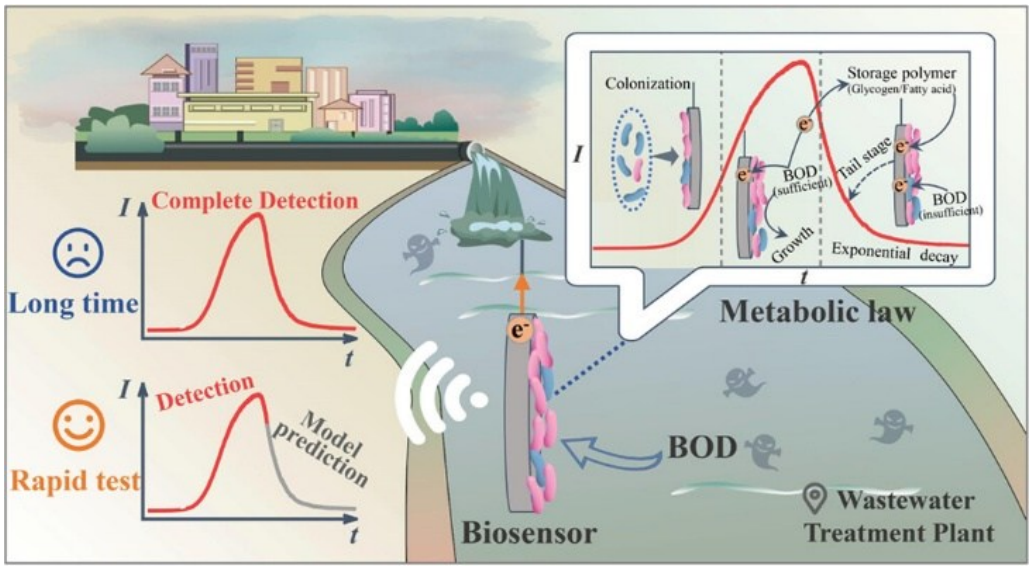
The use of microbial electrochemical systems (MESs), with electroactive biofilms (EABs) as sensing elements, is a promising strategy to timely measure the biochemical oxygen demand (BOD) of wastewater. In MES-based sensors, BOD value is calculated by integrating current over time. However, accumulation of Coulombic yield over a complete degradation cycle is time-consuming. Based on current increase, we recognized a tail stage (TS) on a current-time curve, which exhibited the lowest electron harvesting efficiency and occupied over 50% of the total BOD test duration. Therefore, elucidating the metabolic dynamics of EABs during the TS and rationally predicting the Coulombic yield accumulated in this phase are critically important.

In this study, we investigated the metabolic patterns of pure Geobacter biofilms in the TS and further explored identification and prediction methodologies for the TS in BOD sensors composed of Geobacter-dominated mixed-species biofilms under domestic wastewater BOD measurement conditions. Here, we found that EAB adopted a series of metabolic compensation strategies, including slow metabolism of residual BOD, suspended growth, reduced cell activity, and consumption of carbon storage polymers, to cope with substrate deficiency in TS. The supplementary electrons provided by the decomposition of glycogen and fatty acid polymers increased the Coulombic efficiencies of TS to >100%. The tail current produced by spontaneous metabolic compensation showed a trend of convergent exponential decay, independent of BOD concentration. Therefore, we proposed the TS prediction model (TSPM) to predict Coulombic yield, which shortened BOD measurement time by 96% (to ~0.5 h) with deviation <4 mg/L when using real domestic wastewater. Our findings on current output in TS give insights into bacterial substrate storage and consumption, as well as regulation in substrate-deficient environment, and provide a new perspective for interpreting BOD sensing data.



Graphical abstract

ORAL PRESENTATIONS

Session 9 – Microbial Fuel Cells

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Talk 9-1: A Transcriptional and Metabolite Perspective in Microbial Fuel Cells: the case study of *Pseudomonas citronellolis*

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Abstract

Microbial fuel cells (MFCs) leverage electroactive microorganisms to drive both bioremediation and electricity generation. While *Geobacter* sp. and *Shewanella* sp. have been widely studied, the recently isolated novel strain of *Pseudomonas citronellolis* 620C [1] has exhibited exceptional electrochemical properties [2]. This strain not only generates substantial bioelectricity but also efficiently degrades toxic and persistent oily wastewater, a process enhanced by biofilm formation and the electron shuttle pyocyanin [2]. Additionally, it synthesizes biosurfactants (BSFs) [3], which facilitate extracellular electron transfer (EET) and further support biodegradation.

This study investigates the performance of *P. citronellolis* 620C in a dual-chamber MFC, emphasizing BSF production, hydrocarbon and fatty acid degradation, and the accumulation of high-value byproducts such as polyhydroxyalkanoates (PHAs) [4]. BSF production was confirmed through surface tension kinetics and GC/MS analysis [3], while PHAs and pyocyanin were identified using UV-Vis spectroscopy and GC/MS. Biodegradation efficiency was evaluated using COD and GC/MS, alongside real-time bioelectricity monitoring. Transcriptional kinetics provided insights into key metabolic activities, including hydrocarbon and fatty acid breakdown, PHAs and BSF synthesis, pyocyanin and biofilm formation, quorum sensing, and metabolic adaptations during nutrient depletion. Results demonstrated the rapid co-production of pyocyanin and lipopeptide BSF, an early onset of biodegradation, and the influence of PHAs, biofilm formation, and quorum sensing on electro-bioremediation processes.

This study highlights the significance of understanding microbial behavior under MFC conditions, particularly the interplay between bioremediation, bioelectricity generation, and metabolite production. By leveraging transcriptional kinetics and metabolites monitoring as biomarkers for mechanistic insights, this research advances microbial electrochemical technologies (METs), promoting more effective environmental remediation through strategic optimization at microbiological level.

References:

1. Tsipa, A., Stylianou, K., Papalli, M., Papageorgiou, E., Kyriakou, L., Rigopoulos, I., Ioannou, I., Pinakoulaki, E. 'Iron Stimulated Production and Antimicrobial Potential of a Novel Biosurfactant Produced by a Drilling Waste-Degrading *Pseudomonas citronellolis* Strain', Processes, 9, 686 (2021).
2. Varnava C.K., Persianis P., Ieropoulos I., Tsipa A. 'Electricity generation and real oily wastewater treatment by *Pseudomonas citronellolis* 620C in a microbial fuel cell: pyocyanin production as electron shuttle', Bioprocess Biosyst. Eng. (2024).
3. Varnava C.K., Grenni P., Mariani L., Barra Caracciolo A., Hadjipakkou H., Lefkaritis G., Pinakoulaki E., Chronakis N., Kalogerakis N., Tsipa A. 'Characterization, production optimization and ecotoxicity of a lipopeptide biosurfactant by *Pseudomonas citronellolis* using oily wastewater', Biochem. Eng. J., 205, 109257 (2024).
4. Tsipa A., Varnava C.K., Nastro R. A., Ieropoulos I. 'Biosurfactants, Polyhydroxyalkanoates, and Other Added-Value Products from Wastewater Electro-bioremediation: A New Biorefinery Concept',. doi:10.1007/10_2025_279 (2025).

Talk 9-2: Granular Activated Carbon Tubular Microbial Fuel Cell for Decentralized Greywater Treatment

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Household-scale decentralized greywater reuse systems face significant operational efficiency, treatment performance, and energy sustainability challenges. Among the various emerging technologies, Microbial Fuel Cells (MFCs) offer a sustainable alternative by integrating wastewater treatment with bioelectricity generation. However, due to the cost of the materials, the electrical losses, and the operational conditions, the scalability of these systems is still an unsolved challenge. Granular activated carbon (GAC), a low-cost material, has been proposed as an electrode material for 3D-electrode designs and supports the development of electroactive microorganisms. This study presents the development of a scalable granular tubular MFC (GMFC) utilizing GAC as an electrode material to assess its efficiency in treating synthetic greywater. The GMFC was compared with an aerated granular biofilter (GBF), focusing on effluent quality parameters such as pH, electrical conductivity, turbidity, and soluble chemical oxygen demand (sCOD) to benchmark its performance. The reactors were continuously operated for over a year under varying conditions, monitoring current generation, electrical performance, and evaluating biofilm formation through scanning electron microscopy, epifluorescence microscopy for cell quantification, and microbial community characterization via Illumina MiSeq sequencing.

The results indicated that GMFC and GBF achieved statistically similar sCOD removal efficiencies (>88%) and effluent turbidity levels ranging from 0.34 ± 0.02 to 6.125 ± 0.42 NTU. In terms of electrical performance, GMFCs with non-aerated cathodes exhibited polarization shifts and high but unstable power densities, whereas aerated cathodes provided stable power densities of 8.7 ± 1.7 and 8.2 ± 2.2 mW/m^3. In charge/discharge operation, GMFC performed a 16% enhancement in max current generation. Notably, GMFCs sustained current generation even without organic matter in the medium, implying that adsorbed organic compounds on the GAC contributed to bio-regeneration without additional energy input. Microbial analysis revealed a higher abundance of sessile microorganisms in GMFCs compared to GBFs, with dominant phyla including Proteobacteria, Firmicutes, and Bacteroidetes. Moreover, ecological indices suggested that GMFCs fostered a more diverse and less selective microbial environment than GBFs, enhancing resilience against environmental fluctuations. These findings demonstrate that GMFCs can achieve efficient greywater treatment while consuming less net energy than conventional aerated biofilters, offering a robust and sustainable long-term alternative for decentralized wastewater management.

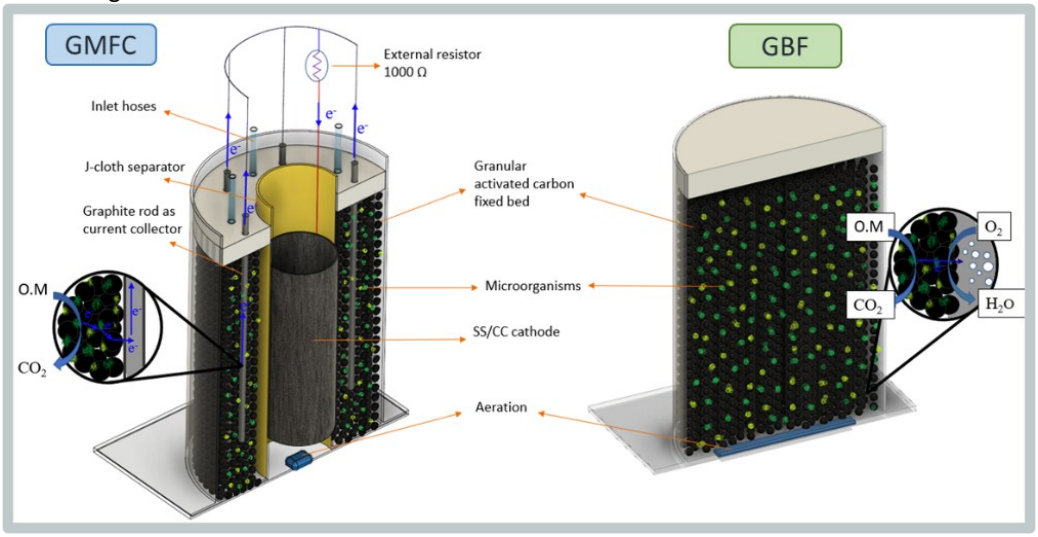

Figure 1: Reactors schematics.

Talk 9-3: A microbial electrochemical system for treating blackwater on board small-size recreational sail- or motorboats: First insights and challenges

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Sewage discharge from maritime pleasure craft is becoming of increasing concern with respect to the pollution of water bodies. Although strict regulations regarding treatment and disposal are set for most of maritime transport [1], vessels with dimensions and occupancy beneath the set limits manage their sewage under milder conditions. For instance, disinfection, comminuting, and raw disposal are allowed and persistent frequent practices. Thus, the development of alternatives for proper wastewater treatment on board smaller craft remain a necessity in order to prevent further pollution. In this context, the aim of this work is to explore the perspectives and challenges of microbial electrochemical systems for sewage treatment on board smaller size recreational sail- or motorboats.

To this end, a two-chamber microbial electrochemical cell was operated in batch mode both under potentiostatic control and in short circuit mode. Anolyte and feeding source of the bioanode consisted of brackish-water based artificial blackwater (salinity of 20 g kg^{-1}_{soln}). Biomass harvested from a running single-chamber cell operated with under same substrate and salinity conditions served as inoculum. The initial chemical oxygen demand (COD) was set to 4,2 g l^{-1}, equivalent to the concentration expected aboard a pleasure craft. In contrast, the cathode was operated as a hybrid submerged/air-cathode employing only brackish water at a salinity of 18 g kg^{-1}_{soln} as catholyte. The reactor was connected to two 500 ml reservoirs containing anolyte and catholyte each. The anode was operated under potentiostatic control at -157 mV vs SCE during biofilm formation to be later operated in short circuit. Chronoamperometry and Coulomb efficiency (CE) were employed to evaluate and compare the electrochemical performance of the cell during operation in potentiostatic control and short circuit mode. Furthermore, anaerobic and aerobic control experiments were performed for comparison. COD removal and sludge production per inlet COD (VSS per COD_{in}) were employed for the evaluation and comparison against controls. Polarization curves of anode and cathode were recorded regularly using linear sweep voltammetry at a scan rate of 0.5 mV s^{-1} while deposits on the cathode surface were characterized via X-Ray powder Diffraction (XRD) at the end of the experiment.

The bioanode acclimation under potentiostatic control was successful, yielding maximum current densities of 0.36 ± 0.03 mA cm^{-2}. CE during this period reached 13 ± 4 % at a COD removal efficiency of 83 ± 6 %. A significant reduction of performance was observed once the operation mode was changed to short circuit, reaching maximum current densities of 0.07 ± 0.01 mA cm^{-2} and CE of 4 ± 1 %. Further analyses using LSV showed a limited performance of the cathode, which affected greatly the current densities that can be obtained in short circuit mode. Close inspection of the cathode surface via XRD showed deposits of up to 99% aragonite, a very stable form of $CaCO_3$, indicating possible surface inactivation. In terms of treatability of the artificial blackwater, a COD removal per day of 821 ± 67 mg O_2 l^{-1} d^{-1} was achieved during the operation of the cell in short circuit whereas the anaerobic control reached only 131 mg O_2 l^{-1} d^{-1} and the aerobic control 510 mg O_2 l^{-1} d^{-1} showing a positive effect of the microbial electrochemical system on COD removal. Finally, sludge production amounted to only 0.08 g VSS per COD_{in}, as compared to 0.7 g VSS per COD_{in} for the aerobic control.

In conclusion, our work shows that under potentiostatic control not only remarkable anodic current densities and treatment efficiencies can be reached, but also very little sludge production is observed as compared to aerobic treatment. However, the low performance of the oxygen reduction cathode, presumably resulting from deposits of Ca-ions which are ubiquitous in seawater, severely limits the operation of the system as an energy-autonomous microbial fuel cell. This limitation has to be addressed for practical application of the concept as a sewage treatment systems aboard pleasure craft operating in brackish- or seawater.

[1] IMO (2019). Prevention of pollution by sewage from ships. Accessed on 31.03.2025. URL: https://www.imo.org/en/OurWork/Environment/Pages/Sewage-Default.aspx

Talk 9-4: *Desulfuromonas* acetoxidans: a marine bacterium to do it all, water desalination and electricity production

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According to the United Nations, freshwater scarcity is a pressing issue that affects nearly 40% of the world's population. Therefore, desalinating the abundant salty water available on the planet is crucial for addressing the global scarcity of safe drinking water, as well as meeting irrigation and industrial demands. The desalination methods available to date are thermal desalination and membrane-based technologies. These methods, besides requiring a great amount of energy, either to heat the water or to pump it through reverse osmosis, also create an environmental issue due to brine discharging, costliness, and potential harm to marine ecosystems.
Bioelectrochemical systems (BES) can tackle both these issues, by harnessing the metabolism of microorganisms capable of utilizing solid electron acceptors or donors and using them for energy production in microbial fuel cells (MFC), chemical synthesis in microbial electrosynthesis (MES), and water desalination in microbial desalination cells (MDC). The interest in MDCs has increased in the last decade, given the ability to desalinate water while producing electricity from renewable and carbon-neutral materials under ambient temperature and pressure.
Although many microorganisms have been successfully applied in BES, most of them are not suited to operate under the conditions required for MDC, since they do not tolerate high salt concentrations. Therefore, marine microorganisms are much better suited for desalination purposes. In experiments where the anode was inoculated with marine wastewater, the marine anaerobic bacterium ***Desulfuromonas acetoxidans*** thrives, making this an interesting candidate for successfully running MDCs. The main goal of this study is to elucidate the best condition to grow *D. acetoxidans* on electrodes to use them in MDCs, showing that this is a good target for larger-scale uses. Different conditions, such as different salt concentrations, carbon sources, applied potentials and stirring, were tested to obtain the highest and most stable current production. Our data demonstrated that this bacterium can grow at salt concentration exceeding 1M, classifying it as a moderate halophilic organism, promising for water desalination coupled with electricity generation from wastewater treatment.

Talk 9-5: Real-life data of Plant-e's SensorStick with local energy-harvesting, for remote peatland ecosystem monitoring in North Pennines National Landscape in the UK

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Under healthy conditions, peatlands form the largest land-based storage of carbon from greenhouse gasses (GHGs). While high water levels sustain healthy natural peatland ecosystems, low water levels lead to increased GHG emissions due to peat oxidation. As a result, ecosystem monitoring, with water level as main pillar, plays a crucial role in maintaining and restoring healthy ecosystems. Proper water-table monitoring is key in the mitigation of GHG emissions under the pressure of climate change, as well as for restoration of natural healthy peatlands to assess the effect of the restauration efforts.

Due to the remote location of many peatland ecosystems, powering wireless sensors in these areas is a particularly challenging task. Currently, these wireless sensors are typically powered by batteries and often do not include a satellite connection. Replacing batteries and manually collecting data in remote locations are some of the biggest barriers to the scaling up of environmental monitoring. Therefore, the Plant-Microbial Fuel Cell (P-MFC) will be used as a sustainable energy source to power satellite-connected sensors in peatland ecosystems. Plant-e recently developed the SensorStick, with a P-MFC and a small solar panel for local energy harvesting, which powers the measurements of a water level sensor and a soil temperature sensor, plus the transmission of data from these sensors to an IoT satellite, making it a fully autonomous and sustainable wireless sensor system that never needs maintenance for the lifetime of the sensors, electronics and communication hardware.

Concept of the SensorStick for wetland ecosystem monitoring through satellite connection, including schematic representation of the underground P-MFC inside the SensorStick (left). Photo of a SensorStick installed in North Pennines National Landscape in the UK, showing the aboveground electrical casing that includes a small solar panel for backup energy-harvesting, a battery for energy storage and a satellite antenna for remote wireless connectivity (center). Impression of the dashboard that shows the monitoring data, energy-harvesting results and battery status of the SensorSticks (right).

In this presentation we will show real-life data from SensorSticks installed in the North Pennines National Landscape, which encompass a complete solution for autonomous and sustainable remote monitoring of the peatland ecosystem. The data consists of average daily water levels, soil temperatures and results of the energy harvesting by P-MFC and small solar cell. The harvested energy is stored on a rechargeable standard lithium battery.

Talk 9-6: Redox-Tuned Ce/Fe-N-C Cathode Catalysts for Efficient Oxygen Reduction Reaction and High-Performance Microbial Fuel Cells

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The sluggish oxygen reduction reaction (ORR) kinetics and high overpotential at cathode electrode present a crucial bottleneck for high-performing microbial fuel cells (MFCs) and hence imperatively require the assistance of an electrocatalyst to boost the reaction kinetics. The transition metal-nitrogen-carbon (M-N-C) type electrocatalysts (M: Fe, Co, Mn, etc.) serve as a viable alternative to Pt/C for MFC applications, but exhibit major drawbacks such as poor stability because of probable metal agglomeration and metal leaching, vulnerability to attack by reactive oxygen species (ROS), especially the hydroxyl radicals diminish the carbon matrix resulting in both carbon oxidation and catalyst demetallation. This study investigates the unique integration of cerium and Fe doped catalysts prepared by ZIF-8 and carbon nanotubes (CNTs) at high-temperature pyrolysis for ORR electrocatalysis [1]. Detailed physical characterization confirmed the formation of well-exposed defect-rich active sites in the catalysts *via* a facile high-temperature pyrolysis at 900°C. In the resultant Fe_xCe_y@N-C catalyst, Ce sites acted as a free-radical scavenger resisting catalyst degradation from ROS generated nearby the Fe centers and hence displayed an outstanding electrocatalytic ORR activity in alkaline and neutral electrolytes, direct four-electron ORR pathway, low peroxide yield (%HO_2^-), and long-term durability compared to monometallic Fe-N-C electrocatalyst (Fig. 1). Furthermore, Fe_1Ce_1@N-C catalyst displayed the peak power density (P_{max}) of 200.2 ± 5.25 mW/m^2 and high stability (>600 h), which outperforms the commercial Pt/C in practical MFC conditions (Fig. 1). The spectacular electrocatalytic ORR activity of the Fe_1Ce_1@N-C catalyst in MFC was attributed to the improved interfacial surface-defects, oxygen vacancies and redox activity of the $Ce^{3+}/^{4+}$ redox couple, lower charge-transfer resistance (R_{ct}) and escalated electron transfer at the exposed catalytically active sites.

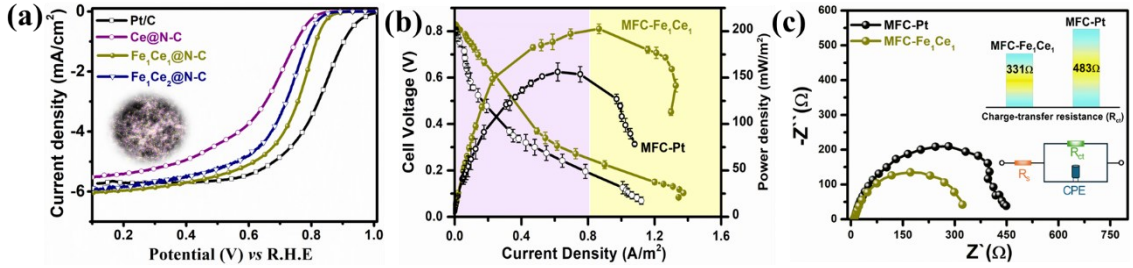

Fig.1. (a) ORR polarization curves recorded in O_2-saturated 0.1 M PBS electrolyte at 1900 rpm, (b) Polarization and power density curves for MFCs, (c) Nyquist plots of MFCs at 0.8 V.

References:

[1] R. Kumar, M. Mooste, I. Zekker, M. Käärik, J. Leis, A. Kikas, A. Treshchalov, J. Kozlova, M. Otsus, J. Aruväli, V. Kisand, K. Kukli, K. Tammeveski, Tailoring highly efficient ZIF-derived FexCey@N–C composite catalysts for oxygen reduction reaction in H-shape microbial fuel cells, *Int. J. Hydrogen Energy* 98 (2025) 793-806.

Acknowledgements

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ORAL PRESENTATIONS

Session 10 - Environmental BES - Bioremediation and Microbiology in Soils

Access to the Live-Q&A of this Session

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Talk 10-1: Magnetite Nanoparticles Enable Self-Constructed Bacterial Networks for Long-Distance Electron Transfer in Soil

Matteo Tucci[1], Marco Resitano[1], Ghada Sellami[2], Habib Chouchane[2], Luca Niccolini[1], Carolina Cruz Viggi[1], Bruna Matturro[1,3], Ugo Marzocchi[4,5], Federico Aulenta[1,3]

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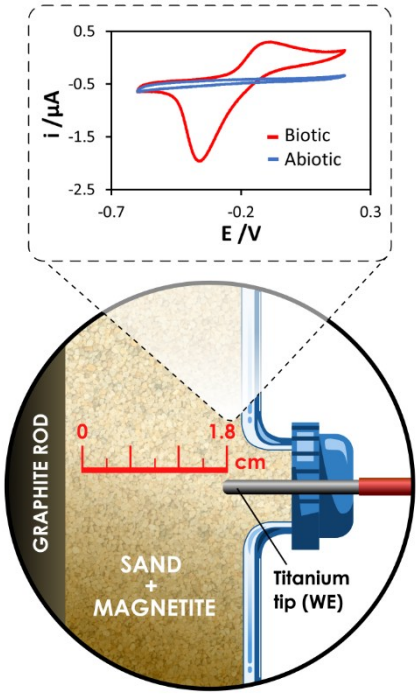

Extracellular electron transfer (EET) enables microorganisms to respire redox-active minerals (e.g., iron and manganese oxides) or solid electrodes, sustaining their metabolism. Some bacteria, achieve long-distance electron transfer (LDET) over tens of micrometers through conductive outer membrane proteins, pili, or both, forming electroactive microbial networks up to 150 µm thick. However, the limited thickness of electroactive biofilms constrains their industrial and environmental applications, particularly in electrobioremediation, where the electrode's radius of influence is critical.

To overcome this limitation, we investigated the role of magnetite nanoparticles in extending LDET within a microbial electrochemical system (MET) deployed in a model soil. Two identical tubular bioelectrochemical reactors were filled with either sand or sand supplemented with 5% w/w magnetite nanoparticles. Graphite anodes were polarized at +0.2 V vs. SHE, and acetate (1 g/L) was supplied as the sole electron donor to prevent direct interspecies electron transfer (DIET). The presence of magnetite nearly doubled the current from acetate oxidation (~5.5 mA vs. ~2.7 mA in sand alone), corresponding to a significantly higher acetate biodegradation rate. Cyclic voltammetry (CV) confirmed the progressive colonization of the anodes by electroactive microorganisms, with significantly higher oxidative currents in the magnetite-containing reactor. To assess the spatial extension of the microbial conductive network, CVs were conducted on a titanium electrode placed at increasing distances from the anode. While no redox peaks were detected in the sand-only reactor and in the abiotic control, clear electrocatalytic activity was observed up to 1.8 cm away from the electrode's surface in presence of magnetite, indicating the formation of an extended conductive bacterial network. These findings demonstrate that magnetite nanoparticles enhance LDET, effectively increasing the electrocatalytic surface and the spatial reach of microbial electron transfer. This approach could significantly improve MET performance in environmental applications, particularly in soil and groundwater electrobioremediation.

1. *Belleville, P.; Merlin, G.; Ramousse, J.; Deseure, J. Characterization of Spatiotemporal Electroactive Anodic Biofilm Activity Distribution Using 1D Simulations. Sci. Rep.* ***2022****, 12, 1–15*
2. *Nakamura, R.; Kai, F.; Okamoto, A.; Newton, G.J.; Hashimoto, K. Self-Constructed Electrically Conductive Bacterial Networks. Angew. Chemie - Int. Ed.* ***2009****, 48, 508–511*

Talk 10-2: Smart Manure: How Erich Kästner's tales of electrosorption and bioelectrochemical degradation of antibiotics and heavy metals provide safe fertilizer to our children

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Livestock manure represents a beneficial resource for fertilization of agricultural land and is used for bioenergy production.[1,2] For instance, 120 million tons of cow and pig liquid manure are annually produced in Germany. However, current on farm manure management results in an accumulation of antibiotics and heavy metals in manure.[1] This poses a risk to human health and the environment through the contamination of soils and aquifers and – similar to a "flying classroom" - promotes the emergence of antibiotic-resistant microorganism.[2] Thus, novel manure management strategies are required to remove these contaminants while preserving the fertilizing properties of manure. Therefore, the project Smart Manure aims to develop a reactor system that electrochemically utilizes greenhouse gases emitted during manure storage, removes antibiotics, recovers heavy metals and aims to evaluate the impact of the decontaminated manure on the soil microbiome and cash crops. Within Smart Manure, we develop a bioelectrochemical system for the removal of antibiotics and recovery of heavy metals via electrosorption and electroactive microorganisms.
Conventional adsorption technologies are either water intensive, require chemical agents or have a high energy consumption to achieve adsorbent regeneration.[3] In contrast, electrosorption on activated carbon felt has the potential to increase adsorption capacity and rate through electrostatic attraction without these drawbacks.[3] Contaminant desorption is facilitated in a "double Lottchen manner" by reversing the electrode charge allowing a low-cost and on-site adsorbent regeneration.[3] In the first reactor system heavy metals and antibiotics are adsorbed at the anode and cathode, according to their charge. After desorption, electroactive microorganisms - just like the moths and rodents of Kästner's "conference of the animals" - degrade antibiotics and facilitate the deposition of heavy. Preliminary experiments indicated, that the simultaneous adsorption of 5 antibiotics from different classes and 4 heavy metals from a mixture is viable. Currently, experiments in an artificial manure matrix are performed to validate these results and test the applicability of the system under more realistic conditions.

In the spirit of "Emil and the Detectives", electroactive microorganisms - cultivated from antibiotics-contaminated manure - join forces in a mixed microbial culture for the degradation of antibiotics in a one-chamber bioelectrochemical system. Subsequently, different antibiotics and mixtures thereof (Amoxicillin, Enrofloxacin, Sulfadiazine, Tetracycline and Trimethoprim) are added to explore the robustness and degradation capabilities of the system. It is demonstrated that various mixtures of antibiotics can be bioelectrochemically degraded and are almost fully mineralized minimizing risks of toxic intermediates. Further experiments are conducted to test the long-term stability of the cultivated mixed microbial culture and its capability to degrade structurally similar antibiotics. Although the capability of electroactive microorganisms to degrade antibiotics was shown several times, we demonstrate that the simultaneous degradation of mixtures of antibiotics is feasible representing a more realistic scenario.[4]

Acknowledgements: This project is part of the PhD cohort Smart Manure financed by the Helmholtz Centre for Environmental Research GmbH – UFZ.

References:

[1] Federal Ministry of Statistics (Germany) on Manure, **2022**
[2] Scholwin, et al., German Ministry for the Environment, **2019**
[3] Xu, et al., Separation and Purification Technology, **2025**, 362, 1383-5866
[4] Tang, et al., Water Research, **2025**, 326, 0043-1354

Talk 10-3: Bioelectrochemical Reactor for the Remediation of Trichloroethylene and Chromium (VI) from Contaminated Groundwaters

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Groundwater contamination by chlorinated solvents, particularly trichloroethylene (TCE), and heavy metals such as hexavalent chromium (Cr(VI)) poses significant environmental and health risks. Traditional remediation approaches, such as pump-and-treat, are often inefficient and costly. In contrast, bioelectrochemical systems (BESs) offer a sustainable alternative by harnessing microbial activity to drive both TCE dechlorination and Cr(VI) reduction. However, the coexistence of these contaminants introduces potential inhibitory effects, particularly on dehalorespiring microbial populations.
The present study focuses on the investigation of a bioelectrochemical process for reductive dechlorination of TCE and Cr(VI)-removal from contaminated water. The bioelectrochemical process consisted of a membrane-less tubular microbial electrolysis cell (MEC) constituted by a granular graphite and an internal graphite granules counter electrode. This configuration ensured a straightforward, adaptable, and cost-effective process design. The MEC has been continuously fed with a TCE and Cr(VI) contaminated solution under the galvanostatic polarization of the system in which the cathode constituted the working electrode. The system was evaluated under various operating conditions, including applying different currents (under galvanostatic mode) and different HRTs. The BES demonstrated remarkable resilience to the presence of Cr(VI), consistently achieving near complete (>99%) removal of TCE and 100% reduction of Cr(VI). Notably, the distribution of dechlorination byproducts varied under different operating conditions (Figure 1), highlighting the dynamic response of the microbial community to process operating conditions. Indeed, despite an initial inhibitory effect of Cr(VI) on dechlorinating bacteria, the process exhibited a strong adaptive capacity, with dechlorination efficiency recovering over time. These findings underscore the potential of BES technology as a robust and adaptable solution for the simultaneous bioremediation of chlorinated solvents and heavy metals in contaminated groundwater.

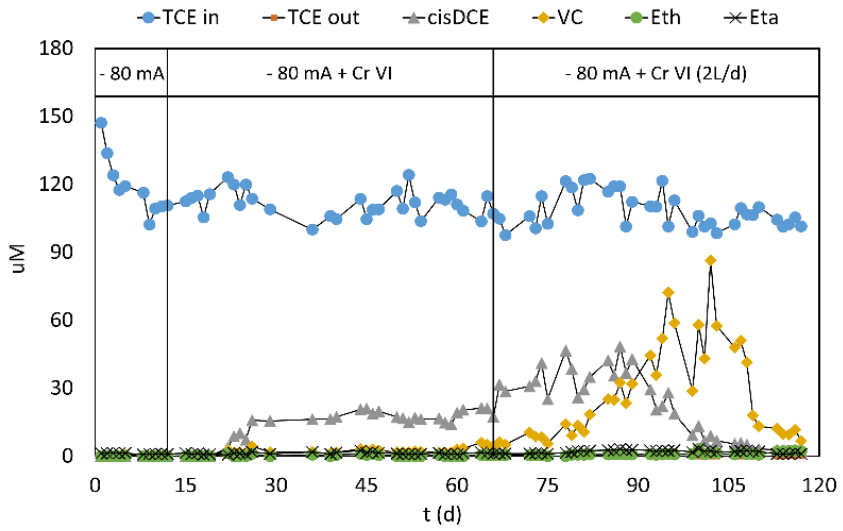

Figure 1. The distribution of dechlorination byproducts varied under different operating conditions.

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Talk 10-4: Enhancing Bioelectrochemical Perchlorate Reduction by Chemical Electrode Modification

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Perchlorate is considered an endocrine disruptor that compromises water sources and human health by blocking the binding sites of iodine to the sodium iodide symporter, causing hypothyroidism. Perchlorate is present in high concentrations in desert environments due to low precipitation and high salinity in the presence of photocatalytic reactions, allowing this emerging contaminant to persist in the soil. Soil samples from the Atacama Desert of Chile contained the higher average perchlorate concentration on Earth, which is 1-2 orders of magnitude higher than in other arid areas. Groundwater samples collected in Chile's arid zones show concentrations of up to 21,600 µg L^{-1} [1]. Thus, in these life-limiting environments, electrotrophic perchlorate-reducing microorganisms (e-PRMs) have been found [2].

In this study, a new e-PRM isolated from the Atacama Desert, *Dechloromonas* sp. CS-1, was evaluated for perchlorate removal in water using a bioelectrochemical reactor (BER) with a chemically modified carbon cloth electrode. Triplicated BERs were operated for 17 days under batch mode conditions with an applied potential of -500 mV (vs. Ag/AgCl). Four conditions were tested to assess the synergistic effect of e-PRMs and the modified carbon cloth electrodes. Inoculated and abiotic chemically modified and not modified carbon cloths were analyzed. Surface analysis (i.e., SEM, XPS, FT-IR, RAMAN spectroscopy) on the electrodes demonstrated a heterogeneous transformation of the carbon fibers by incorporating nitrogen functional groups and the oxidation of the carbonaceous material.

Our results showed that the BERs with the modified electrode and the presence of e-PRMs reached a high cathodic efficiency (90.790 ± 9.157%) among the tested conditions. The perchlorate removal rate was 0.340 ± 0.007 mol m^{-3} $day^{-1,}$ a high value compared with the existing literature considering the absence of an exogenous electron shuttle and the high concentration removed over the operation (>500 mg L^{-1}). Thus, this work shows, for the first time, significant bioelectrochemical reduction of perchlorate by an e-PRM in long-term experiments (i.e., days/weeks), providing information on perchlorate removal and cathodic efficiency. In addition, the observed catalytic enhancement of CS-1 was confirmed by a reduction in the charge transfer resistance obtained by electrochemical impedance spectroscopy. Finally, an electrochemical kinetic study revealed an eight-electron perchlorate bioreduction reaction at about -640 mV (vs. Ag/AgCl). Therefore, our results show the synergistic effect of e-PRM and chemically modified carbon cloth electrodes on perchlorate removal in a BER [3].

References

[1] Vega M., Nerenberg R. and Vargas I.T Perchlorate contamination in Chile: Legacy, challenges, and potential solutions. Environmental Research 164:316-326. https://doi.org/10.1016/j.envres.2018.02.034

[2] Felipe Torres-Rojas, Diana Muñoz, Camila Pía Canales, Ignacio T. Vargas. Bioprospecting for electrochemically active perchlorate-reducing microorganisms. Bioelectrochemistry, 2022, 108171, ISSN 1567-5394, https://doi.org/10.1016/j.bioelechem.2022.108171

[3] Felipe Torres-Rojas, Diana Muñoz, Camila Pía Canales, Samuel A. Hevia, Felipe Leyton, Nicolás Veloso, Mauricio Isaacs, Ignacio T. Vargas. Synergistic effect of electrotrophic perchlorate reducing microorganisms and chemically modified electrodes for enhancing bioelectrochemical perchlorate removal. Environmental Research, Volume 233, 2023, 116442, https://doi.org/10.1016/j.envres.2023.116442.

Talk 10-5: Bioelectrochemical Recovery of Platinum Group Metals from Spent Car Catalysts using *Cupriavidus metallidurans* CH34.

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The increasing demand for platinum group metals (PGMs), has intensified the need for sustainable recovery methods, particularly from secondary sources like spent car catalysts (SCC). Traditional recovery techniques, including pyrometallurgical and hydrometallurgical methods, are energy-intensive, generate significant waste, and pose environmental risks. This study investigates an alternative bioelectrochemical approach using *Cupriavidus metallidurans* CH34 in microbial fuel cells (MFCs) to recover PGMs while simultaneously generating electricity. *C. metallidurans* CH34 is a metal-resistant bacterium with electroactive properties that enable it to interact with metal ions, facilitating their reduction and deposition.

Batch culture experiments were conducted to evaluate the growth and PGM tolerance of *C. metallidurans* CH34 in the presence of SCC. Viable cell counts, bicinchoninic acid (BCA) protein assays, and high-performance liquid chromatography (HPLC) analysis of sodium gluconate utilisation were employed to assess bacterial viability and metabolic activity. Results demonstrated that *C. metallidurans* CH34 could tolerate high SCC concentrations up to 100,000 ppm (10% w/v), maintaining stable growth and metabolic activity. ICP-MS analysis of culture supernatants indicated a decrease in dissolved PGM concentrations compared to abiotic controls, suggesting bioaccumulation or bioreduction of metal ions by the bacteria.

To evaluate the bioelectrochemical performance of *C. metallidurans* CH34, double-chambered MFCs with carbon-based electrodes and a cation exchange membrane were used, with sodium gluconate as the electron donor. Voltage monitoring and cyclic voltammetry (CV) analysis revealed that 10,000 ppm SCC enhanced electron transfer, increasing voltage to 0.43V from 0.37V (control). However, higher SCC concentrations (≥50,000 ppm) reduced performance, with voltage dropping to 0.17V at 100,000 ppm, likely due to metal toxicity or biofilm disruption. Scanning electron microscopy (SEM) confirmed biofilm formation, reinforcing microbial involvement in PGM recovery.

Future work will use transcriptomic and proteomic analyses to better understand the underlying mechanisms of metal recovery and electrochemical activity and to identify key genes and metabolic pathways involved in metal resistance and extracellular electron transfer. Comparative omics studies will provide insights into how *C. metallidurans* CH34 adapts to metal stress and optimises energy metabolism in bioelectrochemical systems. Additionally, a life cycle assessment (LCA) will be conducted to evaluate the environmental impact of MFC-based PGM recovery compared to conventional recovery methods. This research aims to develop a more sustainable, cost-effective, and environmentally friendly approach to recovering critical metals from industrial waste by integrating bioelectrochemical technologies with microbial metal interactions.

References :

1) Yakoumis, I.; Panou, M.; Moschovi, A. M.; Panias, D. Recovery of Platinum Group Metals from Spent Automotive Catalysts: A Review. Cleaner Engineering and Technology 2021, 3, 100112. https://doi.org/10.1016/j.clet.2021.100112
(2) Hughes, A. E.; Haque, N.; Northey, S. A.; Giddey, S. Platinum Group Metals: A Review of Resources, Production and Usage with a Focus on Catalysts. Resources 2021, 10 (9), 93. https://doi.org/10.3390/resources10090093.

Talk 10-6: Microbial electrochemical reduction of vanadate by *Thiobacillus denitrificans* in groundwater

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Microbially mediated vanadate [V(V)] reduction is well accepted as a sustainable approach for remediating V-polluted groundwater. However, this process relies on exogenous electron donors, which is challenging to inject into aquifer. In this study, bioelectrochemical V(V) reduction by autotrophic *Thiobacillus denitrificans* was demonstrated without exogenous electron donor supplementation. At an applied voltage of 0.9 V, 94.5 ± 0.95% of V(V) was removed within 14 d. Insoluble tetravalent V was the main reduction product, distributed both outside and inside of cells at the cathode. Transcriptomics, RT-qPCR and protein quantification analysis collectively suggested that extracellular V(V) reduction was mediated by cytochrome c and extracellular polymeric substances. Intracellular V(V) reduction was catalyzed by sulfate-, chromate-, and nitrate-related reductases and achieved by redox components (NADH, Fe-S clusters, and quinones) within respiratory chain. Particularly, the newly identified metabolic pathways for V(V) reduction, regulated by *aprB* (encoding adenylyl sulfate reductase) and *iscA* (encoding iron-sulfur cluster proteins), were further confirmed through experiments involving genetically modified *E. coli* and protein catalysis assays. This study provides innovative strategy for V(V) bioremediation in groundwater and novel insight into molecular mechanisms of V(V) bioreduction.

Fig. 1. V(V) removal and biomass growth in bioelectrochemical systems inoculated with *T. denitrificans.* Temporal change of V(V) concentration (a) in different systems; (b) under different voltages;

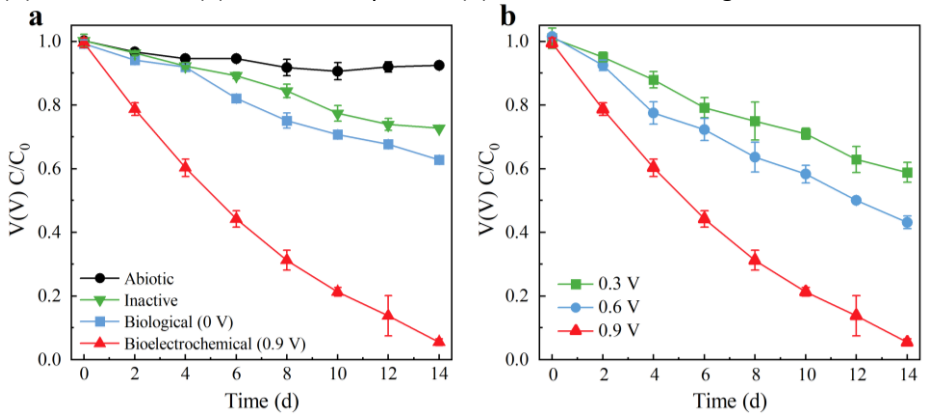
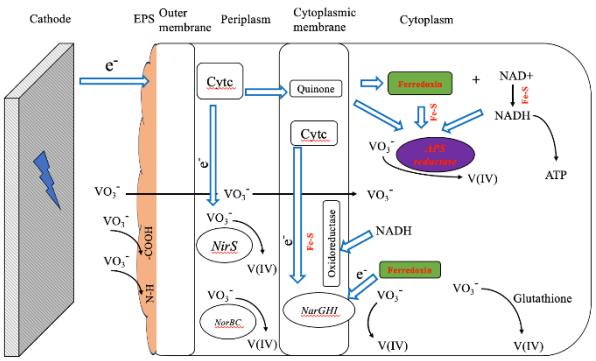


Fig. 2. Mechanism of microbial electrochemical reduction of V(V) by *T. denitrificans* was proposed.



Talk 10-7: Subsurface Electro-biosphere: Electroactive microbes inhabiting iron-rich hot springs

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In this decade, a wide variety of electroactive microbes that can transfer electrons between microbes and minerals, electrodes, or other microbes have been discovered. There are two types of microbial electron transfer reactions: one is electron transport to the solid phase to gain energy through mineral respiration, known as "exo-electrogen", and the other is electron transport from the solid phase as a source of energy for metabolism, known as "electrotroph". These microbial metabolisms could play a role in biogeochemical cycling at the boundary region between oxidative and reductive environments. In particular, a variety of electroactive microbes should inhabit the mineral-rich subsurface environment, exchanging electrons to/from solid minerals; however, this phenomenon remains poorly understood.

In this study, we conducted multi-MetaOmics analyses to search for electro-active microbes in the boundary region between oxidative and reductive areas of iron-rich hot springs. In the bay of Satsuma Iwo Jima (Kagoshima, Japan), iron-rich hot spring water from the seafloor formed iron oxides chimneys (A). In Kowakubi hot spring (Akita, Japan), CO2- and iron-rich spring water (55°C) formed iron-bearing brown (surface) and black (inside) scale on the formation well (B).

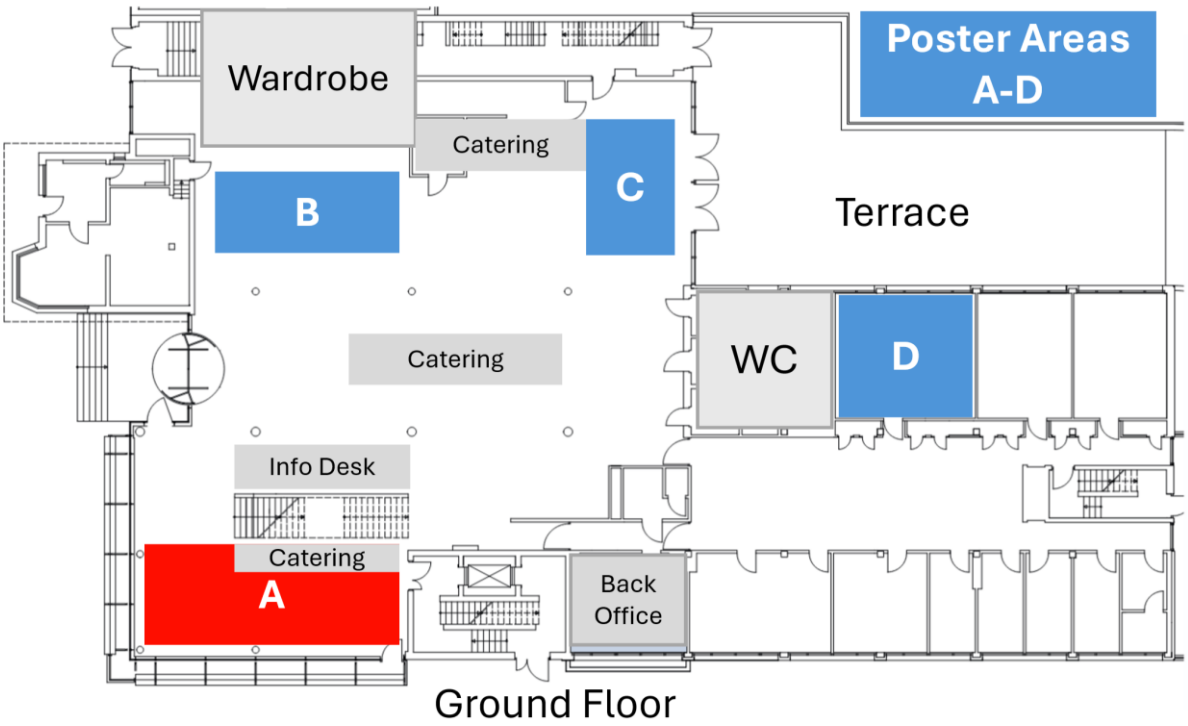
Microbial samples were collected from oxidative, boundary, and reductive regions in these environments, and both DNA-seq and mRNA-seq were performed to obtain a comprehensive set of metagenome-assembled genomes (MAGs) and *in situ* gene expression profiles of each microbe. The results showed that a large number of unknown electroactive microbes inhabit iron-rich chimneys and iron scales. As expected, iron oxidation reactions by Zetaproteobacteria and Gammaproteobacteria were dominant and highly active in an oxidative environment, while iron reduction reactions by Thermodesulfobacterota, Hydrothermarchaeota, and various uncharacterized microbes were dominant and active in a reducing environment affected by subsurface anoxic spring water. Interestingly, all these microbes encoded numerous multi-heme *c*-type cytochromes (MHcytCs), which were highly transcribed under the iron-rich environments. These results suggest that the MHcytCs could play an important role for extracellular electron transfer from/to the solid minerals. These findings suggest that these iron-rich hot spring environments host an "electro-biosphere" originating from subsurface microbiome.

A. Satsuma-iou jima iron rich

B. Kowakubi hot spring

POSTER PRESENTATIONS

Microbial Physiology and Metabolic Engineering
- Area A -



Poster-A01: Bio-electroenzymatic cofactor regeneration of NADPH

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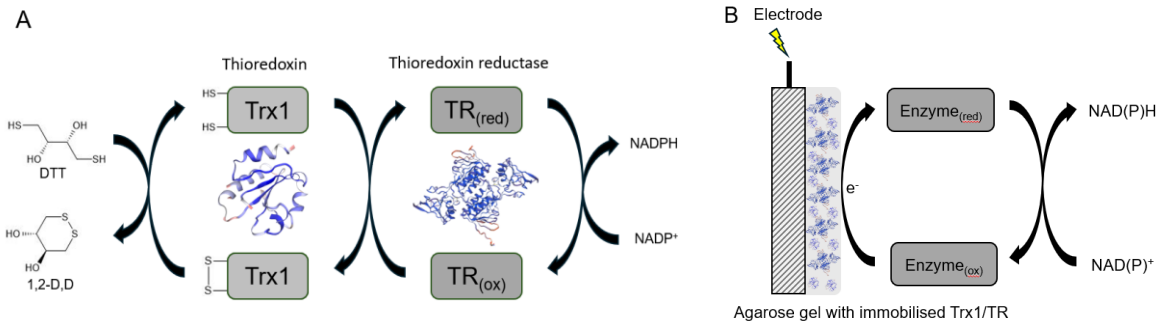
Oxidoreductases are the largest group of enzymes and incorporate a large number of biocatalysts that are relevant to industry. Most of these enzymes are dependent on cofactors such as NAD(P)H, FAD, etc. Hence, there is a need for a robust and stable cofactor regeneration system. The protein/enzyme cascade made of *Thermus thermophilus* thioredoxin (Trx1) and thioredoxin reductase (TR) has shown to regenerate NADPH by using DTT as a reduction equivalent (Scheme 1). The cascade is stable and active at high temperatures (up to 70 °C) and shows a high solvent tolerance. [1]



Scheme 1: **A** Protein-enzyme NADPH regenerating cascade with *Thermus thermophilus* Trx1 and TR. **B** Bio-electroenzymatic setup for the direct reduction of immobilised Trx1/TR for NADPH regeneration.

This research focuses on the application of the Trx1/TR cascade in a bio-electroenzymatic system. In general, electrochemical systems have the advantage of a high efficiency with which electrons are transferred into a reaction (Faradaic efficiency). By enabling a direct electron transfer (DET) of electrons from an electrode directly to the active site of the enzyme, the reaction can be carried out independently of DTT. This eliminates the need for the initial substrate, which decreases the amounts of waste (by-product) generated during the cascade reaction.

Trx1 and TR are immobilised on a carbon felt electrode using an agarose gel. The bio-electroenzymatic system is characterised using spectroelectrochemical tools to measure the potentiostatic conversion of $NADP^+$ dependent on various potentials. The bio-electroenzymatic cascade is then used as a regeneration system for the reduction of acetophenone to 1-phenylethanol which is catalysed by *Lactobacillus brevis* alcohol dehydrogenase (*Lb*ADH).

[1] N. Zhang, B. Müller, T. Ø. Kirkeby, S. Kara, C. Loderer, "Development of a Thioredoxin-Based Cofactor Regeneration System for NADPH-Dependent Oxidoreductases" *ChemCatChem* **2022**, *14*.

Poster-A02: The Difficulty to find the ones! – How to search efficiently for electroactive microbes to create an ensemble like the Thomaner Choir of Leipzig

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The electrobiotechnological application of microorganisms as electrochemically driven biocatalysts is of increasing importance to close the gaps towards a sustainable, circular bioeconomy. Due to the enormous diversity of microorganisms in different habitats such as soil, wastewater, or gut, systematic electrochemical screening of electroactive microorganisms (EAM), is one of the biggest challenges and is currently methodically hampered due to unavailable electrochemical screening tools. As difficult as discovering hidden talents for the world-famous Thomaner Choir in Leipzig is the effective search for EAM to create a microbial ensemble that can close the aforementioned gap.

To tackle this challenge, we developed an electrochemical microwell plate (ec-MP) composed of a 96 electrochemical deepwell plate and a recently developed 96-channel multipotentiostat to investigate 96 independent controllable samples in parallel and real-time [1]. Intensive electrochemical cultivation studies of *Geobacter sulfurreducens* and *Shewanella oneidensis* were performed [2]. With this concept our aim is to develop a commercially available, high throughput screening platform to find “the ones” under the enormous microbial diversity.

The requirements for the throughput screening platform are that electrochemical standard measurements such as chronoamperometry, cyclic voltammetry as well as electrochemical impedance spectroscopy can be carried out reliably in a three-electrode-setup to screen for EAM as efficient and long-term stable biocatalyst in single- or double-chamber systems under anaerobic conditions. An easy-to-use system is a central point of our development to open the bioelectrochemical door to a broader scientific and industrial community. This shall allow the dream become true to succeed in finding a great ensemble that sings the motet on a sustainable, circular bioeconomy.

Funding: This research is funded by the German Federal Ministry of Education and Research (BMBF) under the grant number 031B1378C.

References:

[1] Frank, R., Prönnecke, C., Azendorf, R., Jahnke, H.-G., Beck-Sickinger, A. G., and Robitzki, A. A. (2020). Advanced 96-Microtiter Plate Based Bioelectrochemical Platform Reveals Molecular Short Cut of Electron Flow in Cytochrome P450 Enzyme. Lab. Chip 20 (8), 1449–1460. doi:10.1039/c9lc01220f

[2] Kuchenbuch A, Frank R, Ramos JV, Jahnke HG, Harnisch F. Electrochemical Microwell Plate to Study Electroactive Microorganisms in Parallel and Real-Time. Front Bioeng Biotechnol. 2022 Feb 15;9:821734. doi: 10.3389/fbioe.2021.821734

Poster-A03: Elucidation of potential factors involved in Extracellular Electron Transfer mechanism in *Cupriavidus necator* H16

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Microbial electrochemical technologies (METs) provide sustainable solutions for energy production, wastewater treatment, and environmental remediation[1]. However, their practical applications are limited by low electron transfer (ET) efficiency. Enhancing natural extracellular electron transfer (EET) mechanisms and reengineering EET pathways in industrially relevant bacteria is therefore essential for advancing MET research. *Cupriavidus necator* H16 (*C. nec.* H16), a versatile chemolithoautotroph with industrial relevance, has a poorly characterised EET mechanism. This research investigates the factors influencing EET in *C. nec. H16* and aims to engineer strains with enhanced ET efficiency.

Previous studies across bacterial systems have shown a strong correlation between the aromatic amino acid content of pilin proteins and their conductivity—increased aromaticity enhances EET efficiency[2]. Based on these findings, wild-type (WT) and engineered *C. nec.* H16 PilA variants, incorporating structural truncations and/or aromatic amino acid substitutions, were tested for conductivity improvements. These assessments were carried out using dual-chamber microbial fuel cells (MFCs) with sodium gluconate as the electron donor. Results showed that among the tested strains, T61W, which combines structural truncation with increased aromatic amino acid content, generated the highest potential across all variants. This observation was supported by cyclic voltammetry, where T61W displayed the highest anodic currents. Scanning electron microscopy of the anode electrodes showed that all strains adhered in a dispersed pattern with occasional clumps, rather than forming a continuous biofilm. Therefore, differences in current output were attributed to pilin modifications rather than variations in cell attachment or biofilm formation.

To further evaluate EET efficiency, ongoing experiments assess WT and modified strains using four dual redox mediator systems containing lipophilic and hydrophilic components. Potassium ferricyanide serves as the common hydrophilic reporter mediator. Studies are being conducted under aerobic and anaerobic conditions with glucose and fructose as carbon sources. Following mediator incubation, linear sweep voltammetry of the ferri/ferrocyanide couple is performed, and the plateau current at 425 mV vs Ag/AgCl is used as the analytical signal. The percentage change in oxidative current relative to steady-state current will provide a comparative measure of EET efficiency across strains and under different conditions.

1 A. ElMekawy, S. Srikanth, S. Bajracharya, H. M. Hegab, P. S. Nigam, A. Singh, S. V. Mohan and D. Pant, *Food Research International: A journal of the Canadian Institute of Food Science and Technology (CIFST)*, 2015, **73**, 213–225.
2 M. Vargas, N. S. Malvankar, P.-L. Tremblay, C. Leang, J. A. Smith, P. Patel, O. Snoeyenbos-West, K. P. Nevin and D. R. Lovley, *mBio*, 2013, **4**, e00105-00113.

Poster-A04: Identifying the molecular drivers of electroactivity in mixed-species biofilms

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Microbial electrochemical technologies (MET) provide a vast array of opportunities to address one of today's urgent environmental challenges by enabling the conversion of waste into valuable commodities such as electricity, biofuels, and high-value compounds. The fundamental operation of MET depends on electroactive bacteria that grow on the surface of electrodes, creating a biofilm and performing electron exchange through extracellular electron transfer (EET). Understanding and optimising this process is essential for improving MET performance and recognising its full potential.
While most EET studies are conducted with pure cultures and model organisms, practical MET utilises mixed cultures typically found in waste, which contribute to enhanced performance. The high efficiency of mixed cultures can be attributed to syntrophic interactions and cellular communication processes within the microbial community. This enables the microbes to utilise a wide range of complex substrates, perform EET, and form thicker, more conductive biofilms necessary for efficient power output. To investigate the cellular interaction and communication processes that enhance EET, we are currently conducting studies on co-cultures involving *Shewanella* and various electroactive and non-electroactive bacteria. By modifying the expression levels of key factors influencing EET (e.g., multiheme cytochromes, electron shuttles) and examining their effects on biofilm formation and electroactivity, we aim to uncover the critical factors driving EET in mixed-species biofilms. This will pave the way for developing enhanced versions of electroactive bacteria to accelerate the real-world integration of BES and address pressing societal challenges.

Poster-A05: Electrofermentation of *Corynebacterium Glutamicum* for Glutamate Production

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Corynebacterium glutamicum is a GRAS (Generally Regarded As Safe) bacterium widely employed in industrial amino acid production. Electrofermentation (EF) has emerged as a promising strategy to steer microbial metabolism by modulating redox balances via applied electric potentials (Sriram et al., 2022). While EF has shown promise for enhancing bioproduction in various systems, its application to *C. glutamicum*-based L-glutamate synthesis remains underexplored. This study systematically evaluates how electrochemical stimulation influences the growth and metabolic output of *C. glutamicum*.

C. glutamicum 4157 (CECT) was cultivated in a dual-chamber H-type reactor under controlled electrochemical conditions (Fig. 1). The EF setup featured a cationic exchange membrane, with the working electrode (anode) poised at +1.2 V vs. the counter-electrode (cathode). Both compartments were inoculated to assess the effects of the electric field on cell behavior. Growth kinetics and metabolite profiles were compared between EF and conventional fermentation (control).

Initial findings indicate that anodic conditions enhance *C. glutamicum* biomass accumulation, suggesting a potential electrochemical stimulation of cell proliferation. Although glutamate yields under EF did not yet surpass conventional fermentation, these results highlight the system's capacity to influence microbial physiology. Ongoing work focuses on (1) elucidating the metabolic shifts triggered by electrochemical perturbations via exometabolomics and (2) optimizing reactor parameters (e.g., potential, medium composition) to redirect carbon flux toward target products. By bridging electrochemistry and microbial biotechnology, this work opens new avenues for controlling fermentation outcomes.

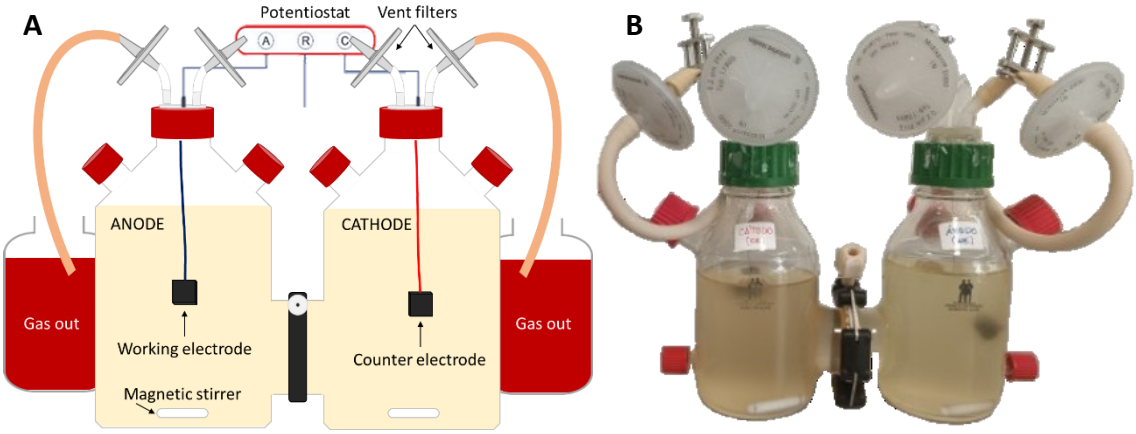

Fig. 1. Schematic (A) and photograph (B) of the H-type electrofermentation reactor.

Sriram, S., Wong, J. W. C., & Pradhan, N. (2022). Recent advances in electro-fermentation technology: A novel approach towards balanced fermentation. *Bioresource Technology*, *360*.

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Poster-A06: Heterologous phenazine production in biotech hosts for bioelectrochemical applications

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In bioelectrochemical systems (BES), microbial metabolism can be linked to electrochemical processes to develop new microbial reaction strategies. Mediated extracellular electron transfer (MET) facilitates this linkage between cells and electrodes via soluble redox mediators, which act as electron shuttles. These mediators permit the utilization of the complete reactor volume.[1] This linkage can be exploited to replace the terminal electron acceptor in a microbe's metabolism, oxygen, with an anode. This would allow for increased growth rates at anaerobic or microaerobic growing conditions, which could be used to boost production of oxygen sensitive or intolerant natural products.

Phenazines are a class of redox mediators natively produced by the bacteria *Pseudomonas aeruginosa*.[1,2] However, since *P. aeruginosa* is an opportunistic human pathogen, biotech processes with the bacteria are problematic, especially when upscaled.[3] Previously, non-pathogenic *Pseudomonas putida* was engineered for phenazine production, but an obligatory reliance on oxygen limits this strain's usefulness.[4,5] To address this host issue, we expressed the phenazine-1-carboxylic acid (PCA) biosynthetic pathway from *P. aeruginos*a PA14 in *Escherichia coli*. The *phz2* gene cluster (genes *phzA2-G2*) was chosen as it has demonstrated higher PCA yield production.[4] Additionally, we co-expressed *phzH*, the phenazine-1-carboxamide (PCN) synthesis gene, from *P. aeruginosa* PA14 in order to compare the effects of PCN's slightly more positive formal potential (E°= 57 mV vs SHE compared to E°= -47 mV vs SHE for PCA) on BES performance.[2] Initial experiments with these PCA and PCA/PCN producing *E. coli* strains were conducted in a 500 mL, single chamber BES, under potentiostatic control, a graphite comb for the working electrode, a graphite block for the counter electrode, and a Ag/AgCl sat KCL reference electrode in a glass chassis.

Results showed that the heterologous production of PCA and PCA/PCN increased electron discharge from the cells to the anode. However, we also showed that phenazine mediated electron discharge to the anode depressed the rate of growth. This could be attributable to phenazines reducing at Complex I in the electron transport chain, disrupting the formation of the proton gradient and decreasing ATP synthesis. To resolve this problem, we will next use our phenazine producing *E. coli* as platforms to evaluate various inner membrane cytochromes. These cytochromes will be tested for their abilities as alternate phenazine reducers to potentially reestablish Complex I's contribution to the proton gradient.

References:
1 Franco A, Elbahnasy M, Rosenbaum MA. 2022. Microbial Biotechnology. 00: 1– 16.
2 Bosire E, Rosenbaum MA. 2017. Front. Microbiol. 8: 892.
3 Simoska O, Gaffney EM, Lim K, Beaver K, Minteer SD. 2021. J. Electrochem. Soc. 168: 025503.
4 Schmitz S, Rosenbaum MA. 2020. ACS Chem Biol. 15: 3244-3252.
5 Franco, A., Chukwubuikem, A., Meiners, C. & Rosenbaum, M. A. 2024 Bioelectrochemistry 157:108636

Poster-A07: Distinguishing the contribution of extracellular electron transfer in the *Desulfovibrio caledoniensis*-induced total corrosion of Q235 carbon steel

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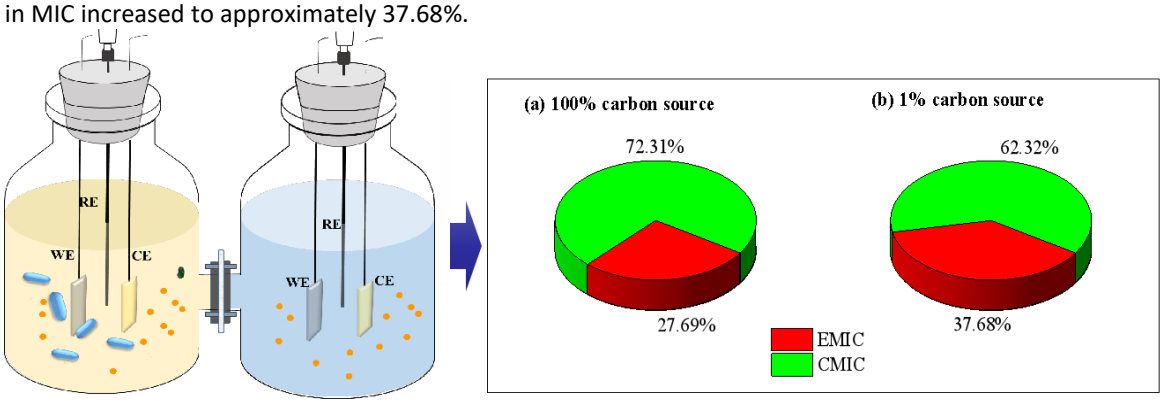
Microbially influenced corrosion (MIC) in anaerobic environments accounts for many severe failures and losses in different industries. Sulfate-reducing bacteria (SRB) represent a typical class of corrosive microorganisms capable of acquiring electrons from steel through extracellular electron transfer processes, thereby inducing severe electrical microbially influenced corrosion (EMIC). Although prior research has underscored the significance of extracellular electron transfer, the contribution of EMIC to the whole MIC has not been comprehensively studied. In this study, Q235 steel coupons were employed in an H-shaped electrochemical cell to conduct electrochemical and coupon immersion experiments, aiming to determine the contribution of EMIC to the overall MIC. The experiments were conducted under two distinct carbon source conditions: 100% carbon source (CS) and 1% CS environments. It was observed that the biotic electrodes exhibited significantly higher cathodic currents, with the most pronounced biological cathodic activity detected in the 100% CS biotic medium. The voltammetric responses of the electrodes before and after changes in the medium confirmed the biocatalytic capability of the attached biofilm in stimulating the cathodic reaction. The proportion of EMIC in MIC was calculated using linear polarization resistance, revealing a trend over time. Additionally, weight loss tests indicated that the contribution of EMIC to the total MIC was approximately 27.69%. Furthermore, the results demonstrated that while the overall corrosion rate was lower in the 1% CS environment, the proportion of EMIC in MIC increased to approximately 37.68%.



Poster-A08: Specific cytochromes and PilA enable extracellular electron transfer in sulfate-reducer *Nitratidesulfovibrio* sp. strain HK-II

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Sulfate-reducing bacteria (SRB) is one of representative microbes under anaerobic environments, deeply relating to carbon and sulfur cycles on the Earth and iron corrosion. Therefore, it is useful for geomicrobiology and industrial applications to understand how SRBs produce energy to survive. SRBs does not become dominants usually but are often found in microbial fuel cells (MFCs). An MFC was constructed with lake sediments as inoculum and lactate as electron donor, which was run under semi-batch culture conditions with 10 Ω as external resistance. The MFC produced high current density over 400 mW m^{-2} anode for around 10 days[1)], and many colonies were isolated from the anode surface of the MFC by roll tube method[2)]. Of those colonies, a black colony was purified several times by streaking on an agar plate under anaerobic conditions. The microbe grew under sulfate-reducing (SR) condition with lactate as electron donor and produced sulfide and acetate, indicating that the microbe was one of incompletely oxygenating SRBs. Full genome analysis indicated that the microbe was affiliated with *Nitratidesulfovibrio* genus, called the isolate strain HK-II. Electrochemical analyses revealed that strain HK-II exhibited activity of extracellular electron transfer (EET) at more positive potential over 0 V. Maximum current density and columbic efficiency were around 250 mA m^{-2} and 80% at 0.6 V and 0.4 V of anode potential, respectively. RNAseq analysis was conducted by comparing SR condition with EET conditions at 0.2 V and 0.4 V of anode potentials. RNAseq analysis results were similar between both EET conditions, whereas it was significantly different between SR and EET conditions, indicating that strain HK-II has different two kinds of survival strategies. By comparing with the SR condition, transcriptional levels of genes coding Rrf2 family transcriptional regulator, NAD(P)/FAD-dependent oxidoreductase, flp family type IVb pilin, ammonium transporter, and the like were over 100-folds higher under EET conditions. These results suggested that the strain HK-II was capable of EET by using specific proteins. PilA plays major role in EET of *Geobacter sulfurreducens* strain PCA. The strain HK-II had three kinds of PilA genes in a cluster and the transcriptional levels of three pilA genes were over 100-folds compared with SR conditions. AlphaFold2 analysis predicted that 3D-structure of three PilAs of the strain HK-II were similar to that of PilA-N of the strain PCA, whereas strain HK-II did not have PilA-C like protein which plays role in EET of the strain PCA. It is reported that strain PCA secrets conductive cytrochromes with the PilA outside of cell wall, which functions as nanowires, resulting in EET[3)]. Full genome sequence data revealed that the strain HK-II had 23 kinds of cytochromes, and RT-qPCR analysis showed that transcriptional levels of 4 kinds of cytochromes were specifically higher under EET conditions at 0.4 V of anode potential than those of SR conditions. Heme stained-SDS PAGE analysis showed that stained band was detected mainly in fractions of cytoplasm with periplasm and inner membrane under SR conditions but was very weak in outer membrane fraction, whereas stained band was strongly detected in outer membrane fraction under EET condition at 0.4 V of anode potential. The size of detected bands was almost similar under SR and EET conditions. RNAseq, RT-qPCR, and Heme stained-SDS PAGE analyses suggested that NapC/NirT family cytochrome *c*, which was similar 3D structure to CymA of *Shewanella oneidensis* MR-1, would play role in EET with PilA in the strain HK-II. The sulfate-reducer strain HK-II senses environmental conditions and regulates metabolisms sophisticatedly, resulting in survival using cytochromes with PilA without sulfate.

1. Suzuki et al., 2018. Bacterial communities adapted to higher external resistance can reduce the onset potential of anode in microbial fuel cells. J. Biosci. Bioeng. 125(5): 565-571.
2. Yui et al. 2025. Rechargeable Biomineral Induced by Sulfate-reducing Bacterium *Nitratidesulfovibrio* sp. strain HK-II. Microbes Environ. (under publication)
3. Gu et al., 2021. Structure of *Geobacter* pili reveals secretory rather than nanowire behavior. Nature 597: 430-434.

Poster-A09: Microbial Fe(III) reduction via novel flavin-based extracellular electron transfer pathway under oxic conditions by Gram positive *Microbacterium deferre* sp. nov. A1-JK

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The *Microbacterium* genus includes metabolically highly versatile Gram positive bacteria that occur in a range of ecosystems. Interactions of *Microbacterium* species with other microbes are known. However, there have been no indications for extracellular electron transfer (EET) in this genus yet. A novel species, *Microbacterium deferre*, was isolated from the oxic-anoxic interface, also known as the microaerophilic veil, of a freshwater sediment that is dominated by multicellular long-distance electron-transferring cable bacteria. We explored the metabolic versatility of *M. deferre* in microaerophilic environments with redox gradients that rapidly change through electrogenic sulphide oxidation activity of the cable bacteria.

In this study, we investigated whether *M. deferre* A1-JK was able to perform EET, which could be used to survive the rapid movements of the oxic-anoxic interface. Combining cyclic voltammetry, differential pulse voltammetry, and chrono amperometry in three-electrode cells and physiological analyses with genomics, we found that *M. deferre* could transfer electrons to electrodes using flavins as mediator. This is the first report of EET within the *Microbacterium* genus. Curiously, we found that oxygen reduction occurred simultaneously with soluble Fe(III) reduction, breaking the conventional model, where Fe(III) reduction only happens after oxygen is depleted. Flavins were found to be secreted and subsequently used to reduce soluble Fe(III) under anaerobic, microaerophilic and atmospheric conditions.

Gram positive bacteria, capable of flavin-based EET (FLEET), all use a highly similar pathway and protein machinery. Genome analysis revealed presence of FmnA, Ndh2, DmkAB, but lacking crucial elements EetAB, PplA of the FLEET machinery (Fig 1). Interestingly, it contained FccA, known from Gram negative *Shewanella oneidensis* as periplasmic electron-conduit in EET. FccA was localized to the cytoplasm in *M. deferre*, suggesting a novel metabolic FLEET pathway (Fig 1). In our model, FccA transports electrons from oxidation processes to the FLEET proteins DmkAB, Ndh2 and FmnA that regulate the electron flow towards flavins. To facilitate quick flavin reduction, *M. deferre* likely has an as of yet unknown periplasmic electron-conduit. The flavins then diffuse through the cell wall to reduce extracellular electron acceptors.

Our results indicate the presence of a novel EET pathway with a new protein. The strain is capable of performing an anaerobic process under fully oxic conditions, showing *M. deferre*'s metabolic versatility, which makes it interesting from a physiological and a bioengineering perspective.

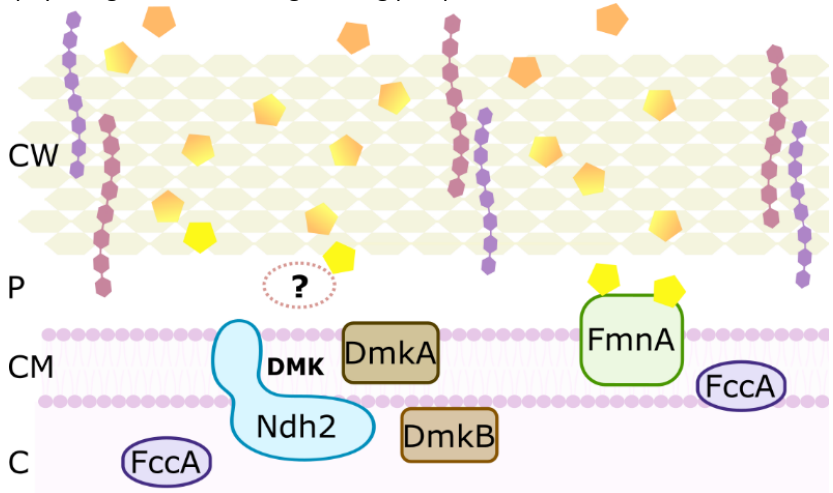

Figure 1: *Potential EET pathway in* M. deferre *A1-JK. Pentagons: reduced flavins, ?: proposed protein, CW: cell wall, P: periplasm, CM: cytoplasmic membrane, C: cytoplasm, hexagonal strings represent teichoic acids.*

Poster-A10: Microbial Hydroponics (Mi-Hy): Circular Sustainable Electrobiosynthesis

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Climate change and global warming, driven by excessive reliance on fossil fuels, pose unprecedented threats to our planet. Addressing these challenges requires innovative and holistic solutions to transform energy production and promote sustainable consumption. One promising alternative is bioenergy, which includes not only biofuels but also electricity generated through Microbial Fuel Cells (MFCs). This cutting-edge technology harnesses the power of microorganisms to oxidize organic compounds in an anode, producing a clean and renewable electric current.

The Mi-Hy European project pioneers the use of organic compounds present in wastewaters and released by plant roots to generate electricity, integrating hydroponic farming—a cultivation system (hydroculture) that delivers soluble nutrients to roots while converting CO_2 and N_2 into biomass. By combining hydroponics with a prosthetic rhizosphere and Microbial Fuel Cells (MFCs), this innovative approach creates a sustainable platform that sequesters carbon as biomass, recovers nitrogen from wastewater, and simultaneously produces electricity. The MFC system will utilize root exudates from hydroponics, targeting a groundbreaking power output of 1 mW per 1 mL, setting a new benchmark for bioenergy technology.

To achieve this goal, Mi-Hy will enhance biofilm formation in the anode through metabolic engineering of symbiotic microbial strains. These biofilms will consist of synergistic bacterial-fungal consortia, optimizing performance through microbial cooperation. This next-generation hydroponic system holds near-future potential for agriculture and urban environments, offering a smart and decentralized infrastructure powered by sustainable organic solutions. Aligned with the EU Missions on Climate Adaptation & Cities, supports urban agriculture, precision gardening, wastewater treatment, renewable energy generation, and even the synthesis of high-value compounds (e.g., vitamins).

Beyond technological innovation, Mi-Hy will engage designers and cross-sector stakeholders in co-creating future applications, accelerating the transition toward healthier, nature-based urban-agricultural ecosystems.

The Mi-Hy project has been funded by the European Innovation Council (EIC) (HORIZON-EIC-2022-PATHFINDERCHALLENGES-01) under grant agreement ID 101114746.

Poster-A11: Isolation of electroactive bacteria from diverse habitats

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Microbial electrochemical devices, including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), have been operated after inoculation with unidentified natural microbial mixtures, such as soil, sediment, digester sludge and wastewater, suggesting that electroactive bacteria (EAB) are present in diverse habitats. Studies have also shown that EAB thrive in guts of animals. Although the presence of EAB can be known by examining current generation in MFCs and/or MECs, taxonomical identification of EAB, such as that based on metabarcoding analyses, is in many cases not conclusive. This would be due in part to the fact that only minor portions of EAB in natural habitats have been isolated and characterized. It is therefore important to isolate yet-to-be identified-EAB in natural habitats.

The present work was conducted to isolate EAB from mud across Japan, sludge in anaerobic digesters and feces of animals. Particular efforts have been made to isolate EAB from a variety of mud, in which sediment-type MFCs (termed mud cells) are used in the initial screening stage to find mud that generates high power outputs. This trial has been conducted in a project under the collaboration with the National Museum of Emerging Science and Innovation and high-school students across Japan. So far, over 400 mud cells have been operated, among which approx. 20 high-power cells were used for the isolation of EAB. In the isolation procedure, anode-adhering microbes were anaerobically grown on agar plates containing mineral medium with acetate as the electron donor and fumarate as the electron acceptor. Colonies, particularly colored colonies, were purified by repeated cultivation on the agar plates and in liquid medium. Taxa of isolates were predicted based on PCR-amplified 16S rRNA gene fragments, and electrochemical activities were evaluated in electrochemical cells (ECs) at a working-electrode (WE) potential of 0 V or +0.4 V vs. SHE. Attempts were also made to isolate EAB from sludge in anaerobic digesters and feces of animals, in which EAB were enriched in air-cathode single-chamber MFCs.

Strains isolated as colonies on the plates were primarily subjected to 16S rRNA gene sequencing, and those that may represent novel taxa were selected. Measurement of electrochemical activities of these isolates identified 6 strains as EAB (Table 1). These EAB are phylogenetically diverse and exhibit relatively high electrochemical activities (in the same EC system, *Shewanella oneidensis* MR-1 attains ~0.3 mA/cm^2, while *Geobacter sulfurreducens* PCA attains ~0.6 mA/cm^2). Among them, we are particularly interested in strain 60473 for its high electrochemical activity. In addition, strain ADMFC1 is interesting, since its closest relative is *Desulfosporosinus acididurans* (88.8% identical in 16S rRNA gene sequence), and it may represent a novel family. Complete genomes of strains 60473, ADMFC1, ADMFC2, ADMFC3 and YSD1 have been determined, which will help identify novel molecular mechanisms for current generation. In addition, we will propose novel taxa for these EAB. Our project will be continued for several more years, in which we will attempt isolation of more novel EAB.

This work was supported by Institute of Fermentation, Osaka (grant number G-2023-1-002). We thank stuffs of the museum and high-school students who attended our project.

Table 1. EAB isolated in this work that may represent novel taxa.

Strain	Habitat	Electrochemical activity[1] (mA/cm^2)	Taxon predicted from 16S rRNA gene sequence
60473	Mud at the shore of lake Suwa, Nagano	1.5	*Geobacter*
ADMFC1	Anaerobic digester, Kanagawa	0.6	*Eubacteriales*
ADMFC2	Anaerobic digester, Kanagawa	0.2	*Sulfurospirillaceae*
ADMFC3	Anaerobic digester, Kanagawa	0.4	*Geovibrio*
YSD1	Mud at the mouse of river Kyobashi, Hiroshima	0.3	*Fundidesulfovibrio*
YA1	Mud in a ditch alongside a fruit farm, Yamanashi	0.4	*Neobacillus*

[1] Current densities (per projection areas of WE) in pure-culture ECs at WE potentials of 0 V (60473) or +0.4 V (others) vs. SHE with acetate as the electron donor.

Poster-A12: Cable Bacteria: Advancing Towards Practical Applications

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Cable bacteria form multicellular filaments up to 3-7 cm in length, extending deep into aquatic sediments. Hereby, they have been discovered in numerous freshwaters and oceans. Cable bacteria oxidize sulphur compounds in the deeper, anoxic sediment layers and transfer the released electrons along their filaments to the oxic zone, where oxygen reduction occurs. By transferring electrons between cells through the shared periplasmic space, cable bacteria can access electron donors and acceptors that are spatially separated. The electron conductivity of cable bacteria filaments is the highest observed in any known biological system [1]. Their unique metabolism, including CO_2 fixation and long-range electron transport, presents significant potential for applications in different fields such as electrochemical bioprocess engineering or biodegradable electronics. However, to harness their full potential, advancements in laboratory cultivation are necessary. Currently, cable bacteria can only be maintained in their native sediment, and standard cultivation and process control methods remain inapplicable.

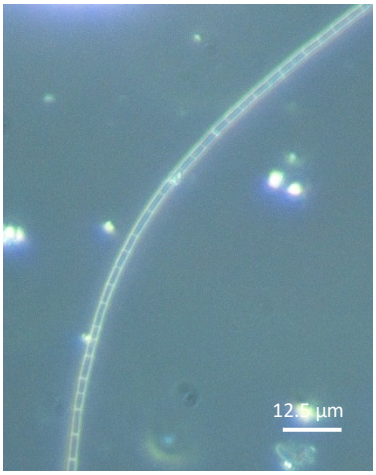

Fig. 1: Microscopic picture of *Electronema aureum* GS.

To enable the application of cable bacteria in bioprocess engineering, the development of suitable cultivation media and reactor systems is essential. A key objective of this study is the design of a synthetic sediment that ensures optimal cultivation conditions and reproducible results. Additionally, a synthetic sediment provides independence from the natural sediment at the extraction site of a given cable bacteria strain. The initial design of the synthetic sediment was based on analyses of natural sediments using ion exchange chromatography, water retention experiments, and literature on cable bacteria metabolism. Preliminary cultivation experiments with *Electronema aureum* GS [2]—kindly provided by Lars Peter Nielsen (Center for Electromicrobiology, Aarhus University)—demonstrated bacterial growth in the synthetic sediment, with an evaluation of different sand types [3]. These cultivations were performed in simple 50 mL vessels, allowing for easy replication and parameter studies, though with limited sample volume and restricted process control. To enable larger-scale cultivation with possible process control, a sediment bioreactor was developed. The reactor, constructed from autoclavable polycarbonate, allows for flexible sampling and sensor integration. Cultivation of *E. aureum* GS in the bioreactor was sustained for over two months, with an increasing bacterial population and colonization reaching sediment depths of at least 5 cm. This setup facilitates controlled growth conditions and establishes a foundation for future applications of cable bacteria, such as in electrochemical bioprocesses.

[1] Meysman FJR, Cornelissen R, Trashin S, Bonné R, Martinez SH, van der Veen J et al. A highly conductive fibre network enables centimetre-scale electron transport in multicellular cable bacteria. Nat Commun 2019;10(1):4120. https://doi.org/10.1038/s41467-019-12115-7.
[2] Thorup C, Petro C, Bøggild A, Ebsen TS, Brokjær S, Nielsen LP et al. How to grow your cable bacteria: Establishment of a stable single-strain culture in sediment and proposal of Candidatus Electronema aureum GS. Syst Appl Microbiol 2021;44(5):126236. https://doi.org/10.1016/j.syapm.2021.126236.
[3] Stiefelmaier J, Keller J, Neupert W, Ulber R: Towards bioprocess engineering of cable bacteria: Establishment of a synthetic sediment. MicrobiologyOpen 2024, 13 (3), e1412. DOI: 10.1002/mbo3.1412.

Poster-A13: Transformation to Regenerative Energy Supply: Biological Methanation as a Pathway to Sustainable Biogas Utilization

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The transition to regenerative energy sources is crucial for mitigating CO_2 emissions and achieving sustainability in industrial energy systems. This work explores the integration of biological methanation as a scalable approach to enhance CO_2 valorization within biogas-based energy systems. A two-chamber electromethanogenesis reactor equipped with 3D-printed activated carbon electrodes was employed to investigate microbial conversion of CO_2 into methane, targeting purity levels above 99% and achieving near-zero CO_2 waste emissions.
Activated carbon monolith electrodes, printed in Nuremberg, were optimized through surface functionalization (e.g., nitrogen doping, hydrophilicity tuning) to promote microbial adhesion and enhance charge transfer. Initial electrochemical testing included non-mediated and mediated cyclic voltammetry to establish baseline redox activity and compare activated versus non-activated materials. Electrochemical impedance spectroscopy (EIS) was used to determine charge transfer resistance (Rct), double-layer capacitance (Cdl), and internal diffusion resistance, linking electrode structure with microbial colonization potential.
Methanobacterium palustre was introduced into the cathode chamber under anoxic conditions with CO_2-saturated catholyte at pH 7.5–8.0. CV and EIS were employed to track biocatalytic activity, biofilm formation, and electron transfer efficiency over time. Mediator-based CV provided insights into microbial metabolic rates. The colonization and biofilm development were analyzed via electrochemical signals and charge transfer profiles. The reactor was supplied with pure CO_2 to validate microbial reduction to CH_4. CV detected microbial electron transfer to CO_2 reduction sites, while gas chromatography (GC) and ion chromatography quantified CH_4 production and monitored residual CO_2 concentrations. Preliminary observations indicate efficient CO_2 conversion and promising methanogenic activity under current conditions. Optimization of electrode potential, pH, and temperature appears to support microbial uptake of CO_2 and enhance methane production. Gas analysis suggests high methane purity with minimal residual CO_2 in the output stream. These results point toward the potential for stable long-term operation and the prospect of achieving near-complete CO_2 utilization.
This study highlights the role of surface-engineered activated carbon electrodes in enabling efficient microbial methanogenesis. Comprehensive electrochemical characterization (CV and EIS), paired with targeted microbial integration and reactor optimization, offers a robust platform for sustainable biogas upgrading and industrial decarbonization through biological CO_2-to-CH_4 conversion.

Poster-A14: Effects of supplementing conductive materials on a clostridial co-culture productivity and gene expression dynamics

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Clostridium comprises multiple species known for their ability to ferment multiple carbon sources into valuable biofuels and platform chemicals, such as ethanol and butanol. Recently, co-cultures of *Clostridium* species harboring complementary metabolisms have gained interest. For instance, the combination of a solventogenic and an acetogenic species can enhance carbon recovery, by capturing the CO_2 by-product of sugar fermentation. However, electron exchange to balance redox reactions often poses a limitation in co-cultures. To address this, the effects of adding two electron conductive compounds – weakly conductive activated carbon, and semiconductor-like magnetite – on the metabolic performance of a consortium containing *C. acetobutylicum* and *C. carboxidivorans* was explored. We investigated whether these materials could act as electron shuttles and enhance fermentative alcohol production by comparing the fermentation profiles achieved with those obtained from each species in pure culture, and analyzing their gene patterns through RNA-sequencing. Magnetite supplementation enhanced the productivity of the co-culture. In contrast, activated carbon had no significant effect compared to the control (Figure 1). Magnetite improved carbon recovery by promoting *C. carboxidivorans* autotrophy. In the co-culture, this led to increased alcohol and acid production, particularly longer-chain compounds (C4 and C6). The observed effects were due to a cooperative behavior, since magnetite supplementation in *C. carboxidivorans* pure cultures resulted in an increased acid production but a significant decrease in alcohol production. This suggests *C. acetobutylicum* was responsible of alcohols synthesis, probably at the expense of surplus reducing equivalents produced by *C. carboxidivorans*. Gene expression analysis confirmed the predicted shift, being *C. carboxidivorans* the main contributor to acid and alcohol production during the first stages of fermentation, whereas *C. acetobutylicum* was primarily responsible for alcohol production at latter stages of fermentation.

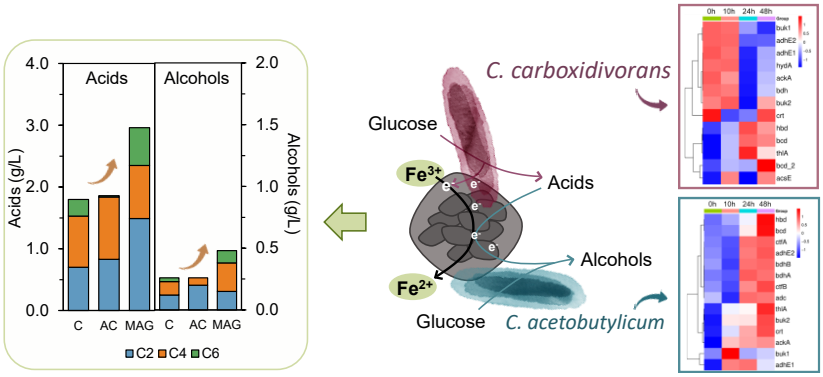

Figure 1. Productivity and schematic reaction in *C. carboxidivorans/C. acetobutylicum* co-culture with magnetite supplementation, and gene expression dynamic of alcohol and acid related genes of both species.

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Poster-A15: Powering *P. putida* – On a Path Towards Understanding Anodic Electro-Fermentation

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Anodic electro-fermentation (AEF) enables the anaerobic cultivation of obligate aerobes by utilizing an anode as the terminal electron acceptor, thereby eliminating the need for an oxygen supply and facilitating process scalability. We've investigated the obligate aerobe *Pseudomonas putida* KT2440 as a promising chassis for AEF applications and analysed its physiology under anaerobic and anode-driven conditions in a bio-electrochemical system (BES) (Weimer*, et al.* 2024).

Glucose uptake pathway analysis using gene-deletion mutants revealed that the metabolism is mainly redirected towards periplasmic glucose oxidation to 2-ketogluconate (2-KG) under BES conditions. Carbon entry into the cytosol occurs primarily via gluconate, with no detectable uptake through the ATP-dependent glucose transporter, indicating that energy limitations restrict ATP-consuming transport. Acetate, as the only significant by-product of the process originating from cytoplasmic metabolism, was only partially produced from the supplied glucose and partially from biomass degradation (Pause*, et al.* 2024).

Microscopy and spectroscopy analyses revealed a significant decrease in cellular phosphorus and polyphosphate (polyP) signals following BES operation, supporting the hypothesis that polyP is mobilized to mitigate the energy shortages.

Finally, quinone-based mediator screening revealed that 1,4-benzoquinone (BQ) significantly enhanced current output and reduced process time; however, its low stability limits continuous operation. Duroquinone (DQ) and anthraquinone-2,6-disulfonate (AQDS) were stable but incompatible with the electron transport chain of *P. putida*, yielding poor performance. Thus, while BQ highlights the potential of *P. putida* in BES, ferricyanide remains the most effective mediator for now.

These results provide new insights into the carbon uptake, energy management, and mediator selection for optimizing AEF processes with *P. putida*.

Bibliography

Pause, L.; **Weimer, A.**; **Wirth, N. T.**; **Nguyen, A. V.**; **Lenz, C.**; **Kohlstedt, M.**; **Wittmann, C.**; **Nikel, P. I.**; **Lai, B.**; **Krömer, J. O.** (2024). Anaerobic glucose uptake in *Pseudomonas putida* KT2440 in a bioelectrochemical system. *Microbial Biotechnology*. 17. e14375. 10.1111/1751-7915.14375.

Weimer, A.; **Pause, L.**; **Ries, F.**; **Kohlstedt, M.**; **Adrian, L.**; **Krömer, J.**; **Lai, B.**; **Wittmann, C.** (2024). Systems biology of electrogenic *Pseudomonas putida* - multi-omics insights and metabolic engineering for enhanced 2-ketogluconate production. *Microbial Cell Factories*. 23. 246. 10.1186/s12934-024-02509-8.

Poster-A16: Turning various ingredients into one sausage:Funneling lignin-derived hydrogenated phenols to adipic acid using recombinant *Pseudomonas taiwanensis* VLB120

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Like tasty sausages, adipic acid is a compound of huge interest as it is a building block chemical, for instance, for the broadly used polyamide nylon6,6. The industrial production of adipic acid relies on fossil-derived raw materials. We aim on replacing the fossil-based feedstock with depolymerized lignin. Yet, this replacement is challenged by the heterogenous and complex nature of lignin.[1] For an efficient industrial application, the different aromatic monomers present in depolymerized lignin need to be streamlined into the one product adipic acid.

We are on the way to solve this issue with a combined electrochemical—microbial process line. Our previous work demonstrated that typical aromatic monomers of depolymerized lignin, including phenol, catechol, guaiacol and syringol can be electrochemically hydrogenated.[2] The emerging aliphatic counterparts can be converted to adipic acid by microbial biotransformation. For this we utilized *Pseudomonas taiwanensis* VLB120 harboring a 5-step enzymatic cascade that was previously developed for the conversion of cyclohexanol to adipic acid.[3]

This work focusses on optimizing the biological funneling of the diverse hydrogenated phenols to one product, namely adipic acid. Therefore, resting cell assays with suspended cultures of *P. taiwanensis* were performed. In addition to cyclohexanol, the conversion of different substituted cycloalkanes to adipic acid was studied.[4] Compared to the previously used substrate cyclohexanol (being gained from electrochemical hydrogenated phenol), converting the lignin-derived substituted compounds to adipic acid reached higher specific activities and was less susceptible to inhibition. We studied the effects of combining different substrates and optimized the ratio and concentration of a mixture consisting of four hydrogenated compounds. The recombinant whole cells converted this optimized mixture to adipic acid with high specific activities (80 U/g_{CDW} during the first hour) and 98 % product yield.

Our results demonstrate how biological funneling can convert substrate mixtures to a single product of interest with high efficiency. It's like making one kind of sausage from different batches of raw materials to always enjoy the same taste!

[1] J. Rios, J. Lebeau, T. Yang, S. Li, M. D. Lynch, *Green Chem.* **2021**, *23*, 3172.
[2] M. C. Morejón, A. Franz, R. Karande, F. Harnisch, *Green Chemistry* **2023**, *25*, 4662.
[3] L. Bretschneider, I. Heuschkel, K. Bühler, R. Karande, B. Bühler, *Metabolic engineering* **2022**, *70*, 206.
[4] L. Seibert, M. Chávez Morejón, R. Karande, F. Harnisch, in preparation.

Poster-A17: Comparative transcriptomics of closely related *Geobacter sulfurreducens* strains uncovers strain-level variations in extracellular electron transfer

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Members of *Geobacter sulfurreducens* are electroactive bacteria (EAB) that transfer electrons to extracellular solid electron acceptors using extracellular electron transfer (EET) pathways, thus enabling current generation in electrochemical cells (ECs). To date, a number of strains affiliated with *G. sulfurreducens* have been isolated, while molecular mechanisms for current generation have mostly been studied using the type strain PCA. On the other hand, studies have also shown that different strains of *G. sulfurreducens* exhibit different levels of electrochemical activities, while limited information is available on molecular bases for strain-level variations.

In the present work, 7 strains (PCA, KN400, YM18, YM35, PL, OSK2A and 60473) of *G. sulfurreducens* for which complete genome sequences are available were subjected to phylogenetic and phylogenomic analyses, showing that strains 60473 and OSK2A are closely related. However, current densities attained by strains 60473 and OSK2A in same EC systems are substantially different (Fig. 1), even though genes of the two strains for catabolic and EET pathways are highly homologous (mostly over 99% in amino acid sequences). In addition, structures of biofilms formed by these strains during current generation are substantially different (Fig. 2). We hypothesized that expression patterns of genes for catabolic and EET pathways differ between these strains and performed comparative transcriptomics under fumarate-respiring (planktonic and biofilm) and current-generating conditions. It is found that expression levels and patterns of genes for outer-membrane cytochromes (*omc* genes) known to be involved in EET are largely different between these strains (Fig. 3), while those for catabolic enzymes are similar. It is therefore suggested that strain-level variations in current generation occur owing to differences in expression levels of *omc* genes. The information obtained in the present study would provide hints for constructing EAB with high electrochemical activities.

This work was supported by Institute of Fermentation, Osaka (grant number G-2023-1-002).

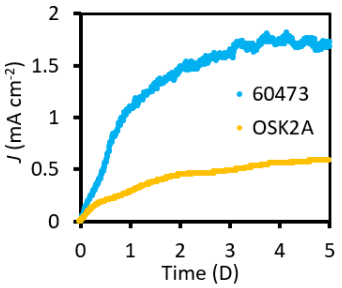

Fig. 1. Current generation by strains 60473 and OSK2A at a working electrode potential of 0 V vs. SHE.

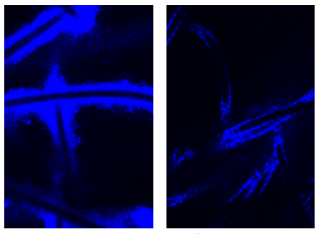

Fig. 2 Structures of current-generating biofilms formed by strains 60473 (left) and OSK2A (right).

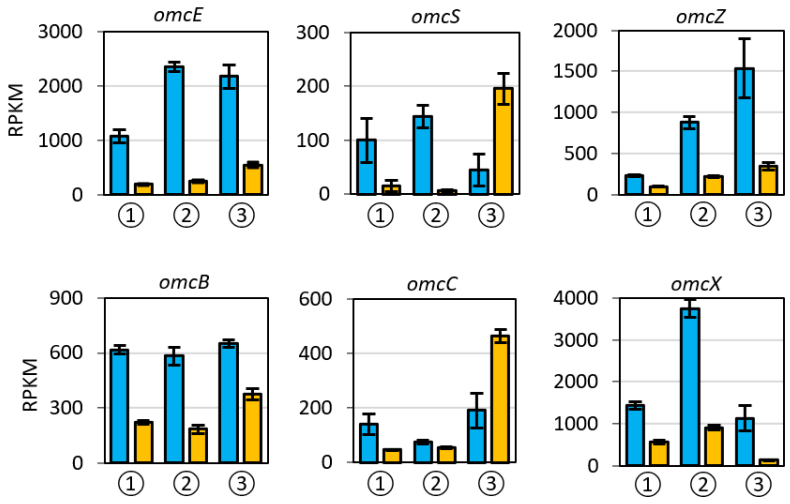

Fig. 3. Expression levels of *omc* genes in strains OSK2A (yellow) and 60473 (blue) as analyzed by RNA sequencing and indicated with RPKM (Reads Per Kilobase of exon per Million mapped reads). ①, fumarate-respiring planktonic cells; ②, fumarate-respiring biofilm cells attached onto graphite; ③, current-generating biofilm cells.

Poster-A18: Exploring stress physiology in *Clostridium ljungdahlii* when applied to microbial electrosynthesis

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Today, the majority of bulk chemicals are produced from fossil hydrocarbon sources. Alternative, sustainable feedstocks such as carbon dioxide from industrial waste gas urgently need to be implemented instead. Acetogens, such as the model organism *Clostridium ljungdahlii*, are capable of fixing carbon dioxide via the Wood-Ljungdahl pathway (WLP) when supplied with hydrogen in a process called gas fermentation (GF) which produces primarily acetate and ethanol. GF is already established on an industrial scale and could serve as an important building block of a future carbon-neutral, circular economy. However, GF suffers from some substantial drawbacks, such as the energy expenditure of pressurizing large fermentation tanks in order to solubilize hydrogen and a starkly limited product spectrum as of now. In contrast to GF, microbial electrosynthesis (MES) supplies cathodic hydrogen directly *in situ* in the solution. Thus, MES has great potential to be used in conjunction with renewable electricity production and, further, does not rely on hydrogen transport infrastructure. However, MES underperforms when compared to GF, in large part due to cell stress caused by exposure to anodic oxygen, high potentials, and limited availability of hydrogen. In attempting to understand and circumvent the effects of cell stress we compared MES and BES side by side using *C. ljungdahlii* as model. Based on the gained insights, we hope to directly improve MES as a bioprocess, but also open an avenue to discover physiological insights and potential new products. In an MES system, we discovered that *C. ljungdahlii* will divert carbon and reducing equivalents from the well-established WLP towards pathways that are less energetically efficient. This allows for elucidation of these pathways, which might not otherwise be active and informs targeted genetic changes for exploiting these reactions to a biotechnological end.[a] In addition, stressed cells will quite literally open up for microscopic insight in ways that would not be possible with healthy cells.

To gain a holistic understanding of the effects of stress within an MES system on *C. ljungdahlii,* we took a multi-pronged approach, investigating morphological changes with different imaging techniques, such as transmission electron microscopy, in conjunction with comparative omics. More targeted explorations of specific stressors or specific changes were performed through immunolabelling or RT-qPCR. So far, this led to the discovery of both novel intracytoplasmic membrane structures, as well as protein-based metabolosomes. Investigating how these subcellular structures relate to different stress factors will not just narrowly benefit MES as a developing technology but also feed into a larger understanding of *C. ljungdahlii* as a model organism for anaerobic biotechnology.

[a]Boto, Santiago T., et al. "Microbial electrosynthesis with Clostridium ljungdahlii benefits from hydrogen electron mediation and permits a greater variety of products." Green Chemistry 25.11 (2023): 4375-4386.

Poster-A19: Metabolism-based Bioelectronic Networks

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In the last decade, artificial intelligence has made enormous progress and has become an integral part of our modern IT. This has also brought the development of customized hardware, so-called neuromorphic systems, to the forefront of research and has led to new developments in solid-state electronics that are closer to their biological counterparts than ever before [1]. However, biological systems are still far superior to technical systems, e.g. in their energy efficiency. Here, metabolism is a key distinguishing feature between living and non-living matter. It is the process by which living beings absorb energy and represents a significant difference to bio-inspired technical systems, such as neuromorphic systems, as the energy is generated externally and supplied to the system. Our project seeks to use this feature by incorporating living matter into neuromorphic architectures, thereby enhancing their adaptability and energy efficiency [2]. Specifically, we cultivate electroactive microorganisms, such as *Shewanella oneidensis*, as biofilms on microelectrode arrays to study their bioelectronic response behavior. We employ a three-electrode arrangement within a continuous batch microreactor, applying different electronic conditions to the system, using chronoamperometry and cyclic voltammetry. The primary focus is to uncover critical operational states within the bioelectronic setup that leads to emergent dynamics behaviors. The electrode chips are fabricated on a dielectric substrate using thin-film technology to enable the additional integration of resistive components. This novel integration of biological metabolism and neuromorphic design represents a promising approach toward increasing the complexity of neuromorphic systems thereby developing more adaptable computing systems.

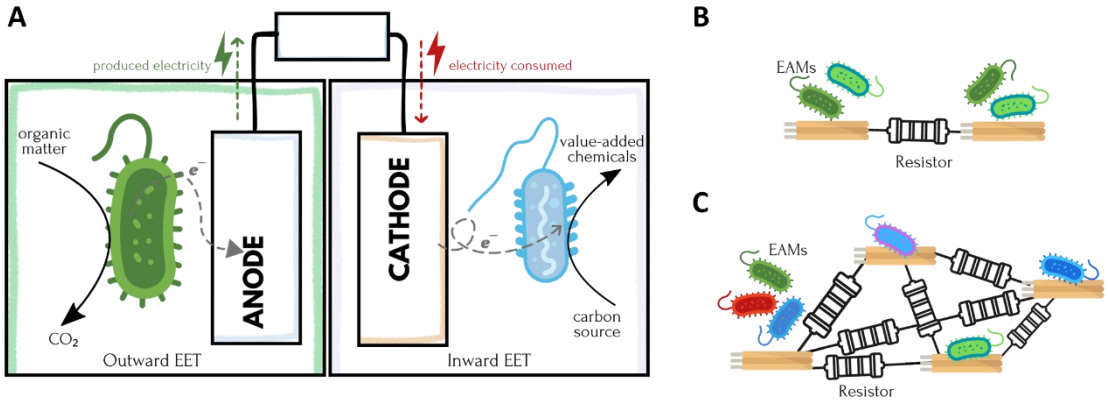

Figure 1: A) Electroactive microorganisms (EAMs) transferring electrons extracellularly to an anode or accepting them from a cathode with direct electron transfer mechanisms. B) Individual bioelectronic cells. C) Network of bioelectronic cells.

References

[1] Marina Ignatov, Martin Ziegler, Mirko Hansen, and Hermann Kohlstedt. 2017. Memristive stochastic plasticity enables mimicking of neural synchrony: Memristive circuit emulates an optical illusion. *Science Advances* 3, 10, e1700849. DOI: https://doi.org/10.1126/sciadv.1700849.

[2] Bruce E. Logan, Ruggero Rossi, Ala'a Ragab, and Pascal E. Saikaly. 2019. Electroactive microorganisms in bioelectrochemical systems. *Nat Rev Microbiol* 17, 5, 307–319. DOI: https://doi.org/10.1038/s41579-019-0173-x.

Poster-A20: Facilitation of DIET by Carbon Pile-Based Brush Boosts Methane Production from Sewage

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Maximizing methane production during anaerobic digestion (AD) of wastewater enhances energy recovery from organic waste, increasing system efficiency and economic viability. One promising strategy to improve methane yields is the promotion of Direct Interspecies Electron Transfer (DIET), a mechanism that enables direct and rapid electron exchange between syntrophic bacteria and methanogenic archaea. DIET can be facilitated by conductive materials or microbial pili, accelerating the degradation of organic matter and boosting methane production. In this study, a carbon pile-based brush (CPB) was employed as a conductive medium to stimulate DIET in anaerobic reactors treating low-strength sewage. Batch experiments were conducted using 200 mL reactors containing a mixture of 10 mL recycled anaerobic sludge and 100 mL primary sedimentation tank effluent. Reactors were set up with and without a 19 cm CPB and run in triplicates. Methane production was monitored by gas chromatography, and the relationship between chemical oxygen demand (COD) reduction and methane generation was evaluated. Microbial community dynamics were analyzed through 16S rRNA gene sequencing. Over a 45-day incubation, reactors containing CPB exhibited significantly enhanced methane production (1.92 mmol/L) compared to the control (0.15 mmol/L). COD degradation was closely correlated with methane yield across all treatments. CPB also supported higher microbial biomass (1.4×10^9 cells/vial) relative to the control (1.1×10^9 cells/vial), with approximately 90% of the cells attached to CPB fibers, suggesting enhanced biofilm formation, a condition favorable for DIET. Microbial analysis revealed a dominant presence of *Geobacter spp*., a known DIET-capable genus, in the CPB-treated reactors. These findings indicate that incorporating CPB as a conductive support is an effective strategy to enhance methane generation from low-organic-content wastewater by promoting DIET, stimulating microbial growth, and improving organic matter degradation.

Poster-A21: Electrosynthesis of glutamate from air using engineered *Acidithiobacillus ferrooxidans*

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Amino acids are major industrial products with large markets and are currently produced by microbial fermentation. However, amino-acid fermentation processes are associated with energy and environmental issues that include the consumption of large amounts of ingredients, such as sugars and ammonia, and the discharge of fermentation residues containing high concentrations of waste organics. Consequently, there are growing interests in developing environment-friendly amino-acid production processes, and we have decided to exploit microbial electrosynthesis for this purpose. A particular focus is placed on the use of an electroactive diazotrophic autotroph, such as *Acidithiobacillus ferrooxidans*, since it is expected that amino acids can be produced from air (CO_2 and N_2) with electricity as the sole source of energy.

In order to demonstrate the above-mentioned idea, we have constructed an engineered strain of *A. ferrooxidans* that expresses a gene encoding a glutamate transporter of *Escherichia coli* under the control of an authentic inducible promotor (Kanao et al., 2023) (termed strain GT) and examined if glutamate is produced in air-supplied inorganic culture media. When strain GT was grown in inorganic medium containing ferrous iron as the sole energy source, glutamate was produced significantly more than that produced by the parental strain. In addition, when strain GT was cultivated in electrochemical reactors equipped with gas-diffusion working electrodes (Fig. 1), glutamate was detected in nitrogen-free inorganic medium only when electricity was supplied to GT at a working-electrode potential of 0 V (vs. Ag/AgCl) (Fig. 2). These results demonstrate that strain GT produces glutamate in nitrogen-free inorganic medium with CO_2 and N_2 in the air as the ingredients and electricity as the energy source. We suggest that the *Acidithiobacillus ferrooxidans* system will serve as a platform for autotrophic microbial electrosynthesis that can produce a variety of valued chemicals from air.

References:
Kanao et al. 2023. J. Biosci. Bioeng. 135, 176-181.

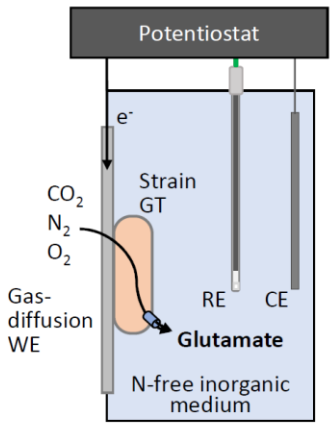

Fig. 1. A diagram showing the structure of an electrochemical reactor (ER) equipped with a gas-diffusion (GD) working electrode (WE).

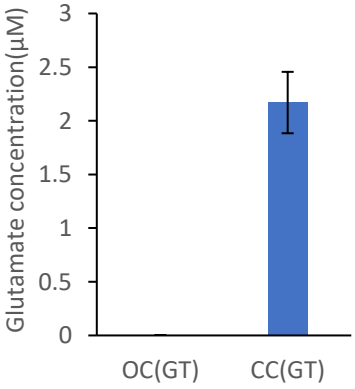

Fig. 2. Production of glutamate in GDWE-ECs containing strain GT (n = 3). OC; open circuit. CC, closed circuit (electricity was supplied).

Poster-A22: Controlling bioanode performance through nutrient concentration ratios: Linking EPS composition to current generation

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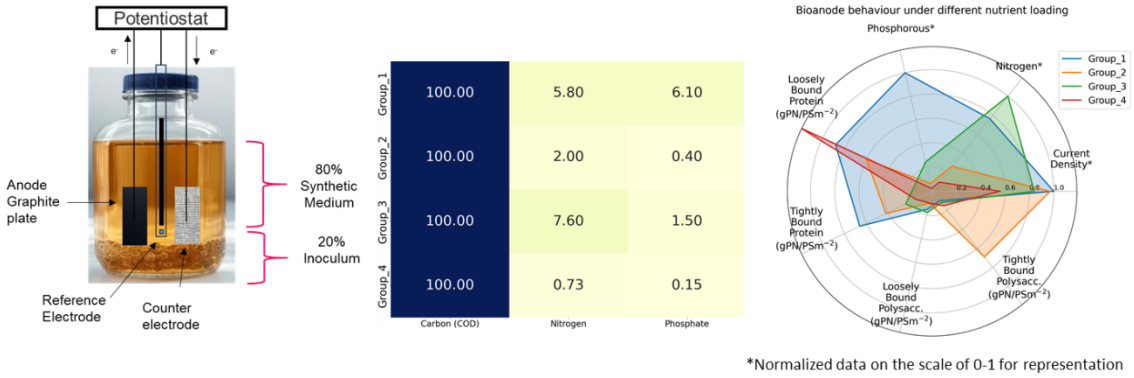
Bioanode performance is closely linked to the structure and electroactivity of electroactive biofilms (EABs) and substrate utilization rate. A key factor influencing the stability and efficiency of these biofilms is the presence of extracellular polymeric substances (EPS) [1]. Although fermentation effluents offer a rich supply of organic matter, they may be deficient in certain essential nutrients like nitrogen and phosphorus [2]. Thus, understanding the effect of nutrients on the EPS formation and energy production can assist in improving bioanode performance and fermentation effluents valorization. In this study, an analytical bioelectrochemical system was subjected to varying carbon (C), nitrogen (N), and phosphate (P) loading ratios (C:N:P) to observe its response in terms of current generation and EPS formation on the bioanode.

An analytical bioelectrochemical system with an effective volume of 600 mL was operated in batch mode with a bioanode polarized at -0.1 V vs SCE. A total of 12 reactors were divided into four groups of triplicates. Sodium acetate was used as the carbon source at a fixed concentration of 1.28 g COD/L, while nitrogen and phosphorus levels were adjusted according to the designated C:N:P ratios for each group. The assigned ratios were G1 (100:5.8:6.1), G2 (100:2:0.4), G3 (100:7.6:1.5), and G4 (100:0.73:0.15). These ratios were chosen on the basis of the actual composition of various fermentation effluents, with G4 being derived from the theoretical nutrient requirements for anaerobic biomass yield.

The study showed that reactors G1 and G2 produced an average current density of 12.8±1.1 and 12.3±2.2 A/m², respectively, while reactor G4 produced a 40% lower current density. This reduced performance under N and P nutrient limitation conditions (G4) may be related to the lower content of tightly bound proteins (TB-PN) in the biofilm. Among different component of EPS, the TB-PN content correlated well with the current generation in each group. Specifically, both TB-PN content and current followed the same decreasing trend: G1 > G2 > G3 > G4. During the third cycle of acetate addition for groups G1, 2 and 3, a release of nitrogen was observed, which was most probably generated from the inoculum's bioflocs. This release coincided with lower current generation in the following cycles of bioanode operation for these reactors. In addition to the highest nitrogen content in G3, the nitrogen release resulted in an inhibiting concentration, which reduced current and TB-PN concentration by approx. 20% and 45%, respectively in comparison to G1 and G2. Therefore, it is essential to control the C:N:P ratio of the substrate as it is found to regulate the biofilms' EPS composition, and promote current generation from the bioanode.



*Normalized data on the scale of 0-1 for representation

References

1. Wang S, Zhi L, Shan W, Lu H, Xu Q, Li J. Correlation of extracellular polymeric substances and microbial community structure in denitrification biofilm exposed to adverse conditions. *Microb Biotechnol*. 13(6), 1889–1903 (2020).
2. Huang J, Yang P, Guo Y, Zhang K. Electricity generation during wastewater treatment: An approach using an AFB-MFC for alcohol distillery wastewater. *Desalination*. 276(1–3), 373–378 (2011).

Poster-A23: Redefining *Clostridium ljungdahlii* Physiology Under Microbial Electrosynthesis Through Multi-Omics Analysis

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Microbial electrosynthesis (MES) allows microorganisms to convert electric energy into valuable compounds using CO_2 as a carbon source. This process is similar to gas fermentation, where oxidation of the externally fed hydrogen gas is the energy (i.e. electron) source instead of the electrolytic hydrogen used in MES. Despite its potential, MES still faces major limitations, such as low efficiency, limited portfolio of low value products, and poor microbial growth, especially in the commonly used H-type electrobioreactors. In this study, we analyzed the physiology of the model acetogen *Clostridium ljungdahlii* grown in gas fermenters compared to H-type electrobioreactors to pinpoint the key stress factors that limit performance MES. We identified the physiological changes happening during MES using transcriptomics, proteomics, and electron microscopy analysis. We showed that the electrochemical setup directly changes the cellular metabolism, the primary CO_2 fixation pathway for acetogen, the Wood-Ljungdahl pathway, showed major changes and imbalance between the carbonyl and methyl branches, in addition to the diversion of the methyl branch towards glycine. MES leads to the activation of the glycine synthase-reductase pathway (GSRP), resulting in a wider variety of products; specifically, ethanolamine and glycine were found to be produced exclusively during MES. Furthermore, our study shows the expression of bacterial microcompartments (BMCs), raising questions about their function during MES. For the first time, we show that *C. ljungdahlii* can natively produce cyanophycin storage granules, which are utilized to support the arginine deaminase (ADI) pathway for ATP generation under MES, pointing to the energy-limited conditions in MES reactors. Our results offer new insights into how MES leads to physiological changes of the biocatalyst, emphasizing the importance of studying the biological side of the process to move forward in its development.

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Poster-A24: Succession of bacterial community during electroactive methanogenic biofilm development under microplastic manipulation

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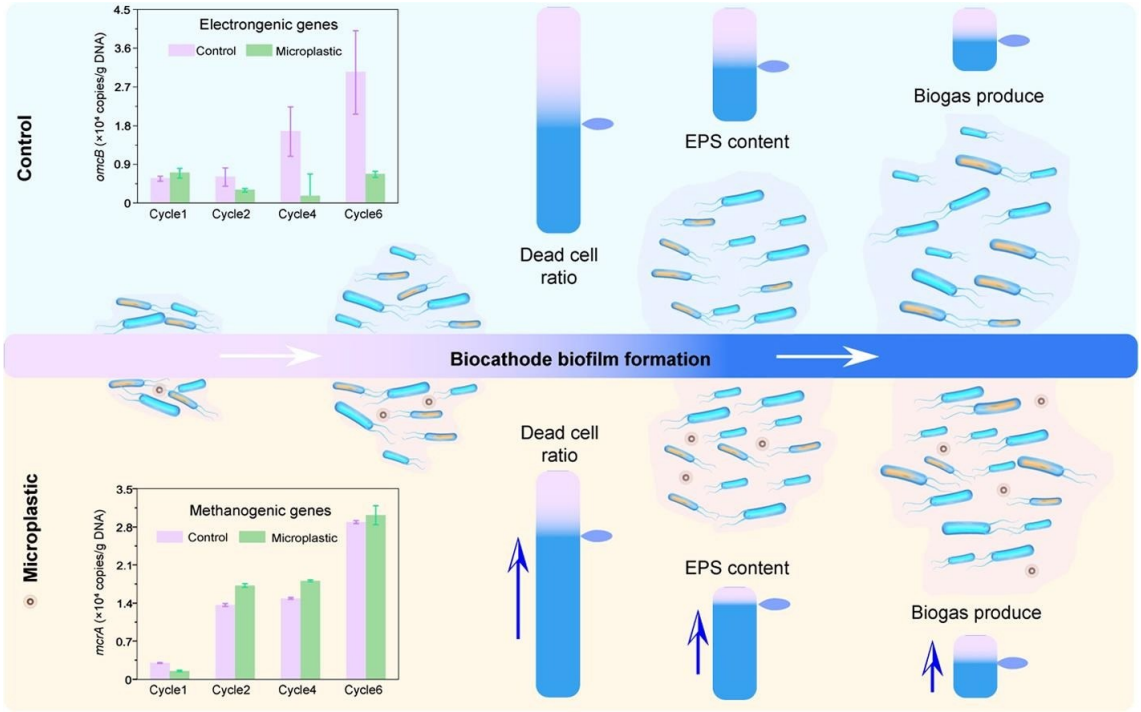
Electrochemical methanogenesis is a promising and reliable process to convert waste streams into CH4, where the electroactive methanogenic biofilms play a key role. However, given that microplastics (MP) have now been spread ubiquitously in the environment, their regulation on the performance and succession of methanogenesis biofilm remains an enigma. Herein, we developed the single-chamber electrochemical methanogenic systems to investigate how poly (ethylene terephthalate) MP (PET-MP) regulates methanogenic biofilm formation and microbial metabolisms. The microbial volume of biofilm formed under MP exposure was similar to that without exposure. However, the live/dead cell ratio of the microbes in the biofilm under the PET-MP exposure decreased significantly ($p < 0.05$). Correspondingly, the richness and diversity of the microbial community in the presence of MP were also lower. Network analysis implied the interspecific cooperation among the microbial communities to cope with the MP stress. Meanwhile, the biofilm produced more extracellular polymer substrates during the biofilm thickening, possibly as a defense against MP invasion. At the gene level, the content of methanogenic gene mcrA was found to positively linearly correlate with the cultivation cycles, both in presence ($r = 0.945$, $p < 0.05$) or absence of PET-MP ($r = 0.913$, $p < 0.05$). The outcomes of this study could provide insights into the practical application of electrochemical methanogenesis technology to upcycle the MP-polluted biowastes and to implement the Power&Waste-to-X concept better.



Poster-A25: Novel haloalkaliphilic *Billgrantia* sp. capable of iron oxidation-dependent nitrate reduction

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Extracellular electron transfer (EET)-based microbial respiration involves the direct exchange of electrons with solid-state electron donors or acceptors. While microbial EET plays a crucial role in various biogeochemical cycles and holds great promise for various applications, current research on EET-capable microbes has largely focused on exoelectrogens from non-extreme environments. Electrotrophs, which utilize insoluble electron sources like reduced minerals or electrodes for respiration, remain comparatively understudied [1]. Investigating extremophilic EET-capable microbes (EAMs), particularly electrotrophs is desired to understand novel respiratory strategies and their roles in the biogeochemical cycling of different elements, besides enabling applied research on energy conversion, wastewater treatment, and bioremediation of contaminated environments [2]. To this end, our ongoing work focuses on nitrate-reducing electrotrophs from the haloalkaline Lonar Lake (India). Nitrate-reducing bacteria (NRB) play a pivotal role in cycling elements like N, Fe, C, etc., especially given the central role of iron in both abiotic systems (as a major crustal element) and microbial metabolic evolution from anoxic to oxic conditions [3]. In line with this, we isolated a novel *Billgrantia* species, named *B. alkalidenitrificans*, under electroautotrophic conditions using Fe(0) as the electron donor and bicarbonate as the carbon source, from cathodic biofilms enriched from Lonar Lake sub-surface sediments [4]. *B. alkalidenitrificans*, a gram-negative facultative anaerobe, exhibits high tolerance to salinity (0–150gNaCl/L, optimum 20g/L), temperature (10–45°C, optimum 37°C), and pH (6.5–11.5, optimum 9.5). Genome comparisons (dDDH 34.3% (d_4) and orthoANI 87.93%) with its closest relative, *B. campisalis* (based on 16S rRNA and whole genome phylogeny), confirmed its novelty. Functional annotation using KEGG BlastKOALA identified key denitrification genes: *napA, napB, narG, narH, narI* (nitrate to nitrite), *nirS* (nitrite to nitric oxide), *norB, norC* (nitric oxide to nitrous oxide), and *nosZ* (nitrous oxide to nitrogen). Genes involved in iron metabolism were also identified, including the iron oxidation gene (*Cyc1*) along with iron transporters (*fbpABC*), heme oxygenases/transporters and storage genes (*bfr*). Experimental assays confirmed Fe(II) oxidation coupled to nitrate reduction under anaerobic conditions by *B. alkalidenitrificans*. In my presentation, I will encompass the comprehensive characterization of the novel strain, unveiling its iron oxidation-linked nitrate reduction capability, thereby broadening the understanding of this respiratory process in extreme habitats.

References

1. Lovley, D.R., et. al. Electromicrobiology: the ecophysiology of phylogenetically diverse electroactive microorganisms. Nat Rev Microbiol, 20, 5–19 (2022).
2. Shrestha, N., et. al. Extremophiles for microbial-electrochemistry applications: A critical review. Bioresour Technol, 255, 318-330 (2018).
3. Ilbert, M., et. al. Insight into the evolution of the iron oxidation pathways. BBA Bioenergetics, 1827, 2, 161-175 (2013).
4. Chaudhary, S., et. al. Electrochemical enrichment of haloalkaliphilic nitrate-reducing microbial biofilm at the cathode of bioelectrochemical systems, iScience, 24, 6, 102682 (2021).

Poster-A26: Electrobiotechnological applicability of *Cupriavidus necator* H16

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Cupriavidus necator H16 is a chemolithoautotrophic bacterium with significant potential for biotechnological application [1,2]. It can thrive on a broad spectrum of organic substrates but is alternatively able to grow on *knallgas*, i.e., mixtures of H_2 and O_2, to fuel the fixation of CO_2 via the Calvin-Benson-Bassham cycle [3]. This enables withdrawal of CO_2 from the atmosphere and facilitates carbon negative production of various compounds. However, explosive gas mixtures bear significant safety issues which is why attempts are made to replace O_2-based respiration with anodic respiration.
This can be achieved using redox mediators which were analysed for their toxicity towards *C. necator*, as well as for their efficiency in anodic respiration [4].
Secondly, attempts are made to exploit one of the formate dehydrogenases (FDH) of *C. necator* in future biotechnological setups for CO_2 fixation. All *C. necator* FDHs naturally work in the presence of O_2 and are thus supposed to be O_2 tolerant. The membrane-bound FDH (FdoGH) was therefore isolated and characterized biochemically and spectroscopically.

(1) Lauterbach, L., and Lenz, O. (2019) How to make the reducing power of H_2 available for *in vivo* biosyntheses and biotransformations. *Curr. Opin. Chem. Biol. 49*, 91–96.
(2) Morlino, M. S., Serna García, R., Savio, F., Zampieri, G., Morosinotto, T., Treu, L., and Campanaro, S. (2023) *Cupriavidus necator* as a platform for polyhydroxyalkanoate production: An overview of strains, metabolism, and modeling approaches. *Biotechnol. Adv. 69*, 108264.
(3) Pohlmann, A., Fricke, W. F., Reinecke, F., Kusian, B., Liesegang, H., Cramm, R., Eitinger, T., Ewering, C., Pötter, M., Schwartz, E., Strittmatter, A., Voß, I., Gottschalk, G., Steinbüchel, A., Friedrich, B., and Bowien, B. (2006) Genome sequence of the bioplastic-producing "Knallgas" bacterium *Ralstonia eutropha* H16. *Nat. Biotechnol. 24*, 1257–1262.
(4) Gemünde, A., Rossini, E., Lenz, O., Frielingsdorf, S., and Holtmann, D. (2024) Chemoorganotrophic electrofermentation by *Cupriavidus necator* using redox mediators. *Bioelectrochemistry 158*, 108694.

Poster-A27: The Extracellular Electron Transfer Mechanism and Phenotype of *Synechocystis* sp. 6803 in Biophotovoltaics System

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Sunlight sustains live on the earth. Converting the energy of sunlight into carbon-free fuel (e.g. hydrogen) is one of the most promising solutions for reaching our climate targets. The key challenge that needs to be overcome is how, for instance, H_2 can be produced sustainably. The natural oxygenic photosystem (from e.g. cyanobacteria, etc) has evolved to be a highly efficient way of capturing solar energy after billions of years of evolution. It can absorb sunlight with a quantum efficiency of over 30% and splits water into oxygen, protons, and electrons, while the latter two provide the perfect source for H_2 production.

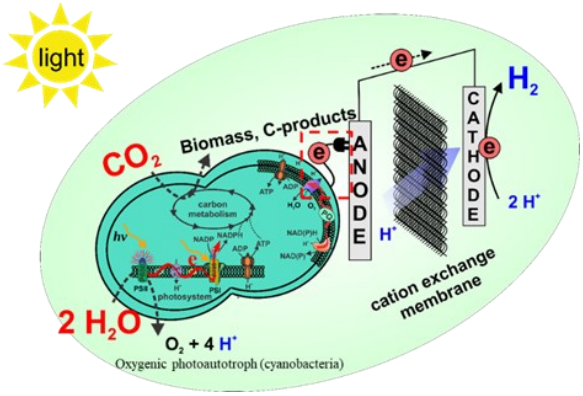
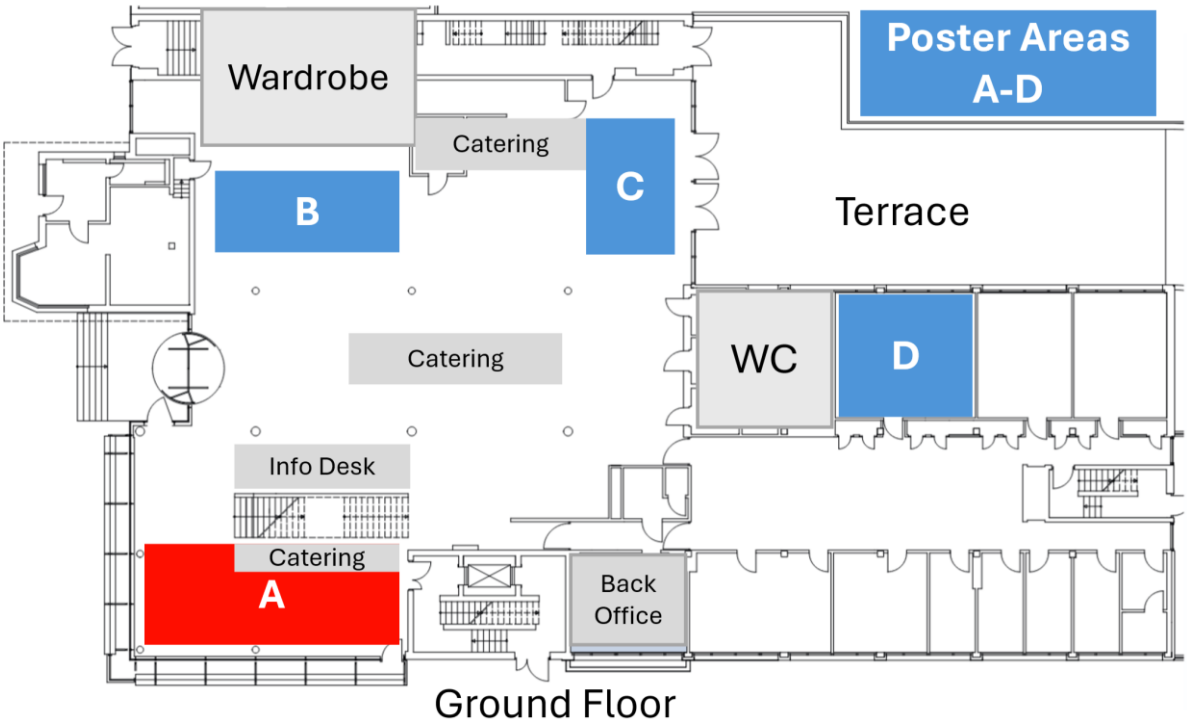


Fig.1 Schematic diagram of BPV system.

Biophotovoltaics (BPV) has been developed in the past decade to utilize the natural oxygenic photosystem for sustainable power production. The photosynthetic electrons, released from water splitting by the photoautotroph, are captured by the anode via the extracellular electron transfer (EET) pathway, which are then transferred to the cathode for e.g. pure hydrogen. CO_2 is consumed for biomass reproduction. A BPV solves the intrinsic problem of oxygen for the photoH_2 production using hydrogenase, and also requires much less energy and resource inputs for H_2 formation compared to the green hydrogen concept.
A significant knowledge gap in optimizing BPV systems is the limited understanding of EET pathways and their impacts on cellular physiology. To address this, we monitored the carbon fixation rate and photosynthetic oxygen exchange to investigate the photosynthetic electron flows in *Synechocystis* sp. PCC 6803 cultivated in a ferricyanide-mediated BPV system. Here we show that EET did not have detectable effects on cell growth, respiration, carbon fixation, or photosystem II efficiency. Nevertheless, the EET process influenced Mehler-like reactions, specifically competing for electrons with the flavodiiron protein flv1/3. This suggests the mediator facilitates photosynthetic electron extraction from ferredoxins downstream of photosystem I. These findings provide important molecular insights into the EET pathways in Synechocystis within BPV systems, offering valuable information for the future optimization of BPV technology.

POSTER PRESENTATIONS

Enzyme BES
- Area A -



Poster-A28: Merging electrochemistry with biocatalysis in one-pot processes

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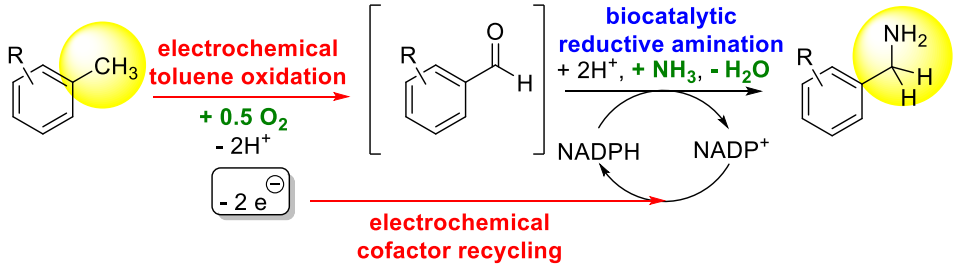
Addressing the enormous potential of one-pot processes in water by combining "different worlds of reaction types", this tandem project focuses on merging electrochemistry with enzymatic reaction steps for direct transformation of toluenes or other alkyl-substituted aromatic compounds in a one-pot fashion to bulk chemicals such as benzylic alcohols and benzylic amines.[1] Today, such alcohols and amines are of high industrial importance but require harsh conditions, hazardous reagents and a high energy demand.[2]

In contrast, the proposed concept enables a one-pot transformation of a methyl group into a CH_2OH or CH_2NH_2 group at room temperature and in water under highly energy-saving conditions, thus representing a transformation, which currently does not exist in organic chemistry. Upon combining electrochemistry and biotechnology such a "*dream reaction*" (defined as chemical transformations, for which no single catalyst exists yet but which are highly desirable) can be realized, which "formally" corresponds to a hydroxylation or amination of an alkyl group just by using air and in the latter case additionally ammonia as a reagent as well as "green energy" (electricity through solar or wind energy) as an energy source. The concept of this target platform technology is shown in Scheme 1.



Scheme 1. Concept of the targeted one-pot process consisting of an initial electrochemical oxidation and subsequent enzymatic reductive process (exemplified for a reductive amination here).

In this presentation we will show our initial studies on the optimization of the electrochemical reaction and selected enzymatic process steps with the target of combining both reaction types.
Electrochemical oxidation of toluenes often takes place in a methanol solution.[3,4] Since methanol is an unsuitable solvent for the enzymatic step water should be used. However, the electrochemical oxidation of toluenes will not take place if the water concentration is too high. Therefore, the oxygen evolution reaction must be suppressed so that the oxidation can take place in an aqueous solution. For the second reaction step both the biocatalytic reduction of benzaldehydes with an alcohol dehydrogenase and the reductive amination using an amine dehydrogenase were already realized with >99% conversion. Among current challenges are the extension of the substrate scope and stability of the enzymes under the reaction conditions of the electrochemical oxidation step in order to realize the desired one-pot process.

References

[1] Y. Hayashi, *Chem. Sci.*, **2016**, *7*, 866.
[2] a) L. Heuer, *Ullmann's Encyclopedia of Industrial Chemistry*, **2000**, *5*, 385-387. b) F. Brühne, E. Wright, *Ullmann's Encyclopedia of Industrial Chemistry*, **2000**, *5*, 357-364.
[3] H. Wendt, S. Bitterlich, E. Lodowicks, Z. Liu, *Electrochim. Acta*, **1992**, *37*, 1959–1969.
[4] U. Griesbach, J. Botzem, F. Stecker, *EP 2 616 424 B1* **2011** (BASF).

Poster-A29: Development of an Electrochemical System for Coenzyme F_{420} Using Immobilized F_{420}-Dependent Sulfite Reductase

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Introduction

Coenzyme F_{420} (F_{420}) is a physiological electron carrier in methanogenic archaea, playing a central role in methane-generating metabolic processes. The reduced form of F_{420} ($F_{420}H_2$) has attracted attention for developing enzymatic reduction systems of recalcitrant substrates due to its lower standard redox potential (-340 mV vs. SHE) [1]. F_{420} does not directly react with electrodes, but recently, we successfully established an electrode reaction system for F_{420} by utilizing F_{420}-dependent sulfite reductase (Fsr) and an appropriate mediator [2]. In this study, we (1) developed Fsr and the mediator immobilization methods and (2) evaluated various electrode materials suitable for applications of this system, such as biosensors for methanogenic activity and efficient F_{420} reduction technologies.

materials & methods

Fsr and appropriate mediators were immobilized on a carbon mesh electrode that allows light transmission by using a hydrogel polymer and crosslinking agents. To demonstrate the electrochemical redox reaction of F_{420}, the spectroelectrochemical analysis was conducted (Fig. 1). Absorbance changes at 420 nm (ΔAbs) were monitored during cyclic voltammetry (CV) because only the oxidized form of F_{420} strongly absorbs light at 420 nm. In this experiment, Fo, which is an analog of F_{420} and exhibits functionality equivalent to F_{420}, was used instead of F_{420}.

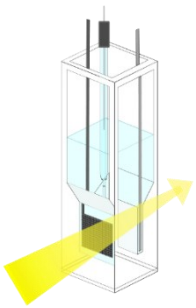


*Figure 1: **Spectroelectrochemical cell. WE: Fsr-immmobilized carbon mesh electrode, RE: Ag/AgCl, CE: Pt.***

Results

We successfully developed an electrode immobilized with Fsr and redox mediator and demonstrated the electrochemical redox reaction of F_{420}. As the applied potential was scanned negatively during the CV, the ΔAbs at 420 nm decreased, indicating the proceeding of F_O (F_{420}) reduction. Conversely, the absorbance increased during the positive scanning, indicating the F_O oxidation (Fig. 2). The redox potential calculated from the inflection points of the ΔAbs was -0.560 V (vs. Ag/AgCl/sat. KCl), which is comparable with the standard redox potential of F_{420} (-0.540 V vs. Ag/AgCl/sat. KCl). The developed Fsr-immobilized electrode operated continuously for 24 hours and kept functioning at temperatures up to 65°C. These features are advantageous for industrial applications and the development of the F_{420} biosensor.

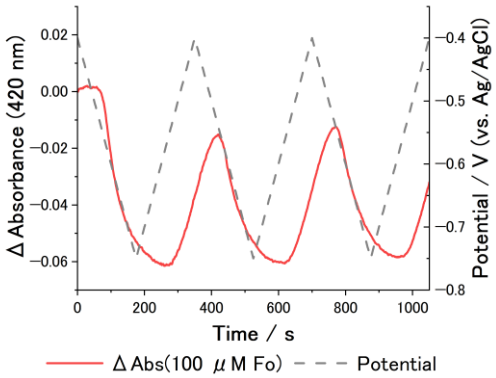
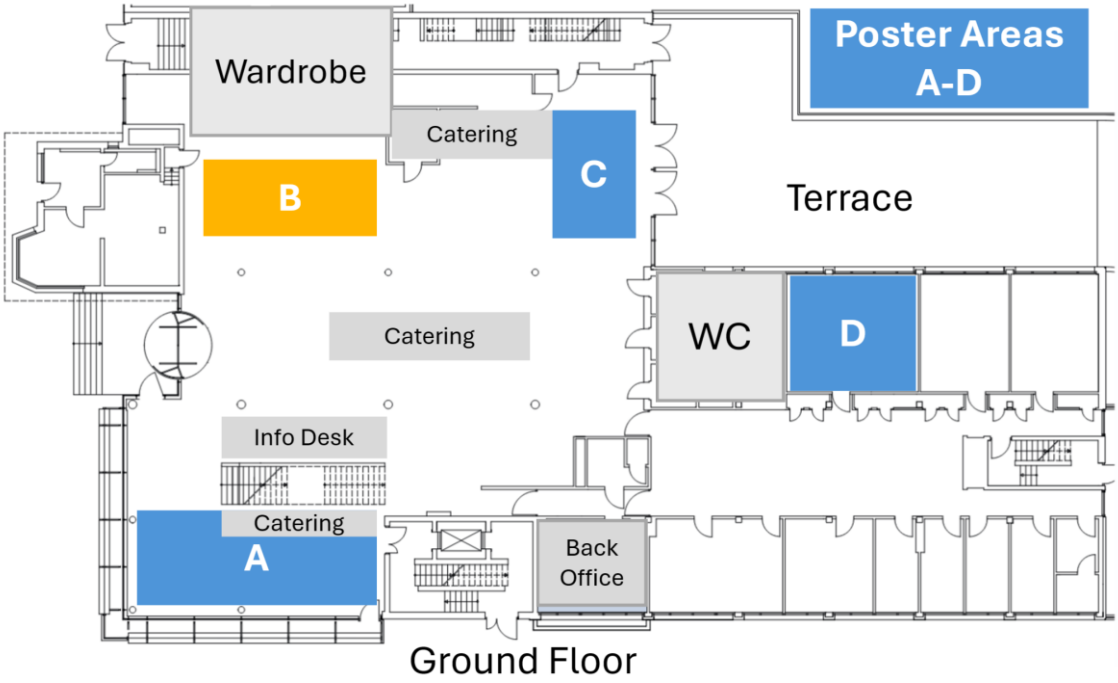


***Figure 12:** Absorbance changes at 420 nm during the CV on Fo using the Fsr-modified electrode. The change in ΔAbs corresponding to the applied potential demonstrates the progression of the electrochemical redox reaction of Fo.*

(1) R. Grinter, *et al.*, FEMS microbiology reviews, **45**, fuab009 (2021).
(2) S. Furota, *et al.*, Bioelectrochemistry, **164**, 108922 (2025).

POSTER PRESENTATIONS

Electrochemistry and Systems Engineering
- Area B -



Poster-B01: Screening of redox mediator interaction with *Vibrio natriegens* combining electrochemical and spectrophotometric analysis in a miniaturized bioelectrochemical system

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Vibrio natriegens is one of the fastest-growing organisms known[1], with a highly dynamic metabolism that has sparked interest due to its potential to increase the efficiency of biotechnological processes. However, do its remarkably high growth rate, conventional gassing strategies may fall short when controlling for oxygen supply during scale-up[2]. Therefore, alternative final electron acceptors might help to achieve controlled conditions in a stirred tank bioreactor setup.

Initial studies demonstrated that *V. natriegens* possesses the necessary molecular machinery to transport electrons beyond its cell wall through a pathway involving CymA, PdsA and MtrCAB[3]. Additionally, studies conducted within our group demonstrated, for the first time, that *V. natriegens* can interact with ferricyanide in a bioelectrochemical system (BES). This resulted in a significantly enhanced electron transfer efficiency—by a factor of 30 compared to a mediator-free cultivation and influenced the metabolite profile[2]. Bioelectrochemical systems might offer a suitable alternative to conventional gassing due to the significantly higher solubility in the cultivation media of some redox mediators compared to oxygen gas and the virtually endless supply of oxidized mediator through its electrochemical oxidation at a poised anode. While a previous study identified methylene blue as the most effective mediator for a microbial fuel cell with *V. natriegens* among seven different mediator candidates[4], no comprehensive screening has yet been conducted to compare methylene blue, ferricyanide, and other promising redox mediators demonstrated for *V. natriegens* and other gram-negative bacteria.

To address this, the present study employs a miniaturized BES setup[5], consisting of eight individual systems coupled to a photometer to systematically investigate the interaction of suspended *V. natriegens* cells with different redox mediators. The cells were first cultivated in shake flasks, then washed and resuspended in a carbon- and nitrogen-free medium, either with or without a redox mediator. After equilibration in cuvettes, glucose was added, allowing the mediator reduction rates and total turnover number to be determined via chronoamperometry and photometric analysis. This approach enables the identification of the most suitable redox mediator for establishing a mediated BES with *V. natriegens,* using glucose as the electron donor, while reducing the effort and resources needed to conduct this screening.

1. Eagon, R. G. *Pseudomonas natriegens*, a marine bacterium with a generation time of less than 10 minutes. J Bacteriol 83, 736–737 (1962).
2. Gemünde, A., Gail, J. & Holtmann, D. Anodic Respiration of *Vibrio natriegens* in a Bioelectrochemical System. *ChemSusChem* 16, e202300181 (2023).
3. Conley, B. E., Weinstock, M. T., Bond, D. R. & Gralnick, J. A. A Hybrid Extracellular Electron Transfer Pathway Enhances the Survival of *Vibrio natriegens*. Appl Environ Microbiol 86, e01253-20 (2020).
4. Gong, Z., Xie, R., Zhang, Y., Wang, M. & Tan, T. Identification of Emerging Industrial Biotechnology Chassis *Vibrio natriegens* as a Novel High Salt-Tolerant and Feedstock Flexibility Electroactive Microorganism for Microbial Fuel Cell. *Microorganisms* 11, 490 (2023).
5. Gemünde, A., Gail, J. & Holtmann, D. Redox mediator interaction with *Cupriavidus necator* – spectroelectrochemical online analysis. *Electrochemistry Communications* 162, 107705 (2024).

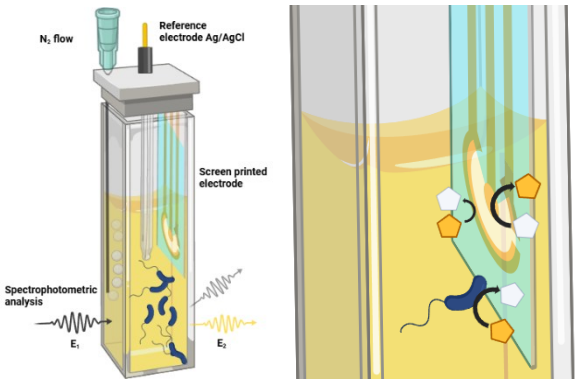

Figure 1: Schematic representation of the used system.

Poster-B02: Real-Time Monitoring of Electroactive Biofilms Using Torsional Quartz Crystal Microbalance

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Electroactive biofilms are widely studied for applications in energy generation, wastewater treatment, and bioproduction. The electrical current produced by these biofilms is proportional to their metabolic activity, and their efficiency is influenced by the film's structural organization. Thus, assessing film thickness and mechanical properties, such as softness, is essential for an optimized performance. The quartz crystal microbalance with dissipation monitoring (QCM-D) provides a powerful, real-time, and label-free method for monitoring mass changes and mechanical properties of biofilms, as shifts in resonance frequency and bandwidth offer insights into biofilm dynamics, growth, stability, and response to environmental stress.
However, the traditional thickness-shear QCM has limitations when studying biofilms, as the penetration depth of the shear wave emanating from such resonators' surfaces is less than 1 µm. Since biofilms are often tens of micrometers thick, the wave does not fully penetrate the film and instead quickly dissipates within it. To address this, torsional quartz crystal microbalance (TQCM) has been used as an alternative. Torsional resonators oscillate via torsional deformation with a resonance frequency three orders of magnitude lower than conventional QCMs (in the KHz range), significantly increasing shear wave penetration and enabling deeper biofilm analysis [1] (Figure 1).
Similar to thickness-shear QCMs, torsional resonators can be utilized as electrochemical QCMs (EQCMs). By integrating an electrochemical setup, they enable simultaneous measurement of electrical current, frequency, and bandwidth shifts. This allows for real-time correlation between biofilm thickness and electrochemical activity, providing a more comprehensive understanding of biofilm processes [2].
Our investigations suggest that changes in biofilm mechanical integrity can be directly linked to variations in its response to environmental and electrochemical stimuli. In particular, observed shifts in frequency and bandwidth not only correlate with biofilm growth and metabolic activity but also serve as sensitive indicators of biofilm stress and softness—key parameters for assessing biofilm health and functionality. This dual-mode measurement capability, combining real-time electrical and acoustic monitoring, highlights the potential of torsional EQCM in offering deeper insights into biofilm behavior beyond conventional electrochemical analysis.

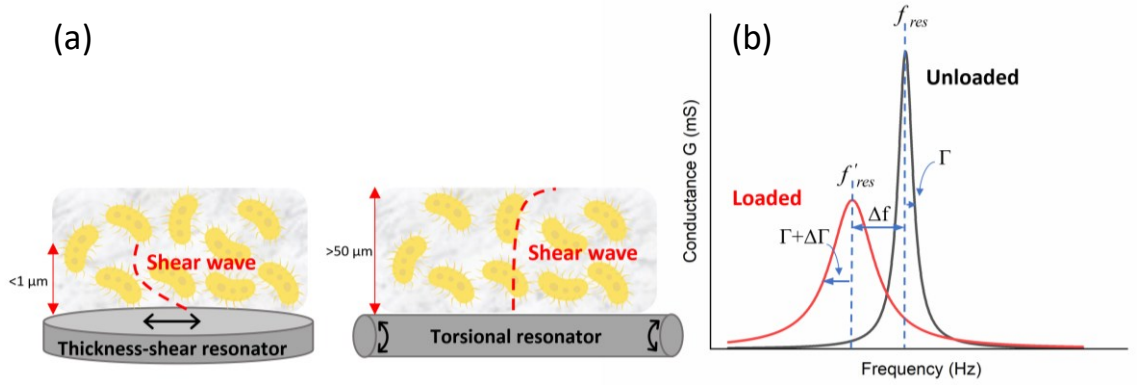

***Figure 1**: a) Comparison between thickness-shear QCM and torsional QCM. b) The graph shows the resonance frequency shift by Δf (f_{res}+ f'_{res}) and bandwidth increase (Γ→Γ+ΔΓ) due to film deposition and energy dissipation.*

[1] Phillipp Sievers and others, 'Use of Torsional Resonators to Monitor Electroactive Biofilms', *Biosensors and Bioelectronics*, 110 (2018), pp. 225–32, doi:10.1016/j.bios.2018.03.046.
[2] Philipp Sievers and Diethelm Johannsmann, 'Environmental-Stress-Induced Increased Softness of Electroactive Biofilms, Determined with a Torsional Quartz Crystal Microbalance', *Analytical Chemistry* 2019, 2019, doi:10.1021/acs.analchem.9b03204.

Poster-B03: Modeling of Microbial Corrosion of Carbon Steel due to Electrochemically Active Sulfate-Reducing Bacteria

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Sulfate-reducing bacteria (SRB) are microorganisms responsible for severe microbially influenced corrosion (MIC) episodes. Recent findings have shown that SRB can use extracellular electron transfer (EET) to corrode steel in the environment.

In this work, we used mathematical modeling to represent the effect of the EET on pitting corrosion of carbon steel plates and the subsequent changes in surface topography. A mechanistic mathematical model of MIC by SRB through EET was developed and implemented using a hybrid differential-discrete model to accomplish this objective. The model was programmed using Python to facilitate access and future applications. The developed model used available data from experiments in the literature to describe the phenomenon and define stoichiometric and kinetic parameters.

The model properly represents the morphology of an early biofilm of SRB and the change in the surface topography due to corrosion. Simulation results of biofilm development, weight loss, and maximum pit depths were similar to experimental evidence reported in the literature for the same tested conditions. The model reveals that the main parameters that control MIC are the maintenance coefficient of SRB, the initial planktonic cell concentration, and the probability of surface colonization. The results of this work highlight the necessity of including EET mechanisms to estimate the impact of SRB on MIC events.

Reference

Javiera Anguita, Gonzalo Pizarro, Ignacio T. Vargas (2022). *Mathematical modelling of microbial corrosion in carbon steel due to early-biofilm formation of sulfate-reducing bacteria via extracellular electron transfer*. Bioelectrochemistry, Volume 145, 2022, 108058, https://doi.org/10.1016/j.bioelechem.2022.108058.

Poster-B04: Tuning copper catalyst for methanol production like DJ Müller and Maichel sculpt electronic beats

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The SmartManure consortium aims to address the challenges associated with untreated manure on farms. The SmartManure cohort of four PhD-students intends to address manure management from storage to land applications, thereby developing sustainable solutions for on-farm manure utilization.[1] This sub-project has the goal to address the greenhouse gas emissions from the manure, with a waste to energy approach by converting the CO_2 component of the emissions to a high-energy compound methanol.

To curb the rising CO_2 emissions, Carbon Capture and Utilization (CCU) technologies are increasingly being investigated. Electrochemical CO_2 reduction reaction (eCO_2RR) is of significant interest within these technologies as one of the most economical ways to convert streams of waste CO_2 to valuable compounds. Up to now, eCO_2RR has been extensively researched for C_1-compounds such as formate and CO; however, for higher energy compounds such as methanol, the technology still remains to be established.[2]

Methanol formation from eCO_2RR undergoes via a multistep and multielectron pathway, thus a selective and active catalyst is needed to facilitate this reaction. Through eCO_2RR, methanol can be produced either via CO or HCOOH as intermediates, and a catalyst having an intermediate affinity for CO can drive the CO_2 molecule for methanol formation (Figure 1).[3]

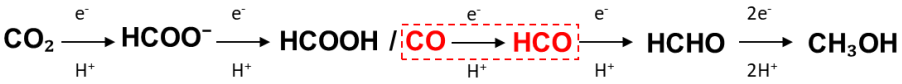

Figure 1: Suggested pathway for methanol formation via eCO_2RR

Copper-based catalysts, including alloys and oxide layers, are discussed to be promising in promoting alcohol formation in eCO_2RR and can be optimized for methanol selectivity. Thus, this study aims to systematically analyse copper as a catalyst in eCO_2RR using electrodeposition for catalyst preparation and evaluating product composition under varying operational parameters. Further, catalyst modifications will be tested by preparing copper-based alloys or stable copper oxide layers to target methanol production. The reactions conditions will be optimized to develop an efficient electrochemical CO_2-to-methanol conversion technology, supporting efforts toward a circular carbon economy.

References

1. https://www.ufz.de/index.php?en=50492
2. Qiao, Jinli, et al. "A review of catalysts for the electroreduction of carbon dioxide to produce low-carbon fuels." *Chemical Society Reviews* 43.2 (2014): 631-675. DOI: https://doi.org/10.1039/C3CS60323G
3. Liu, Yanrong, et al. "Recent progress on electrochemical reduction of CO2 to methanol." *Current Opinion in Green and Sustainable Chemistry* 23 (2020): 10-17. DOI: https://doi.org/10.1016/j.cogsc.2020.03.009

Poster-B05: A Midsummer Night's Dream: Ensembling microbiology and electrochemistry for bio-based fuel production

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A sustainable circular economy can be achieved by linking different economic sectors, for example electric power production and storage with the production of chemicals and fuels [1]. A promising approach to create this link is through electrobiorefineries that combine microbial and electrochemical conversions into one process line [2]. One example is the electrochemical conversion of microbially produced medium-chain carboxylic acids (MCCA) [3] via Kolbe electrolysis to *n*-alkanes that serve as drop-in fuels [4] (Fig. 1).

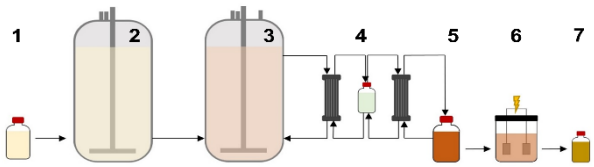

Fig. 1: Simplified process scheme of an electrobiorefinery producing drop-in fuel (7) from renewable resources (1) via one- or multistep microbial conversion (2-3) combined with electrochemical conversion (6). The interface is an extraction process (4-5).

Thereby, it is crucial to investigate different process parameters like electrode material or substrate composition for efficient process management of the Kolbe electrolysis. We showed that the traditionally used electrode material, pure platinum (Pt), can be replaced by platinized titanium, achieving a comparable Coulombic Efficiency (*CE*) at significantly lower costs [5].
For these developments *n*-hexanoic acid (C_6) has served as model, as electrolysis of other MCCAs has limitations due to solubility and agglomeration issues [6]. However, during microbial conversion mixtures of MCCAs are produced. We investigated the suitability of different MCCA and mixtures thereof as substrate for Kolbe electrolysis [7]. Interestingly, when a mixture of MCCAs was used, the *CE* surpassed those obtained with single acids and overcomes their respective limitations and drawbacks.
Furthermore, the transfer from batch experiments to a commercially available stack flow cell for large-scale application was successfully established [8].

References

[1] de Vasconcelos B.R., Lavoie J.M. Recent advances in power-to-X technology for the production of fuels and chemicals. *Front. Chem.*, 2019, 7: 392.
[2] Harnisch F., Urban C. Electrobiorefineries: Unlocking the Synergy of Electrochemical and Microbial Conversions. *Angew. Chem. Int. Ed.*, 2018, 57, 10016.
[3] Spirito C.M., et al. Variability in n-caprylate and n-caproate producing microbiomes in reactors with in-line product extraction. *mSystems*, 2024, 9 (8): e00416-24.
[4] Urban C., et al. Production of drop-in fuels from biomass at high selectivity by combined microbial and electrochemical conversion. *Energy Environ. Sci.*, 2017, 10: 2231-2244.
[5] Neubert K., et al. Platinized Titanium as Alternative Cost-Effective Anode for Efficient Kolbe Electrolysis in Aqueous Electrolyte Solutions. *ChemSusChem*, 2021, 14: 3097-3109.
[6] Urban C., Harnisch F. Deterioration of Aqueous n-Octanoate Electrolysis with Electrolytic Conductivity Collapse Caused by the Formation of n-Octanoic Acid/n-Octanoate Agglomerates. *ChemElectroChem*, 2017, 4: 1378–1389.
[7] Neubert K., et al. Hetero-coupling of bio-based medium chain carboxylic acids by Kolbe electrolysis enables high fuel yield and efficiency. *ChemSusChem*, 2022, 15: e202201426.
[8] Rosa L. F. M., et al. Electrolysis of medium chain carboxylic acids to aviation fuel at technical scale. *Fuel*, 2024, 356: 129590.

Poster-B06: Investigating the Characteristics of Irreversible Reduction Substances Generated by Co-culturing *Chlorella* and *Shewanella* on Carbon-Felt Electrodes and Their Application in Bio-Cathodes

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Introduction:

The extracellular-electron-transfer(EET)-capable bacteria can reduce oxidized metal minerals, and it is confirmed that solid-state-electron-acceptors utilization is growth associated, requiring EET to complete the respiration. Two EET processes are typical: (i) indirect EET involves electron shuttles which can be produced by microorganisms themselves or added experimentally; (ii) direct EET occurring when microorganisms physically contact the electrode, relying on c-type cytochromes or bacterial nanowires. As for photoelectrochemical conversion, microbial mat from hot spring and lake sediments were used in previous reports. In our previous experiments, *Chlorella sorokiniana* SU-1 and *Shewanella decolorationis* NTOU1 were co-cultivated on carbon-felt anodes with potentials of +0.4 V (vs. Ag/AgCl) applied, while under the substrate-free conditions, a reduction current peak at –0.2 V (vs. Ag/AgCl) were observed using cyclic voltammetry (CV) one day after inoculating the algal and bacterial cells. Under co-culture conditions and with acetate supplementation, a reduction current signal of –14.7 mA was observed at –0.2 V on the second day after acetate depletion(Fig. 1).

Experimental:

C. sorokiniana SU-1 and *S. decolorationis* NTOU1 were co-cultured on the carbon felts, with acetate as a substrate. Chronoamperometry, high-performance liquid chromatography (HPLC), and CV were utilized.

Results and Discussion:

When the light sources and aeration were turned off for a period, the –0.2 V (vs. Ag/AgCl) signal weakened but is still detectable. While repeated CV scanning showed the signal been shifted to –0.3 V (vs. Ag/AgCl), and it may vanish after re-adding glucose. Accordingly, the reducing substance is hypothesized to be irreversible, neither oxygen nor a photosensitive compound, and may interact or be suppressed with reducing substrate such as glucose. With both *C. sorokiniana* SU-1 and *S. decolorationis* NTOU1 inoculated on the cathode at 0 h, the current increased from –0.41 mA to –0.89 mA by the 5th h. Acetate was added at 12, 15, and 18 h in sequential concentrations of 2, 1, and 0.5 mM. The identical results showed that when acetate were added, the cathodic current was 30-60% reduced but recovered after substrate depleted (Fig. 2). The CV results shows that the reducing signal presented only after substrate depletion. but immediately disappeared after acetate addition.

Reference

Kouzuma, A., and K. Watanabe. 2015. Exploring the potential of algae/bacteria interactions, *Current Opinion in Biotechnology*, 33: 125-29.

Keywords: *Chlorella sorokiniana* SU-1, *Shewanella decolorationis* NTOU1, extracellular electron transfer mechanism, algal-bacterial symbiosis, dual-chamber photosynthetic bioelectrochemical cell

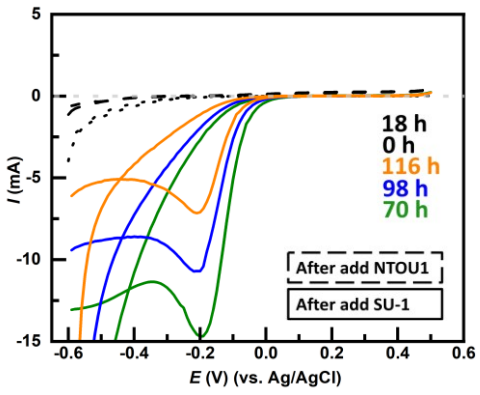

Fig. 1 CV graphs for reducing compound

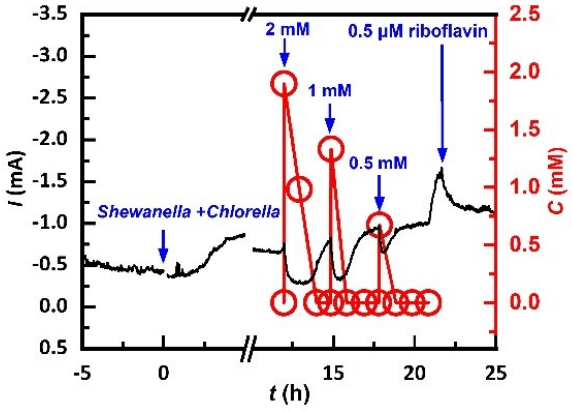

Fig. 2 Acetate addition affecting cathodic current

Poster-B07: Field Deployment of an Embedded System for State of Health monitoring of Wastewater-Fed Microbial Fuel Cells

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Introduction. Microbial fuel cells (MFCs), which rely on living organisms, inherently exhibit performance fluctuations over time. As dynamic systems, they are susceptible to variations in key operating parameters such as pH, conductivity, carbon source composition, toxicants, and temperature. Hence, it is crucial to assess their state of health (SOH) by continuously monitoring essential energetic and electrochemical parameters, including ohmic resistance, activation resistance, capacity, open-circuit voltage, and cumulated electric charge. Tracking the evolution of these parameters plays a vital role in enabling real-time diagnostics, troubleshooting, and ensuring reliability as well as overall system performance.
This study describes and publishes, for the first time, the employment of a low-cost embedded system for online monitoring of energetic and electrochemical parameters in a ceramic MFC under real conditions.

Material and methods. Experiments were carried out on a tubular ceramic MFC installed in the effluent of the primary clarifier, which also served as the sole inoculation source, at the wastewater treatment plant, WWTP, Ölbachtal (Ruhrverband, GER). The MFC was connected to an embedded system (**Figure 1**) comprising both hardware and software at market-ready stage (TRL 8), designed to monitor energetic and electrochemical parameters. Electrochemical parameters were measured every 10 minutes using a modified parameter estimation routine (Littfinski et al., 2021). To date, SOH monitoring has been successfully conducted for a period of >4 months without any need for adjustment or service.

Results. Following inoculation (~10 days), SOH monitoring of energetic and electrochemical parameters reveals substantial fluctuations due to changes in wastewater composition. **Figure 1** illustrates these variations representative of cell voltage and ohmic resistance, notably influenced by rainfall events. For instance, dilution effects reduce electrical conductivity (EC), while the ohmic resistance increases from 873 Ω to 962 Ω within 100 minutes. Since ohmic resistance and EC are inversely related ($R_{ohmic} = 1/EC \cdot K_{MFC}$), such trends are expected. The near-future outlook of this pioneering technology will enable SOH monitoring for fault detection, including membrane or electrode deterioration and progressive MFC aging.

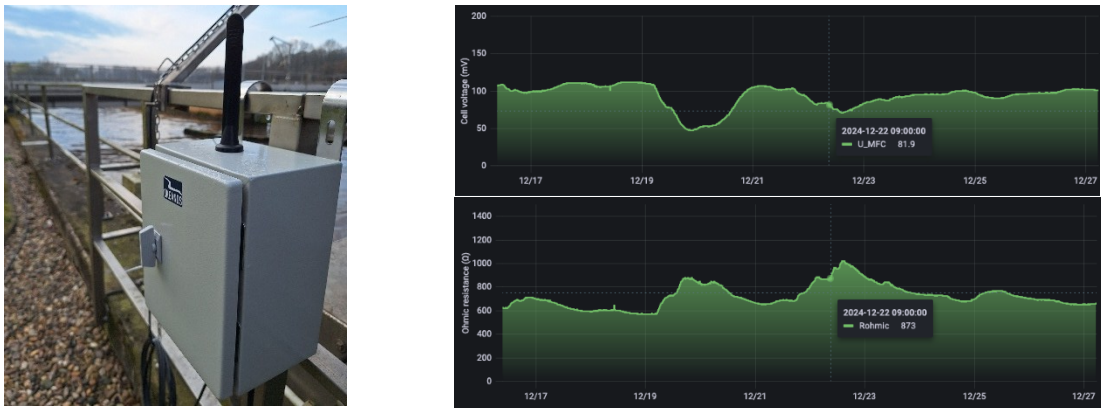


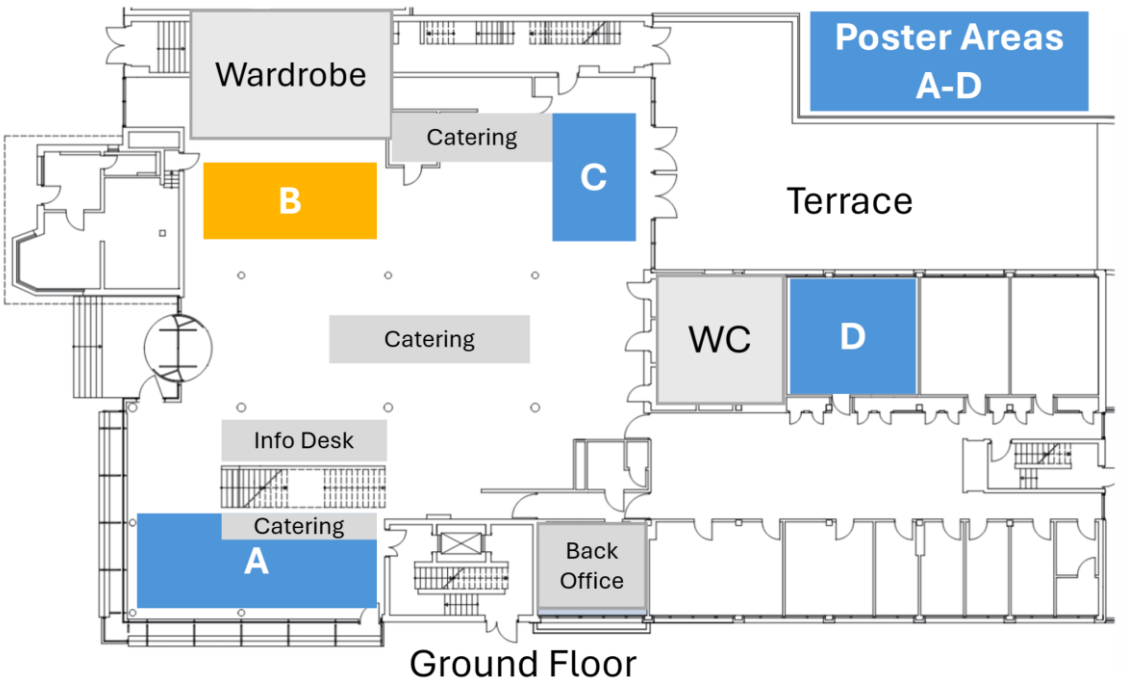
Figure 1: Picture of the installed embedded system (left) on the WWTP Ölbachtal (Ruhrverband, GER) and a representative screenshot of web interface (right).

References

T. Littfinski, E. Nettmann, T. Gehring, S. Krimmler, J. Heinrichmeier, E. Murnleitner, M. Lübken, D.Pant, M. Wichern, 2021. A comparative study of different electrochemical methods to determine cell internal parameters of microbial fuel cells. Journal of Power Sources 494, 229707.

POSTER PRESENTATIONS

Microbial Electrolysis Cells
- Area B -



Poster-B08: Analysis of a bioelectrochemical system for sustainable hydrogen production and wastewater treatment

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Bioelectrochemical systems (BES) combine electrochemistry with the metabolism of electroactive microorganisms for energy production. In Microbial Electrolysis Cells (MECs) electroactive bacteria grow building a biofilm on the surface of a conductive anode, which serves as electron acceptor. The electroactive microorganisms oxidize organic matter to CO_2 under anaerobic conditions and the electrons obtained in the process are transferred from the anode to the cathode through an electrical circuit **[1]**. The cathodic reaction is the H_2 formation through H_2O reduction under alkaline conditions, along with the application of an external potential. In the present work a saline compartment is added in the MEC reactor and filled with $NaHCO_3$ to avoid acidification of the biofilm (limitation) in the anodic chamber, as bicarbonate migrates from saline compartment due to the electric field in the system.

This work, which is part of the Regenera Project (MISIONES 2019, CDTI), presents the BES-BioH_2 system, a laboratory scaled reactor based on a MEC system **[2]** with the purpose of obtainingi biohydrogen with lower energy requirements than conventional electrolysis, using the energy produced by electroactive microorganisms and employing wastewater as organic matter source.

The electrochemical operation of the BES-BioH_2 reactor is analysed to obtain the potential diagram of the system and its energy analysis is performed. Additionally, the microbial population is assessed and correlated to the microbial electrochemical behaviour of the system. The results obtained allow us to determine the optimal operational conditions that would allow us to obtain H_2 and remove COD from wastewater with lower energy requirements than conventional processes, what is achieved operating the system at current densities below 0.4 mA/cm^2 with synthetic wastewater.

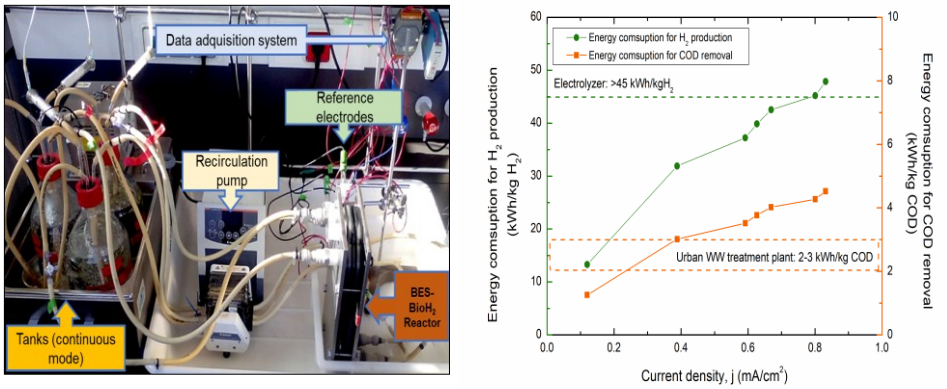

Figure 1. Left: Experimental setup and experimental conditions for BES-BioH_2 system. Right: Potential diagram for the BES-BioH_2 system using synthetic wastewater.

Bibliography:

[1] Luo, H.; Jenkins, P.E.; Ren, Z. Concurrent desalination and hydrogen generation using microbial electrolysis and desalination cells. Environ. Sci. Technol. 2011, 45, 340-344.

[2] Ramírez-Moreno, M.; Esteve-Núñez, A.; Ortiz J.M. Desalination of brackish water using a microbial desalination cell: Analysis of the electrochemical behaviour. Electrochim. Acta 2021, 388, 138570.

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Poster-B09: Detecting and controlling biofilm thickness in microbial electrolysis cells by in-situ biofilm monitoring

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The anodic biofilm morphology plays a key role in the current production and efficiency of bioelectrochemical systems (BES). Electroactive biofilms can be considered as counter-diffusional, with the electron donor (e.g., substrate) being supplied from the bulk liquid while the substratum acts also as electron acceptor (e.g., anode). Limitations of the diffusional penetration depth of the substrate, along with limitations in the electron transfer distance of electroactive bacteria suggest the existence of an optimal biofilm thickness in the trade-off between high biomass accumulation and the avoidance of transport limitations. Controlling the biofilm thickness to its optimum might be key for a long-term and stable operation of BES in wastewater treatment.

In this study, the development of the electroactive biofilm morphology was monitored daily by means of optical coherence tomography (OCT) in single-chamber microbial electrolysis cells (MEC) constructed as mesofluidic flow cells (n=5) with carbon-polypropylene anodes (A_{an} = 20 cm²) and stainless-steel cathodes. The electroactive biofilm was cultivated in a recirculatory system from a preconditioned mixed wastewater culture for up to 80 days under anaerobic conditions at a constant anodic potential of 0 mV vs. SHE. The cultivation medium was continuously replenished (~ 500 mg COD/d) to avoid limiting growth conditions.

A maximum current density of approximately 3.5 A/m² was reproducibly found at a mean anodic biofilm thickness in the range of 100-150 µm in all five MECs. Beyond the maximum current density, a deteriorating performance of the MECs was associated with the growth of a secondary biofilm layer, dominated by supposedly non-electroactive microorganisms, which became visible in volumetric OCT scans. This layer led to increased diffusion pathways of the substrate, along with an increased substrate consumption and thereby reduced the substrate availability to the electroactive biofilm in the proximity of the anode. The resulting substrate limiting conditions for the electroactive biofilm decreased the current densities of the MECs by up to 70 %. To recover the electroactive performance of the MEC biofilm control mechanisms (e.g., shear force increase, N_2-scouring) were applied to remove the secondary biofilm layer. Hereby, especially the scouring with N_2 bubbles through the flow channel showed significant sloughing of the biofilm on the anodes. The sloughing process predominantly effected the secondary (non-electroactive) biofilm layer at the top of the biofilm and showed a more efficient biofilm removal of fluffy biofilm structures. After N_2-scouring events, a rapid increase of the produced current density in the MEC was noted up to 77 % (2.7 A/cm²) of the maximum current densities.

Poster-B10: Sustainable Hydrogen Production Valorizing Organic Waste through Microbial Electrolysis Cells: A Comparative Study of Substrate Impact

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Introduction

Hydrogen is widely regarded as a clean energy carrier, yet current production is predominantly fossil-fuel based and energy-intensive, resulting in significant CO_2 emissions. An innovative solution is provided by Microbial Electrolysis Cells (MECs), which employ electroactive microorganisms to oxidize organic substrates, thereby reducing the required potential to +0.187 V in comparison with +1.23 V of alkaline water electrolysis requirement for acetate oxidation. In addition to their energetic advantages, MECs can utilize waste substrates derived from the acidogenic fermentation of the solid fraction of municipal waste. This capability not only enhances the sustainability of hydrogen production but also offers an integrated approach to waste treatment, effectively addressing the management of these complex effluents. A novel MEC configuration featuring a central anode chamber flanked by dual cathode chambers further optimizes current conversion efficiency and stabilizes electrochemical performance. In this work, by using different fermented-real substrates, the technology's potential in both renewable energy production and environmental remediation was emphasized.

Material and methods

The laboratory-scale MEC consisted of three identical Plexiglas chambers (0.86 L each) with internal dimensions of 17 cm × 17 cm × 3 cm. The central bioanode was flanked by two cathode chambers, each separated by a CMI International exchange membrane (Membrane International, USA). The cathode chambers were equipped with two 316 stainless steel sheets (RS Components), each having a surface area of 176.46 cm², while granular graphite (<4 mm) served as filler in the bioanode. The anode chamber operated under continuous flow, working with different organic loading rates (OLRs) with either a synthetic feed or a real-fermented solution from the acidogenic fermentation of organic waste, whereas the cathode chambers operated in batch mode. Throughout the experiments, an Ag/AgCl reference electrode (saturated KCl, E°' = 199 mV vs. SHE) was placed in each chamber for potential control. Graphite granules were connected to the electrical circuit via graphite rods and titanium wires, and a potentiostat applied a potential of +0.2 V vs. SHE (selected based on previous studies [1]), configuring the anode as the working electrode.

Results and Discussion

After an initial biomass acclimatization period, the MEC was operated for a total of 205 days. Daily COD measurements permitted to calculate the anodic coulombic efficiency (CE), while gas-phase analysis confirmed nearly 100% hydrogen production for all cases investigated. Following this result, the cathodic capture efficiency (CCE) resulted in around 100% for all substrates fed to the reactor. However, the system produced an average current of approximately 95, 51, and 227 mA, at OLR equal to 4 gCOD/Ld, for synthetic and real-fermented substrates (types A and B), respectively. The overall energy efficiency resulted in 110 ± 11%, 55 ± 4% and 39 ±34%, respectively. This behaviour was attributed to the lower substrate conductivity of the real-fermented solutions (2.6 mS/cm vs. 6.1 mS/cm for synthetic one) and a higher voltage drop (ΔV_{synth} = –1.35 V; ΔV_{FermA} = –2.5 V; ΔV_{FermB} = –4.0 V). In addition, potentiodynamic (cyclic voltammetry) and potentiostatic techniques were employed to characterize biofilm activity, offering a non-destructive and precise method to monitor MEC operation. These results demonstrate that the new configuration is more energy-efficient than conventional single-chamber setups, pointing out the potential for high performance using waste-derived substrates, supporting their sustainable valorization.

References

1. Zeppilli, M.; Villano, M.; Majone, M. Microbial Electrolysis Cell to Enhance Energy Recovery from Wastewater Treatment. *Chem Eng Trans* **2016**, *43*, 2341–2346, doi:10.3303/CET1543391.

Poster-B11: Targeted Application of Microbial Fuel Cells for Enhanced Disinfection and Recalcitrant Pollutant Removal in Wastewater Treatment

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Microbial fuel cells (MFCs) have attracted considerable interest as a sustainable technology capable of simultaneously generating bioelectricity and treating wastewater. Their anaerobic operation offers the advantage of reducing aeration costs typically associated with conventional treatment, thereby improving overall energy efficiency. However, key barriers remain in achieving stable effluent quality and in enhancing power output to levels sufficient for practical application. This study explores a targeted utilisation of low-power MFCs, focusing on their potential to support advanced treatment functions such as the degradation of recalcitrant pollutants and the enhancement of disinfection performance in the stream of wastewater treatment process. We applied the electricity generated from MFCs to drive UV-based disinfection and further integrated it with in situ hydrogen peroxide generation to develop an advanced oxidation process. In addition, an electro-chlorination system was introduced as an alternative to UV, enabling a more sustainable approach that replaces conventional chlorine-based disinfection. By coupling the inherent energy production of MFCs with these downstream processes, we propose a strategy that may contribute to the broader applicability and commercial viability of MES technologies in real-world wastewater treatment systems.

Poster-B12: Anodic Biofilm Enrichment for the Oxidation of Slow-to-Degrade Carboxylic Acids: Towards an Integrated DF-MEC System

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Dark fermentation (DF) is a biotechnological process according to which organic waste substrates are degraded by microorganisms in absence of oxygen to produce bio-H_2 as gaseous effluent. However, fermentative H_2 production provides only a partial oxidation of the organic substrate, due to thermodynamical limitations. Indeed, 2/3 of the carbon and the H_2 of the substrate are converted to microbial metabolic byproducts which, unutilized, represent a disposal burden and a waste of energy[1]. A possible approach to maximize H_2 recovery from organic waste, is the integration of DF with Microbial Electrolysis Cells (MEC), wherein electroactive microorganisms (EAMs) at the MEC anode are able to oxide the organic compounds present in the DF effluent producing electric current that allows further H_2 generation at the MEC cathode[1].
According to evidence from literature, the predominant components of DF effluents are acetic, propionic, butyric, and lactic acids along with alcohols (e.g., ethanol). Propionic and butyric acids are among the most slow-to-degrade carboxylic acids[2] and low current densities are observed when they are used as feeding for the anode biofilm.
In the present study, a substrate acclimatation strategy was performed with the aim to select electroactive microbial consortia able to oxidize specific slow-to-degrade substrates (*i.e.*, propionic or butyric acid). The objective was to obtain an anode microbial community more resilient utilizing real organic feedstock (i.e., DF effluent) as substrate, allowing the optimization of MEC performance (higher COD removal efficiency, higher current density generation and, finally, higher H_2 recovery).
Experiments were conducted in H-type cells consisting of gas-tight borosilicate glass bottles (each having a working volume of 200 mL) separated by a ®PFSA D170-U Proton Exchange Membrane. All experimental conditions were tested in triplicate for each of the substrates under investigation. Throughout the experiments, conducted at room temperature, the bioelectrochemical cells were maintained under continuous stirring. At the beginning of the experimentation, the anode chamber of each cell was inoculated with aerobic sludge (1.9 g_{VSS}/L) from a municipal wastewater treatment plant (WWTP). The bioelectrochemical set-up consisted of a three-electrode configuration with the anode potential set at +0.20 V *vs.* SHE. The anode consisted of a carbon felt electrode (3cm x 6cm) and the cathode of a stainless-steel mesh electrode (3cm x 6cm). Propionic or butyric acid, both at 0.5g_{COD}/L, were used as sole substrates. Different acclimation cycles were conducted and current generation, COD removal efficiency, and Coulombic efficiency were used as monitoring parameters. With reference to the first acclimation cycle, propionic acid - fed cells were the most performing reaching ~70% of COD removal efficiency and ~35% of Coulombic Efficiency. Butyric acid was the most recalcitrant, but a second acclimation cycle is ongoing to confirm this hypothesis.
Moreover, to explore higher microbial diversity, as further investigation, different inocula, such as sediments and digestate, will be tested, posing particular attention to the evaluation of the synergy between the planktonic community and the anode electroactive biofilm.

1. Marone et al., Int. J. Hydrogen Energy, 2017, doi: http://dx.doi.org/10.1016/j.ijhydene.2016.09.166.
2. Flayac, et al., In: Das, D. (eds) Microbial Fuel Cell. Springer, 2018, doi:10.1007/978-3-319-66793-5_4.

This research was funded by the European Union – NextGeneration EU from the Italian Ministry of Environment and Energy Security

Poster-B13: Integration of microbial electrochemical system and electrodialysis for production of high-purity hydrogen peroxide

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Hydrogen peroxide (H_2O_2) is one of the most powerful and versatile oxidizing agents. It is widely used as an essential chemical in various industrial and environmental fields, including wastewater treatment, pulp and paper manufacturing, textile bleaching, and medical disinfection. Among the commercial production methods, the anthraquinone process is the most commonly used, offering high yield and industrial stability. However, it requires complex reaction steps and large-scale, high-cost equipment. In addition, the extensive use of organic solvents poses significant environmental risks, raising concerns about the long-term sustainability of the process. Accordingly, alternative production methods that are both environmentally sustainable and cost-effective are increasingly required. Microbial Fuel Cell (MFC) is a sustainable bioelectrochemical system in which electrons generated during the microbial oxidation of organic matter flow through an external circuit to produce electricity. At the cathode, depending on the conditions, oxidation reactions can lead to the formation of either water or hydrogen peroxide. In this study, hydrogen peroxide was produced by inducing a two-electron oxygen reduction reaction (ORR) in a MFC that generates electricity while utilizing organic matter in wastewater. To enhance the purity of the hydrogen peroxide produced, electrodialysis technology was integrated into the system and experimentally evaluated.

Poster-B14: Harnessing the S^0/H_2S Redox Cycle for Low-Potential Microbial Electrolysis Cell with *Desulfurella amilsii*

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Hydrogen, a decarbonized energy carrier with exceptional energy efficiency, is considered a critical replacement for fossil fuels in residential and industrial sectors. Microbial electrolysis cells (MECs) have been identified as a potentially viable solution to this problem (see Kadier *et al.*, 2020), with the capacity to convert organic waste into hydrogen. Exoelectrogenic microorganisms oxidize organic matter and transfer electrons to unconventional acceptors such as Fe(III) oxides or an anode, enhancing cathodic hydrogen production in MECs. The performance of MECs can be further enhanced by the utilization of polyextremophilic strains (Kadier *et al.*, 2020; Quehenberger *et al.*, 2017). Typically, exoelectrogenic and acetoclastic microorganisms are enriched by poising the anode potential to approximately +0.4 V_{SHE} (Kadier *et al.*, 2020). However, H_2S undergoes abiotic oxidation to S^0 at lower applied potentials (Qi *et al.*, 2011). Consequently, the utilization of S^0-reducing bacteria could facilitate current generation through an indirect H_2S/S^0 redox cycle. *Desulfurella amilsii* $TR1^T$, isolated from the metal-rich Río Tinto River (Florentino *et al.*, 2016), is acid-, heat- and salt-tolerant and encodes a Type IV pilus associated with exoelectrogeny in *Geobacter spp.* (Ye *et al.*, 2022). This study explores its exoelectrogenic potential in MECs and the feasibility of a low-potential MEC sustained by an active S^0/H_2S cycle.

D. amilsii was cultured in saline media with acetate as the electron donor and S^0 as the electron acceptor. MECs employed graphite anodes poised at +0.1 or +0.5 V_{SHE} with or without S^0 supplementation. Cell growth, current generation, and gas evolution (N_2, O_2, H_2, CO_2, H_2S) were monitored during experiments.

Under sulfur-reducing conditions (+0.1 V_{SHE}), *D. amilsii* degraded 3.83 mM acetate at a maximum rate of 35.26 $\mu M \cdot h^{-1}$, generating an anodic current density of 68.32 $mA \cdot m^{-2}$. However, the low coulombic efficiency (7.39%) highlights significant electron flux partitioning toward S^0 reduction rather than anodic electron transfer, consistent with known metabolic priorities of sulfur-reducing bacteria. The observed biofilm thickness of 30–50 µm (CLSM data) is comparable to the thickness of *Geobacter sulfurreducens* biofilms at higher potentials (+0.2–0.4 V_{SHE}) (Ye *et al.*, 2022).

For anode-only respiration (-S^0), biomass precultured with S^0 was transferred into S^0-free medium at +0.5 V_{SHE}. Four successive 1/10 dilutions were performed to minimize residual S^0 from the inoculum. A 10,000-fold dilution of residual S^0 increased coulombic efficiency to 35.84%, confirming *D. amilsii*'s capacity for direct electron transfer and generating an anodic current of 120.90 $mA.m^{-2}$. However, the thinner biofilm (~15 µm) with isolated "mushroom" structures (~40 µm) indicated suboptimal electrode colonization compared to S^0-rich conditions. The reduced acetate degradation rate (132.83 $\mu M.h^{-1}$ *vs.* 14.54 $\mu M.h^{-1}$) suggests metabolic stress under S^0 limitation. The observed 3.4-fold excess H_2S production (0.53 mM *vs.* 0.16 mM expected) in S^0-limited media suggests: (i) Electrochemical deposition of S^0 from HS^- oxidation at the anode ($HS^- \rightarrow S^0 + H^+ + 2e^-$); or (ii) Functional cycling of S^0/H_2S via *D. amilsii*'s sulfur reducing enzymes.

This study demonstrates the exoelectrogenic capacity of *D. amilsii* and the feasibility of a low potential (+0.1 V_{SHE}) polyextremophilic acetic MEC supported by an active S^0/ H_2S cycle. Future research should focus on the evaluation of hydrogen production in the cathode chamber under oxygen-limited conditions.

Poster-B15: Cellulose-to-Energy via Bioelectrochemical Conversion in Microbial Electrolysis Cells (MECs)

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In the pursuit of sustainable and circular energy systems, microbial electrolysis cells (MECs) have emerged as a promising bioelectrochemical technology to convert organic waste into valuable energy carriers, such as hydrogen. Among various waste streams, disposable paper towels constitute a significant fraction, primarily composed of pure cellulose, a polysaccharide rich in chemically stored energy. Rather than being discarded or incinerated, this cellulose-rich material presents an opportunity for renewable energy recovery. Through microbial electrolysis, specialized, enriched microbial communities or isolated cellulose degrading bacteria can catalyze the biodegradation of cellulose, coupling it to electrochemical hydrogen production with *Geobacter sulfurreducens*, a well-studied model organism. This approach not only contributes to effective waste valorization, but also aligns with efforts to produce clean hydrogen from sustainable feedstocks, ultimately advancing both waste management and green energy generation.
To explore this potential, environmental samples were collected from diverse environmental sources and enriched towards their cellulose degradation capabilities. Enrichment was conducted at 30-35°C under static, anaerobic conditions using minimal salt media supplemented with cellulose as the sole carbon source. After multiple enrichment steps the mixed cultures were able to completely degrade 1 g L^{-1} of cellulose tissue within 7 days. However, the enriched mixed cultures did not show electrochemical potential in MECs. Co-cultivations of these enriched cultures with *G. sulfurreducens* (DSM 12127) at 35 °C, 350 rpm in MECs lead to efficient cellulose degradation of 1 g L^{-1} as well as current densities of up to 0.7 mA cm^{-2}. To gain further knowledge of the underlying processes of microbial cellulose degradation in MECs, we are aiming for defined co-cultivations of isolated cellulolytic bacteria and *G. sulfurreducens*. Therefore, cellulolytic strains were isolated from different enriched cultures with established protocols and characterized for their cellulolytic activity as well as for the metabolites released during the degradation process. Suitable candidates were identified using MALDI-TOF and will further on be used in co-cultivations with *G. sulfurreducens* in MECs to check for cellulose degradation and current as well as hydrogen production.

The authors gratefully acknowledge financial support from Volkswagen Stiftung.

Poster-B16: Real-Time Control of Microbial Electrolysis Cells for Energy-Efficient Wastewater Treatment

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In response to growing environmental concerns and the global shift toward sustainable, resource recovery frameworks, Microbial Electrolysis Cells (MECs) have emerged as a promising next-generation wastewater treatment technology. These bioelectrical systems harness microbial metabolism to simultaneously degrade organic pollutants and produce useful by-products such as hydrogen—all while operating at significantly lower energy costs compared to conventional methods.

A key performance indicator in MECs is the electrical current generated through microbial activity, which provides real-time insight into system efficiency, pollutant levels, and microbial health. However, most current MEC implementations rely on static voltage inputs, lacking the ability to dynamically respond to real-time fluctuations in current, which can limit overall performance.

To address this challenge, we present a novel Real-time Management Unit (RMU) capable of simultaneously monitoring and controlling the applied voltage and current across up to 20 individual MECs using a single microcontroller unit (MCU). The RMU integrates a PID-based control strategy to maintain optimal voltage levels for each MEC channel and employs LoRaWAN for low-power, long-range wireless communication. Initial testing on pilot-scale setups demonstrated the RMU's ability to perform dynamic, remote voltage control while continuously capturing current data in real time. This system represents a significant step towards establishing the framework for more scalable, intelligent control for energy-efficient wastewater treatment.

Poster-B17: HUMANFORHYDRO project: Integrating waste derived Ligno-Humic-Like (LHLs) compounds in bioanodes for bioelectrochemical hydrogen generation

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Introduction

The management of wastewater treatment and the products generated in the process has become an actual problem worldwide. Recently, sewage sludge attracted huge attention due to its potential as a source of conventional biofuel and new materials. The most common strategy for sludge valorization requires a preliminary separation of the different components (i.e., lipid fraction from fibre components), with specific upgrading treatment in separate steps. With the lipid valorization to obtain fatty acid alkyl esters, a concomitant enhancement of cellulose, sugars and proteins was obtained with the production of valuable chemicals or biogas. However, in order to achieve a full valorization of sewage sludge, it is necessary to study a component not yet properly exploited, consisting of ligno-humic-like (LHL) compounds. Despite they constitute about 4-20% of the total solids, up to now, the potentialities of LHL compounds have not been extensively investigated. The HUMANFORHYDRO project will propose and demonstrate innovative methods for the valorization of LHLs compounds, lowering the environmental impact deriving from sludge disposal and in line with the principles of Circular Economy. In detail, two novel and very promising approach will be presented for biohydrogen production from renewable resources: the direct use of LHL compounds as "booster" of the electrocatalytic activity of electroactive microorganisms in bioelectrochemical processes and the design and application of novel supported Ti, Fe catalysts to be applied into photocatalytic processes. In the frame of HUMANFORHYDRO project, the direct utilization of ligno-humic-like (LHL) compounds to boost the electrocatalytic activity of a MEC will be assessed in a bioanode coupled to an abiotic cathode for hydrogen production. The LHLs compounds deriving from the treatment of sewage sludge will be used in combination with graphite-based electrode to evaluate their potential use as sustainable “booster” of the bioelectrochemical oxidation of organic substrates which partially sustain the hydrogen production in the cathodic chamber of the MEC. The specific effects of the LHLs compounds addition to the bioelectrochemical interphases, i.e. the MEC’s anode, will be determined by the adoption of cyclic voltammetry (CV) and Electrochemical impedance spectroscopy (EIS). The use of LHLs compounds as “boosters” for the electrocatalytic activity of bioelectrochemical systems will offer an alternative strategy to the common use of this fraction in soil fertilizer. The use of LHLs compounds will offer new strategies for bioelectrochemical technologies. When evaluating the performance of novel catalytic materials incorporating humic acids against traditional catalysts, it is essential to assess the key operational parameters of the MEC. For instance, the anodic and cathodic coulombic efficiencies can provide insight into the electron transfer dynamics and the interfacial reactions occurring at the electrodes. Additionally, the overall energy efficiency of the system provides a critical indicator of the process's viability and sustainability. Preliminary assessments suggest that humic-acid-based catalysts may enhance electron transfer kinetics, yielding improved coulombic efficiencies compared to conventional catalysts. Comparative analysis could offer a robust framework to quantitatively evaluate the advantages and limitations of incorporating waste-derived catalytic materials in sustainable hydrogen production processes.

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Poster-B18: Electrochemical characterization and biofilm morphology of *Desulfuromonas acetexigens* in microbial electrolysis cells

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In light of finite fossil resources and increasing global energy demand, microbial electrolysis cells (MECs) are being explored as sustainable technologies for hydrogen production. The electroactive Deltaproteobacterium *Desulfuromonas acetexigens* has gained attention as a potential biocatalyst due to its unique metabolism: it cannot utilize hydrogen as an electron donor, which prevents internal hydrogen recycling and favours high Coulombic efficiencies. This aim of this study was to apply *D. acetexigens* in lab-scale MECs and compare its electrochemical behavior and biofilm morphology with the model organism *Geobacter sulfurreducens*.

Both organisms were cultivated under identical hydrodynamic conditions in flow-cell MECs with a planar graphite working electrode of 20 cm^2. The influence of key parameters, such as flow velocity and inoculation density, on biofilm formation and current generation were investigated. Biofilm morphology was assessed non-invasively via optical coherence tomography (OCT), providing insight into quantitative parameters, including spatially resolved thickness, volume and anode surface coverage. The biofilms of *D. acetexigens* developed faster, reaching significant current generation after approximately 4 days, while *G. sulfurreducens* showed a lag phase of 8 days. Despite similar maximum current densities ($\bar{J}_{max,\ Des2B}$ = 425 µA cm^{-2}; $\bar{J}_{max,\ Geo1A}$ = 412 µA cm^{-2}), electron transfer limitations appeared at lower average biofilm volumes of *D. acetexigens* ($\overline{BV}_{\bar{J}_{max}}$ ≈ 10 – 25 $\mu m^3\ \mu m^{-2}$) compared to *Geobacter* ($\overline{BV}_{\bar{J}_{max}}$ ≈ 40 $\mu m^3\ \mu m^{-2}$).

Phylogenetic analyses were used to taxonomically classify the microbial consortia present in individual cultures. The results indicate that, despite the strong abundance of homoacetogenic and clostridial contaminants, the current density can be exclusively attributed to the extracellular electron transfer of the introduced electroactive bacteria, indicating a niche dominance of these target organisms. The production of short-chain fatty acids, determined in some cultivations could be explained by the metabolic interaction of contaminants and target organisms. Based on these results, an electrode-mediated syntrophic ethanol degradation by *D. acetexigens* and the homoacetogenic, ethanol-utilizing *Sporomusa sphaeroides* is postulated in this work.

Notably, a Coulombic efficiency of 0.96 ± 0.03 was achieved by *D. acetexigens*, confirming that hydrogen was not oxidized. These findings suggest that *D. acetexigens* combines favorable electrochemical characteristics with robust biofilm formation, making it a promising candidate for application in microbial electrolysis cells and bioelectrochemical systems (BES) for circular and sustainable energy generation.

Poster-B19: From lab to field: Assessing the performance and economic challenges of a 1 m^3 microbial electrolysis cell in a wastewater treatment plant

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Scaling up microbial electrolysis cells (MECs) from laboratory to real-world applications involves significant technical challenges that limit their commercial viability. This study presents the design, construction, and long-term assessment of a 1 m^3 MEC operating at a municipal wastewater treatment plant in El Prat de Llobregat (Barcelona, Spain). The primary objective was to evaluate the performance and stability of MEC technology under real conditions.

Experiments conducted with synthetic wastewater demonstrated that the MEC could produce hydrogen efficiently across a wide range of applied potentials (ΔV from 0.4 to 1.4 V). Hydrogen production rates reached a maximum of 8.59 L m^{-2} d^{-1} at ΔV = 1.4 V, significantly higher than previously reported values in pilot-scale double-chamber MECs. Importantly, efficient hydrogen generation at low voltages (≥0.4 V) confirmed the potential for energy-efficient MEC operation at a large scale. Subsequent trials with real urban wastewater highlighted MEC robustness, achieving continuous hydrogen production (average of 3.45 L m^{-2} d^{-1}) despite substantial fluctuations in wastewater composition and operational temperature. Operational stability was maintained for over 250 days, during which the MEC achieved an average chemical oxygen demand (COD) removal efficiency of 34 ± 5% and up to 51 ± 4% at hydraulic retention times (HRTs) of 1 and 2 days, respectively.

The scalability assessment confirmed that increasing the reactor volume to 1 m³ did not significantly compromise MEC performance compared to previous pilot-scale trials (>100 L). A detailed preliminary techno-economic analysis was conducted, incorporating hydrogen revenue, electricity consumption, and capital expenses. The analysis indicated that current MEC performance with UWW could achieve electricity cost reductions up to 2.5-fold compared to conventional activated sludge systems. Nevertheless, profitability remains highly dependent on external market conditions for electricity and hydrogen prices. Results emphasized the critical importance of minimizing voltage losses and hydrogen leakages to enhance economic viability. Furthermore, material costs emerged as the primary economic barrier, representing 99% of total expenses and overshadowing the benefits derived from hydrogen recovery and reduced electricity consumption. In conclusion, the successful scale-up and stable long-term operation of the MEC demonstrated substantial progress towards large-scale implementation of MEC technology. Addressing the identified economic and technical bottlenecks, particularly through cost-effective materials and operational optimizations, will be essential to realize the full potential of MECs.

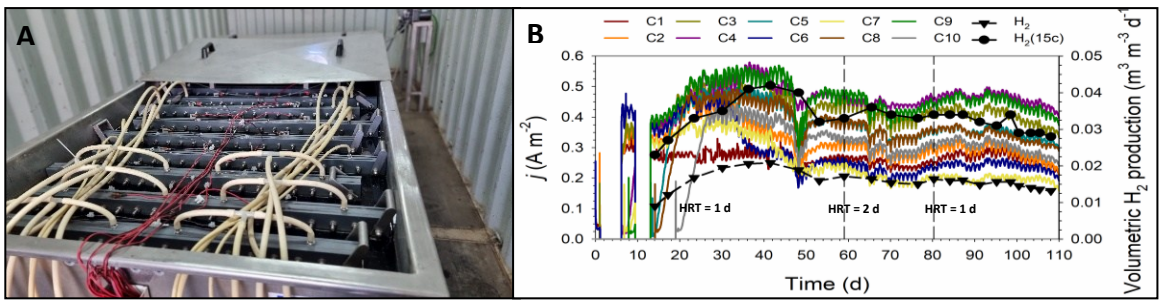

Figure 1: A) Overview of the 1 m^3 MEC pilot
plant, and B) Current density (j) and volumetric hydrogen production rate (H_2) during the continuous operation of the pilot plant (10 cassettes) with real wastewater. $H_{2(15c)}$ refers to the estimated hydrogen production rate considering the maximum capacity of the MEC (15 cassettes yielding peak hydrogen production). Dashed lines represent changes in the HRT.

Poster-B20: Integrated Microbial Desalination and Microbial Electrolysis Cell for Improved Desalination and Wastewater Treatment

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Abstract

The conventional approach of desalination, which relies heavily on energy and high pressure, underscores the compelling benefits of bioelectrochemical systems. This shift in focus is spurring exciting research into sustainable technologies like microbial desalination cells (MDCs). The MDC stands out as a revolutionary, self-sustaining approach that effectively addresses both wastewater treatment and desalination needs. By combining the MDC with a microbial electrolysis cell (MEC), we can significantly enhance desalination efficiency while improving chemical oxygen demand (COD) removal. Moreover, this integration opens avenues for resource recovery, including hydrogen (H_2) gas production, positioning this hybrid bioelectrochemical system as a highly advantageous solution for a wide range of applications.

The study involves two set-ups at laboratory scale, one as control MDC and the other as the integration of MDC and MEC using three glass jars, each with a volume of 30 mL, designated as the anode, desalination, and cathode chambers (Fig. 1 (a, b)). The anode and desalination compartments were separated by an anion exchange membrane (Ralex AMHPES), while a cation exchange membrane (Ralex CMHPES) divided the desalination and cathode sections. Prior to use, the membranes were pretreated by soaking them in a diluted hydrochloric acid solution (0.1 M HCl) and a diluted basic solution (0.1 M NaOH) for 24 hours to facilitate activation. Carbon felt, with a diameter of 4 cm, was employed as both the anode and cathode materials. The cathode was coated with a powdered alloy catalyst ($LaNi_5$) (Aldrich) using polymethyl siloxane as the binder. The anode chamber was inoculated with 10% volume of secondary sludge following heat shock treatment and was supplied with a sucrose feed (3000 mg/L of COD) to simulate synthetic wastewater in control and integrated systems. The desalination chamber was initially set with a NaCl concentration of 35 g/L, similar to seawater conditions, while the catholyte consisted of a buffer solution made up of KH_2PO_4 (3 g/L) and Na_2HPO_4 (6 g/L) in the MDC-MEC, while aeration was provided in the control MDC. The experiments were conducted after acclimatization of 12-15 days for an applied cell voltage of 0.4 V and compared with that of control MDC.

The integrated MDC-MEC system resulted in a significant improvement in COD removal, achieving 85 ± 0.2% compared to 77 ± 0.1% for the control MDC. Additionally, desalination efficiency was higher in the MDC-MEC, recorded at 91 ± 3.2% versus 85 ± 0.4% for the control MDC. Moreover, the average daily salt removal rate for the MDC-MEC was 10.61 ± 0.37 g/L.d, alongside a desalination rate of 132.6 mg/h, both surpassing the control MDC's rates of 9.91 ± 4.67 g/L.d and 123.8 mg/h, respectively. Thus, the Integration of MDC-MEC shows improved desalination and COD removal efficiencies, highlighting its potential for further future works such as H_2 recovery, target contaminant removal, etc.

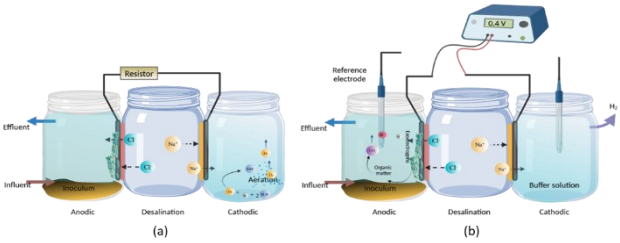

Fig 1: Lab-scale (a) control MDC and (b) MEC-MDC integration

Poster-B21: Potential-Assisted Hydrogen Production by *Clostridium pasteurianum* DSM525: The Impact of Iron Nanoparticle

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Iron is a key element for H_2 production by the genus *Clostridium*, as it is a cofactor of enzymes directly or indirectly involved in the H_2 evolution, such as hydrogenase, NADH:ferredoxin oxidoreductase (NFOR), and pyruvate:ferredoxin oxidoreductase (PFOR). In this work, iron was added as nanoparticles (NP) to a traditional fermentation (TF) and to an electrofermentation (EF) system with glucose as a carbon source (10 g/L). Iron NP supported on carbon black (NPCB), NPCB without iron (NPCB-0), and NPCB immobilized on the electrode (NPCB-I) were added to the TF and EF systems (in triplicates). Carbon cloth and platinum wire electrodes were introduced into the EF flasks and connected to an external potential source fixed at 0.4 V during the assays. All the fermentative assays were carried out in 120 mL penicillin-type flasks containing 100 mL of culture medium, bubbled with N_2 before closing them with a rubber stopper and aluminium seal. The assays were inoculated with a 12-hour culture of *Clostridium pasteurianum* DSM 525, such that the initial optical density at 600 nm (OD) was 0.1. The flasks were agitated at 120 rpm and maintained at 37°C for 24 hours. The gas volume produced during the assays was measured by connecting tubes from the fermentation systems to an inverted flask containing 500 mL of 1.25 mol/L NaOH. The H_2 volume was considered as the volume of NaOH displaced. To ensure that gas evolution was only due to the metabolism of the microorganism and not due to the electrolysis of water or the culture medium, control tests were performed under the following conditions: culture medium with the application of 0.4 V; culture medium + NPCB with the application of 0.4 V; and culture medium with Carbon Black (CB). In none of the control tests was NaOH solution displacement detected, indicating no H_2 generation. After 24 hours, the TF+CB tests yielded lower H_2 volumes than TF alone (30 mL and 173.5 mL, respectively), suggesting inhibition of CB on the fermentation. Compared to TF, all EF conditions resulted in greater H_2 volumes. The application of 0.4 V increased the H_2 volume until 12 hours of fermentation, yielding 145.0 mL and 178.5 mL of H_2 for EF and EF+NPCB, respectively. However, at the end of 24 hours, EF alone reached 330.0 mL of H_2. In the EF+NPCB-I increased H_2 volume compared to the NPCB in suspension (248 mL and 232.5 mL of H_2, respectively). When NPCB was added to the TF, in suspension or immobilized in an electrode (without voltage), an increase in substrate consumption and cell concentration was detected, resulting in higher H_2 volume compared to the TF alone. This effect may suggest that NPCB increased H_2 production mainly due to enhanced microbial growth. However, the EF condition without NPCB promoted the largest volume of H_2 but showed a low OD increase, low glucose consumption, and low concentration of soluble products compared to the other conditions. Therefore, only the application of a potential difference without NP was able to rise H_2 production by *C. pasteurianum*, probably by modulating metabolism. The reduction of the coenzyme NAD^+ to NADH by the applied potential, combined with the oxidative action of the NFOR enzyme, may have led to increased H_2 evolution without increasing glucose consumption. Such assumptions will be investigated by quantifying the expression of genes related to H_2 formation, such as those encoding hydrogenase, NFOR, and PFOR.

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Poster-B22: Influence of operational factors on the performance of a the long-term operated 2025 cm² flat-plate microbial electrolysis cell

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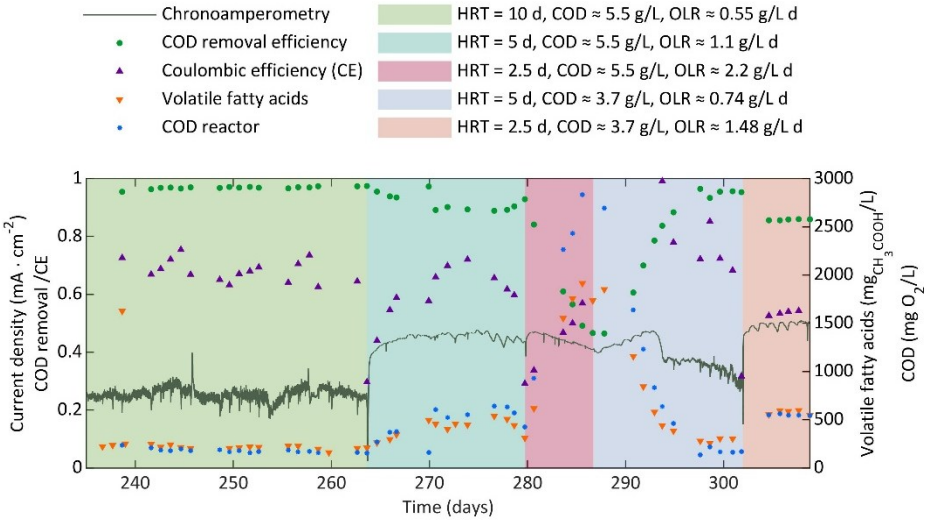
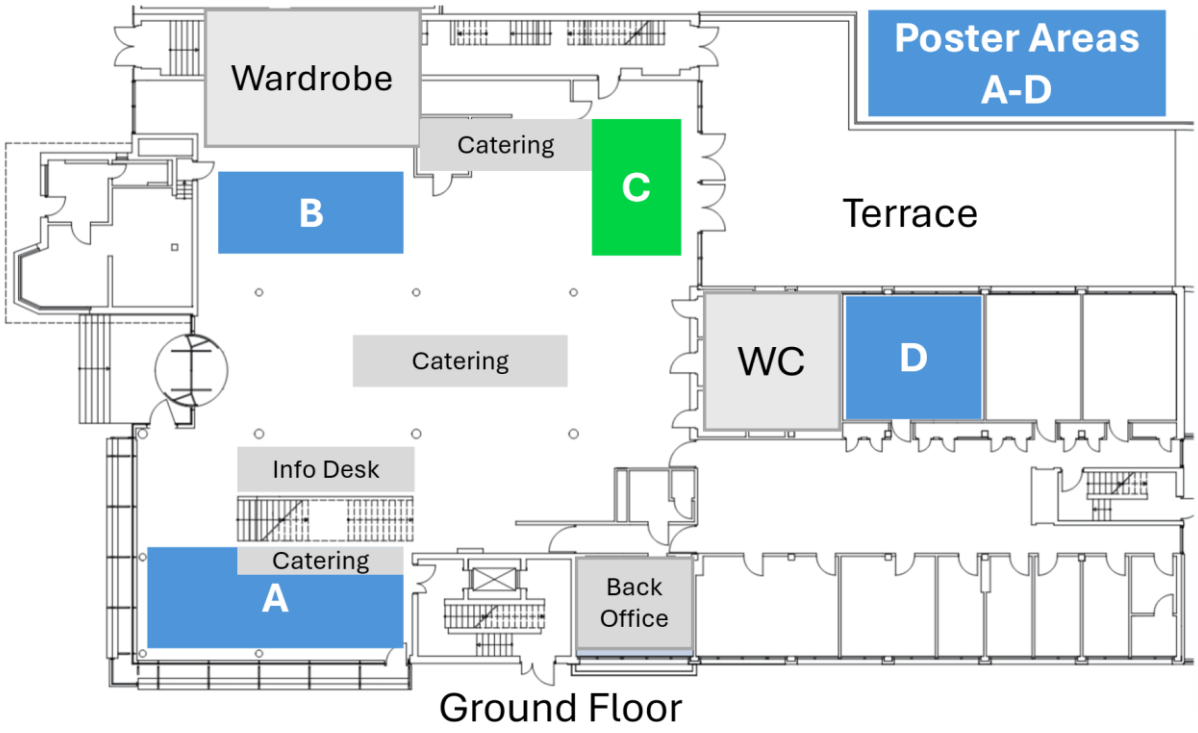
Aim of this work is to evaluate the long-term operational characteristics of a novel upscaled 2025 cm^2 flat-plate microbial electrolysis cell (MEC) under various conditions. The MEC consists of a 434 stainless steel wool anode (RAKSO, Oscar Weil GmbH, Lahr, Germany), a bipolar membrane (Membranes International Inc.), and a 316l stainless steel mesh cathode (Haver and Boecker OHG, Oelde, Germany). As catholyte a HCl solution (pH 1–4) is continuously recirculated. Initially, the anode was operated in fill-and-draw mode during 100 days using acetate and later beer, as substrate. Subsequently, it was transitioned to chemostat operation under different conditions. For evaluation the 10 L anolyte is recirculated through the anode chamber at a constant flow rate, with the system operated at three hydraulic retention times (HRTs: 10, 5, and 2.5 days) and two chemical oxygen demand (COD) inputs (5.5 and 3.7 g L^{-1}; made from fermented beer in a buffered salt medium as a complex substrate) with the resulting organic loading rates (OLRs). The anode is polarized at -350 mV vs. SCE.



The above Figure presents the evolution of main operational parameters over the time. When the OLR is 0.55 g L^{-1} day^{-1} or 0.74 g L^{-1} day^{-1}, COD removal exceeds 98 %, resulting in an effluent COD of ~170 mg O_2 L^{-1}. However, the current density remains relatively low (0.25 - 0.30 mA cm^{-2}), with a coulombic efficiency (CE) of around 70 %. These results are observed at HRT = 10 days with 5.5 g O_2 L^{-1} COD input, and HRT = 5 days and 3.7 g O_2 L^{-1} COD input. When the ORL increases to 1.1 g L^{-1} day^{-1} (HRT = 5 days, COD input = 5.5 g O_2 L^{-1}), the current density increases markedly to 0.45 mA cm^{-2} with a CE of 60 % and a COD removal close to 90 %. However, as a consequence the effluent COD exceeds 600 mg O_2 L^{-1}. At an OLR of 1.48 g L^{-1} day^{-1} (HRT = 2.5 days and COD input of 3.7 g O_2/L) the current density increases further to over 0.5 mA cm^{-2}, while COD removal and CE decreases to 85 % and 55 %, respectively. Contrary to expectation, the effluent COD is slightly lower than before, amounting to 545 mg O_2 L^{-1}. This suggests that HRT and COD influent may influence performance beyond their combined effect as OLR. At the highest OLR tested (2.2 g L^{-1} day^{-1}), the COD accumulates in the anolyte to effluent levels approaching 3000 mg O_2 L^{-1}, while COD removal drops drastically below 50 %. Therefore, low OLR, up to 0.74 g L^{-1} day^{-1}, enable effluent COD values close to discharge limits, while higher OLRs (up to 1.48 g L^{-1} day^{-1}) may be preferred to maximize current production. Finally, the fluctuation level of the current appears inversely related to volatile fatty acid concentrations and COD levels in the anolyte.

POSTER PRESENTATIONS

Electromethanogenesis
- Area C -



Poster-C01: Scaled-Up Electromethanogenesis in a Labyrinth-Flow Reactor for Efficient Biogas Upgrading

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Biogas upgrading is crucial for enhancing quality and usability, particularly for grid injection and sustainable energy applications. While physicochemical methods are widely used, they are resource-intensive, making microbial electrochemical technologies, such as electromethanogenesis (EMG), a promising carbon-neutral alternative. EMG utilizes direct electron transfer and in-situ hydrogen production to convert CO_2 into methane, improving biogas purity. In this study, anaerobic granular sludge (AGS) was employed as a cathodic biocatalyst in a 10-liter two-chamber bioelectrochemical reactor, achieving CO_2 capture and conversion without external chemical input. When the system was operated at 5V, the methane content in biogas was successfully upgraded to over 90% during the half year continuous operation, demonstrating its potential for sustainable and efficient biogas upgrading. The catholyte was liquid digestate which could also act as the buffer to control the pH in the neutral pH. The average volatile fatty acids (VFAs) concentration on catholyte was 12.3±1.7mg/L. As a pre-pilot scale study, it demonstrates the practical applicability of this technology for large-scale implementation, offering a viable alternative to conventional physicochemical methods while contributing to carbon-neutral energy solutions.

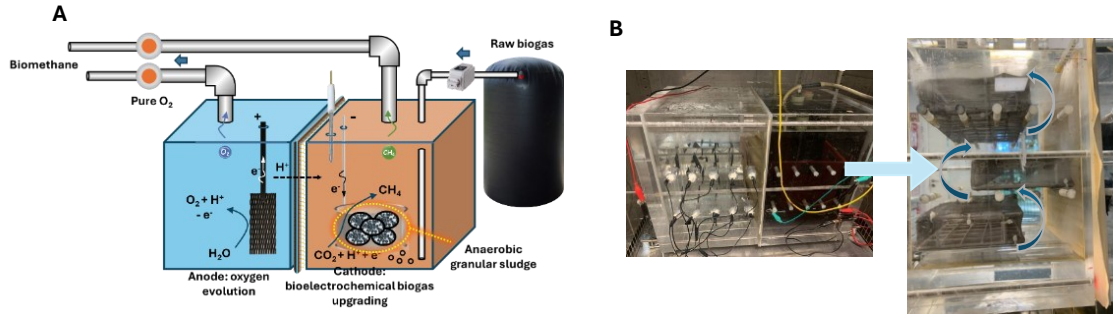

Figure 13:A) State of the art two-chamber bioelectrochemical reactor design, and B) 10-liter two-chamber Labyrinth-flow bioelectrochemical reactor

Poster-C02: Advancing Electromethanogenesis: Biomethane Production from Industrial Anaerobic Digestion Substrates

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Electromethanogenesis (EMG), which combines Anaerobic Digestion (AD) with electrochemical systems, is emerging as a promising approach to enhance biomethane (CH_4) production. This technology could play a crucial role in achieving the European Union's target of producing 370 TWh of CH_4 by 2030. Over the past 15 years, significant advances have been made, particularly in improving CH_4 production performance using various electrode materials and synthetic substrates such as acetate. However, experimental data on the efficiency and underlying mechanisms of EMG with real industrial AD substrates remain limited, especially regarding the role of the anodic biofilm in organic matter degradation.

In this context, our initial experiments investigated the use of real AD feedstock (digestate, manure, slurry, straw, and food waste) in single-chamber EMG reactors equipped with a three-electrode system. Our results demonstrated that cathodic electroactive microorganisms significantly enhance the kinetics of reduction reactions and current generation at the cathode. A subsequent study explored CH_4 production mechanisms, whether hydrogen-mediated or not, by comparing EMG reactors polarized at different cathodic potentials with conventional AD reactors. Daily monitoring included quantitative (gas flow meter) and qualitative (MicroGC) biogas analysis, as well as the quantification of Chemical Oxygen Demand (COD) and Volatile Fatty Acids (VFAs) to assess substrate degradation. Additionally, microbial community dynamics were analysed using Next-Generation Sequencing (NGS). Although no significant increase in CH_4 production was observed under the tested conditions, EMG effectively reduced hydrogen sulfide (H_2S) emissions, a toxic biogas contaminant. Cathode polarization had little impact on CH_4 production, but non-polarized systems exhibited higher H_2S levels, highlighted EMG's potential for co-pollutant mitigation.

Further investigations were carried out through two comparative experimental campaigns assessing the correlation between different carbon-based electrodes (carbon felt and carbon brushes) and a mixture of digestate and real substrates at varying proportions. The focus was on evaluating CH_4 production performance and the role of the anodic biofilm in the degradation of real substrates. Monitoring included daily biogas volume measurement, detailed biogas composition analysis, COD balance, current density, and regular cyclic voltammetry to characterize the reaction kinetics at EMG cell electrodes. Experiments using carbon felt electrodes revealed that, over multiple successive biogas production batches, EMG reactors consistently outperformed conventional AD reactors in CH_4 production, regardless of substrate ratios.

These findings suggest that the primary challenges of EMG lie in the development and maintenance of highly electroactive bioanodes capable of efficiently degrading real substrates, rather than in biocathodic mechanisms. This conclusion contrasts with previous reports in the scientific literature. These discoveries raise critical new questions: Could optimizing anodic biofilm development be the key to enhancing EMG performance? Furthermore, could cathodic biofilms or bioelectrodes help mitigate the biogeneration of undesirable by-products and pave the way for novel industrial applications?

References

1. Flores-Rodriguez, C., Nagendranatha Reddy, C. & Min, B. Enhanced methane production from acetate intermediate by bioelectrochemical anaerobic digestion at optimal applied voltages. *Biomass Bioenergy* **127**, 105261 (2019).
2. Amrut Pawar, A., Karthic, A., Lee, S., Pandit, S. & Jung, S. P. Microbial electrolysis cells for electromethanogenesis: Materials, configurations and operations. *Environ. Eng. Res.* **27**, 200484–0 (2020).

Poster-C03: Performance of a CH_4-producing Microbial Electrolysis Cell fed with real organic waste streams

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Methane-producing microbial electrolysis cells (MECs) represent a promising technology to combine the valorization of organic waste streams with the biological biogas upgrading. Indeed, biogas calorific value is lower that natural gas (CH_4 > 98%)[1] and in a two chamber CH_4-producing MEC methanogens drive, in presence of H_2, the cathodic CO_2 reduction into CH_4 resulting in a CH_4-enriched biogas (Figure 1). The energy input required for the cathodic electrochemical H_2 production is partially provided by the biologically-driven oxidation of organic (waste) substrates at the MEC bioanode. However, the complex composition of real feedstocks often poses critical challenges to the bioanode performance.
In this research, the bioanode ability to oxide two real feedstocks was evaluated in terms of current generation, COD (Chemical Oxygen Demand) removal efficiency, and coulombic efficiency (CE). The two adopted feedstocks consisted of the liquid effluent deriving from a pilot-scale acidogenic fermenter treating food waste (Run I) and of the liquid effluent from a full-scale anaerobic digestor treating the organic fraction of municipal solid wastes (Run II). In both runs the cathode performance was monitored under either abiotic or biotic (after methanogens inoculation) conditions. The MEC was operated with the anode potential controlled at +0.20 *vs* SHE (Standard Hydrogen Electrode) to promote the establishment of an electroactive anodic biofilm from an activated sludge. In Run I, the bioanode achieved a COD removal efficiency of 42±11% and 38±4% for the abiotic and biotic cathode phase, respectively; resulting in a stable current generation of 37±3 mA and 40±3mA for the abiotic and biotic cathode phase, respectively; which corresponded to a nearly complete chemical to electric energy conversion (i.e., CE of ca.100 %). In Run II, the presence of particulate substances (about 30% of the overall COD) in the digestate likely led to feedstock accumulation[2] and slow hydrolysis in the anode chamber, resulting in a difficult assessment of the effective COD removal efficiency. As for the cathode performance, H_2 and CH_4 production rates in Run I accounted for 24±1 mEq/d and 8±1 mEq/d, respectively; with a corresponding cathode capture efficiency (CCE) of 73±8% (for H_2) and 23±5% (for CH_4). Similar values were observed in Run II, with production rates of 22±3 mEq/d (for H_2) and 11±2 mEq/d (for CH_4), corresponding to a CCE_{H2} of 79±16% and a CCE_{CH4} of 33±5%. Overall, the obtained results point out that while the bioanode performance is strongly influenced by the nature of the influent feedstock, the cathode performance remains unaffected, highlighting the flexibility of CH_4-producing MECs in treating real organic streams.

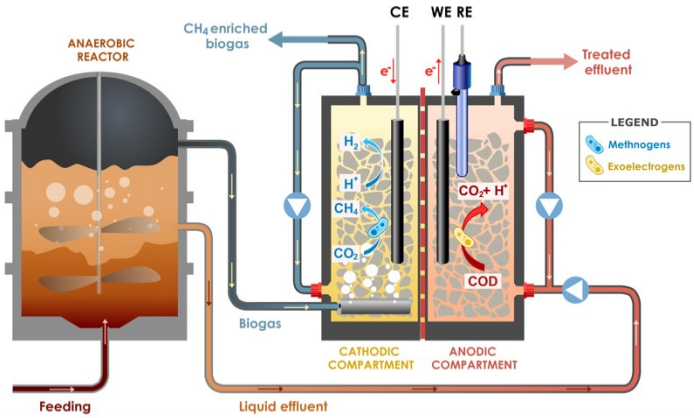

Figure 1. Scheme of a two-chamber CH_4-producing MEC treating real organic streams

1 S. Fu, I. Angelidaki and Y. Zhang, *Trends Biotechnol*, 2021, 39, 336–347.
2 F. Wu, J. Xie, X. Xin and J. He, *Front Microbiol*, 2022, 13, 999647.

Poster-C04: Pre-enriched electrodes, bioelectrochemical processes, and biomass retention collectively enhance methane production in integrated microbial electrolysis cell-anaerobic digestion

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Integrating microbial electrolysis cells (MECs) with anaerobic digestion (AD) enhances system performance by increasing organic removal rates, methane (CH_4) production, and CH_4 concentration in biogas. This study systematically examined the effect of different microbial electrolysis cell (MEC) components (i.e., anode, cathode, and suspension) on CH_4 production in MEC-AD system. The impact of pre-enriching the anode with *Geobacter sulfurreducens* and the cathode with hydrogenotrophic methanogens on system performance was also evaluated. CH_4 production was 14±4% higher in reactors with pre-enriched electrodes (PEE) than virgin electrodes (VE) under both open and close circuit conditions (OC & CC), demonstrating the advantages of electrode pre-enrichment. Closed circuit reactors (PEE-CC & VE-CC) notably exceeded open circuit reactors (PEE-OC & VE-OC) in CH_4 production by 16.5±2%, underscoring the importance of bioelectrochemical processes. The VE-OC reactors produced 12±2% more CH_4 than anaerobic digesters without electrodes (AD-NE), which recorded the lowest CH_4 production (306±5 mL), highlighting the role of biomass retention on electrode surfaces. The suspension contributed most significantly to CH_4 production in all MEC-AD reactors (43-62%), followed by the cathode (21-32%) and the anode (7-19%). *G. sulfurreducens* and *D. acetexigens* dominated the anodes of PEE-CC and VE-CC reactors, respectively, while *Methanobacterium* was prevalent on the cathode. *Methanosarcina* and *Methanosaeta* were widespread across all suspension samples. The detection of 19 diverse methanogen species underscores the metabolic diversity of the system, supporting hydrogenotrophic, acetoclastic, and electrotrophic methanogenesis. The high commonality of operational taxonomic units across the anode, cathode, and suspension illustrates the interconnected microbial ecosystems and vital interactions among biofilms and planktonic communities that facilitate fermentation, electroactivity and CH_4 production. Collectively, these results highlight the synergistic effects of electrode pre-enrichment, bioelectrochemical processes, and biomass retention in enhancing AD performance by improving system stability and robustness.

Poster-C05: Continuous conversion of CO_2 to CH_4 by bioelectrochemical methanation: influence of dissolved CO_2 and ion crossover

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Bioelectrochemical methanation (BEM) is an emerging power-to-gas technology, also serving for carbon capture and utilization (CCU). A BEM unit typically consists of two electrodes, an anode and a cathode, inserted into two adjacent chambers of a bioelectrochemical cell. The biocathode converts electricity and CO_2 into methane (CH_4). In previous studies, bicarbonate was added to the catholyte as a source of CO_2, rather than delivering gaseous CO_2 directly to BEM cells, as it would be required in real industrial applications. Indeed, the challenge arises from the limited solubility of CO_2 in the water-based electrolyte, making it difficult to dissolve CO_2 at the required rate. In this study, three double-chambered BEM cells were assembled in the laboratory. Each one consisted of an abiotic anode performing water-splitting (9 cm^2) and a stainless-steel wool biocathode (100 cm^2), separated by a cation exchange membrane (CEM). The three cells were hydraulically operated in parallel: the anode chambers (200 mL each) were recirculating the anolyte at 200 mL min^{-1} to an external buffer tank of 2 L, while the cathode chambers (200 mL each) were recirculating the catholyte at the same flow rate to a 5 L tank. In this tank, CO_2 gas was injected at 20 mL min^{-1} when the concentration of dissolved CO_2 fell below 20%. The BEM cells were operated electrically at a constant current density of 12 A m^{-2}. An optimal CH_4 production rate of 0.4-0.6 L-CH_4 L^{-1}cathode chamber d^{-1} was achieved, corresponding to a dissolved CO_2 concentration in the catholyte between 10% and 20%, while ensuring a high quality of the produced outflow gas (>90% CH_4) (Figure 1). Additional analyses were performed to characterize the system. Microbiological characterization assays were conducted on some catholyte samples, collected from the bulk of the catholyte, to monitor the expression of targeted methanogenic genes *hdrA* and *mcrA*. Ion chromatography and inductively coupled plasma spectroscopy were used to evaluate ion crossover between the anode and cathode chambers of the cells, through the CEM.
These findings demonstrate the feasibility of continuous CO_2-to-CH_4 conversion in BEM systems, highlighting key operational parameters for optimizing efficiency and gas quality.

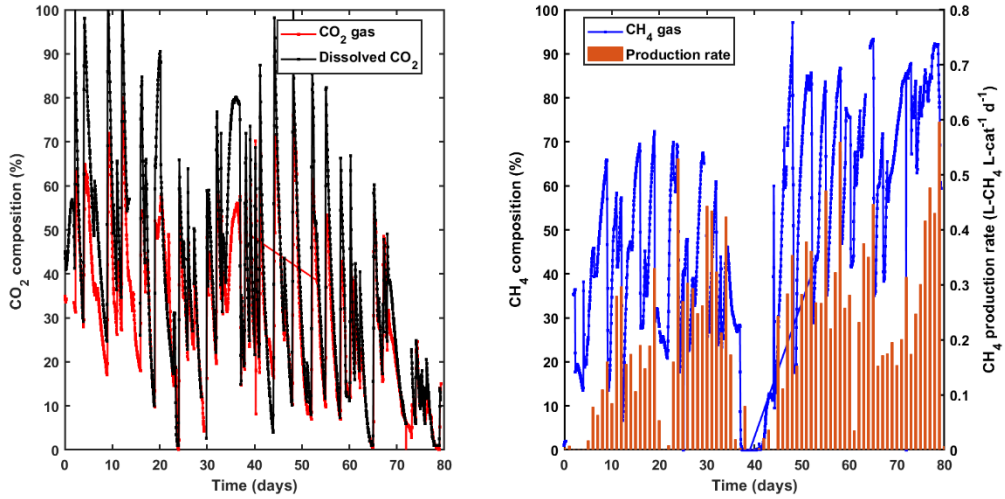

Figure 1. *The left panel illustrates the concentration of CO_2 in the gas (red) and liquid phase (black). The right panel shows the concentration of CH_4 in the gas phase (blue) and the specific CH_4 production rate (orange bars).*

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Poster-C06: Electromethanogenesis in membrane-less reactors using brewery wastewater

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Bioelectrochemical systems are an option for biofuel production using wastewater by taking advantage of the chemical energy stored within the organic matter present in the wastewater stream. Electromethanogenesis, also known as methane electrosynthesis, enables the generation of biogas with higher concentrations than conventional anaerobic digestion (50-60% vs 70-80%). This study evaluated methane production using brewery effluent and assessed two cathode materials, stainless-steel and nickel foam, within a membrane-less bioelectrochemical reactor. Four acrylics cylindrical bioelectrochemical reactors made of acrylic were used, with nickel foam and stainless-steel mesh as cathodes. Graphite felt was the anode material, and titanium wire was used for the connections. The substrate was brewery effluent, and reactors were inoculated with anaerobic granular sludge (20 g/L). Experiments were conducted under potentiostat control, maintaining an anodic potential of +200 mV vs. Ag/AgCl to stimulate a selection pressure. Two control setups were included: reactors without electrodes and open circuit voltage controls. All experiments were conducted at 30 ± 1 °C with continuous stirring. Two effluent samples from the brewery industry were evaluated: 2.97±42 g COD/L and 4.26±0.15 g COD/L. Electrical activity was observed after two days of operation indicating the growth of exoelectrogenic bacteria on the anode. Methane purity and production varied across setups, with stainless steel mesh outperforming nickel foam but falling short of controls without electrodes. The methane concentrations in the biogas were 81 % and 68 % with stainless steel and nickel foam cathodes, respectively. Methane production was 353±43 mL $CH_4/L_{reactor}$ using stainless steel mesh and 298±32 mL $CH4/L_{reactor}$ using nickel foam. Organic matter removal, measured as COD, showed lower concentrations in electromethanogenesis reactors (83±3 %) than in the controls without electrodes (95±3 %), possibly due to the presence of intermediate metabolites. In conclusion, while stainless steel mesh showed promising results, the performance of electromethanogenesis using brewery effluent was inferior to conventional anaerobic digestion, possibly due to the production of intermediate metabolites.

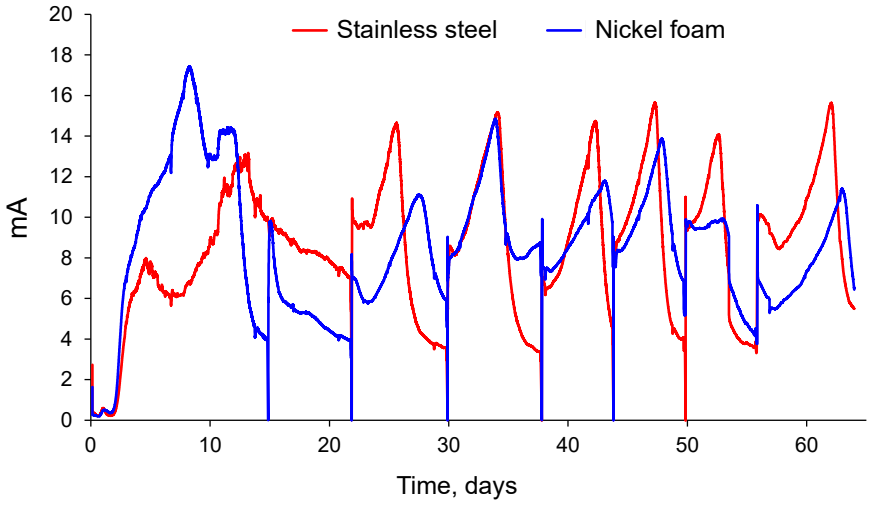

Fig.1. Chronoamperometry of electromethanogenesis evaluating brewery effluents with two different cathodes (stainless steel vs nickel foam).

Poster-C07: Optimization of Flow Dynamics in a Double-Chamber Electromethanogenesis Reactor using CFD Simulation

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Bioelectrochemical systems (BES) have emerged as a promising approach for enhancing anaerobic digestion (AD) processes, particularly in biogas upgrading and power-to-gas applications. In two chamber BES reactors, the oxidation of organic matter occurs at the bioanode, while the carbon dioxide is reduced to methane by electromethanogenic archaea at the biocathode, through the application of an external voltage
Computational Fluid Dynamics (CFD) is a valuable instrument for developing mechanistic models. It facilitates the simulation and prediction of factors such as fluid flow, mixing conditions, chemical species transport, among other parameters. This capability is crucial for the design, evaluation, and optimization of efficient bioreactors, including AD-BES systems.
This study investigates fluid dynamics within a double-chamber BES reactor for biogas upgrading, with a particular focus on the cathode chamber, shown in figure 1. For this purpose, a digital twin of a BES reactor was created using ICEM 19.1 software for the reactor geometry and meshing and Fluent 2024 R1 for the fluid dynamic simulations. For validation, the simulation results are compared with experimental data.

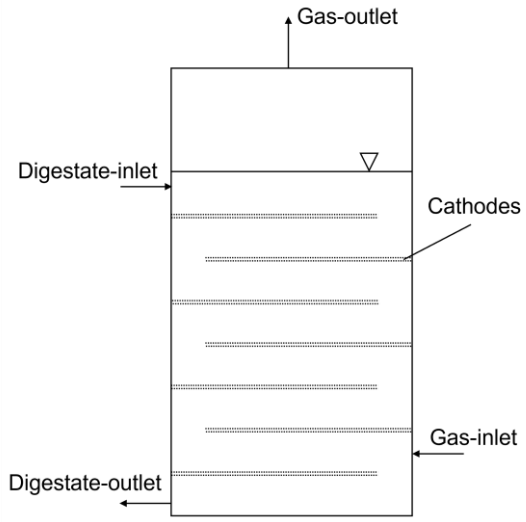

Figure 1: Schematic representation of the cathode chamber used in the study.

This study highlights the interplay between reactor geometry, operating parameters, and flow dynamics, shaping overall performance. Through advanced flow simulations, key hydrodynamic aspects, such as velocity distribution, turbulence characteristics, and mixing behavior are examined. The findings underscore the strategic influence of cathode arrangement in prolonging reactant residence time and reveal the effects of gas and digestate feed variations on reactor flow and consequently BES efficiency.

Keywords: Anaerobic digestion,biogas upgrading, bioelectrochemical system, energy storage, computational fluid dynamics

Poster-C08: The role of Granular Activated Carbon (GAC) in Microbial Electrolysis Cell integrated Anaerobic Digestion Systems

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Microbial electrolysis cells (MECs) integrated anaerobic digestion (AD) systems enhance methane (CH_4) production by promoting electromethanogenesis under applied voltage and direct interspecies electron transfer (DIET). Several studies have shown that MEC-AD integration led to improved CH_4 output, kinetics, and process stability (Chen et al., 2016). However, the MEC-AD integration alone may be insufficient to fully harness DIET pathways. Emerging evidence suggests that adding conductive materials such as granular activated carbon (GAC) not only supports DIET but also engages the reactor's bulk volume in electron transfer. To evaluate this potential, a comprehensive experimental design incorporating 16 batch reactor configurations was implemented, including systems amended with fresh GAC or biofilm-attached GAC (BioGAC), tested under varying electrode types (bare vs. biofilm-coated) and the presence or absence of applied voltage. Experiments were performed in phosphate buffer saline (PBS) and a salt media.

Random Forest (RF) analysis, a supervised machine learning method, was used for comparison of reactor performances based on three performance parameters, namely, CH4 production rate, CH4 production yield, and lag time, which were used as responses in the RF method. RF generated a normalized Inc.NodePurity value for each of the five factors (BioGAC, fresh GAC, Bioelectrodes, Bare Electrodes, and Voltage) for a given reactor performance parameter (Fig. 1). The higher the normalized Inc.NodePurity value, the higher the factor's importance on a given reactor performance parameter (Che et al., 2011).

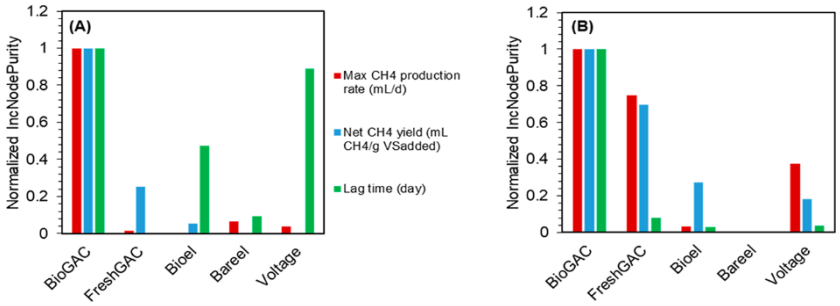

Figure 1. Importance of different factors on reactor performance with (A) PBS medium and (B) salt medium.

Reactors amended with BioGAC consistently outperformed other configurations. In PBS medium, the BioGAC-amended AD-MECs exhibited the highest methane yields of 318 mL CH_4/g VS_added—approximately 4.4 times greater than the control. Moreover, BioGAC reduced lag time and increased CH_4 production rates, even under inhibitory phosphate-rich conditions (Fig.1 A). RF analysis confirmed BioGAC as the most influential factor in improving reactor performance. Further, applying a voltage significantly accelerated start-up under stress conditions, likely by boosting electron transfer mechanisms.

References

Chen, Yu, B., Yin, C., Zhang, C., Dai, X., Yuan, H., Zhu, N., 2016. Biostimulation by direct voltage to enhance anaerobic digestion of waste activated sludge. RSC Adv. 6, 1581–1588
Che, D.; Liu, Q.; Rasheed, K.; Tao, X. Decision Tree and Ensemble Learning Algorithms with Their Applications in Bio- informatics; In Software Tools and Algorithms for Biological Systems 2011; Springer: New York, NY Advances in Experimental Medicine and Biology.

Acknowledgements

Funding received from the METU Scientific Research Projects Coordination Unit (No. 10776) and the Science Academy via BAGEP award.

Poster-C09: Coupling Electromethanogenesis and Sulfide Oxidation in MES: H_2S removal and microbial community dynamics in the reactors

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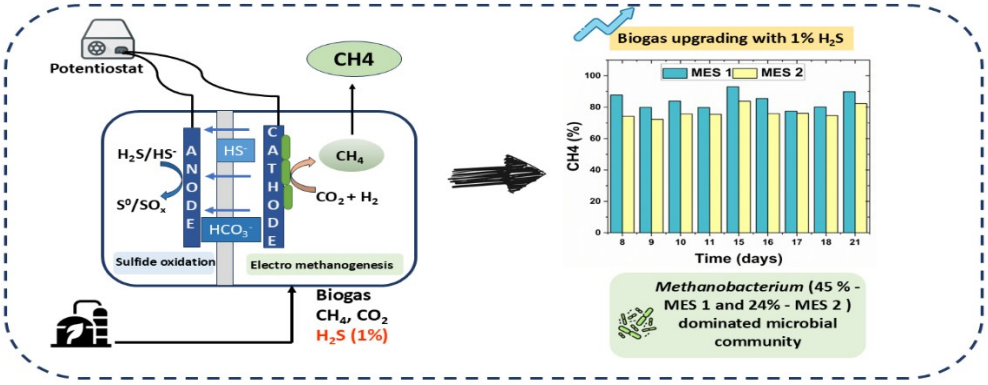
The growing demand for low carbon energy carriers like biomethane is driving the transition to cleaner energy systems. As a key step, biogas upgrading is essential to produce high purity methane (>95%) by removing CO_2 and H_2S to meet natural gas grid standards. Microbial electrosynthesis (MES) offers a promising power to methane approach by linking renewable electricity with methane production [1]. However, H_2S as biogas impurity is largely overlooked in previous MES studies [2]. This study demonstrates a single step MES system that integrates CO_2 to CH_4 conversion via electromethanogenesis and H_2S removal through electromigration and subsequent anodic sulfide oxidation. We have set two H-type MES (1L), separated with an anion exchange membrane with stainless steel mesh as cathode material, instead of the commonly used carbon based, to target directly H_2 evolution and thus higher CH_4 production rates. Cyclic voltammetry (CV) was performed to identify the H_2 evolution potential under our system conditions, using a modified methanogenic medium and inoculum sourced from an active, low-rate MES. The reactors were operated in fed batch mode, initially with 99.9% pure CO_2 to establish electromethanogenesis and subsequently switched to CO_2(40%)/CH_4(60%) (29 days) and CO_2/CH_4/H_2S (1%) (32 days) to investigate the biogas upgrading. Applied cathode potentials were progressively lowered during CO_2 operation (-0.850, -0.900 -0.950 V vs Ag/AgCl).

During CO_2 operation, abiotic H_2 evolution at the cathode supported microbial acclimatization, leading to CH_4 and VFA production. A clear shift from acetogenesis to methanogenesis was observed after day 45, marked by a decline in acetate concentration to zero. Maximum CH_4 production rates of 7.26 and 6.47 L m^{-2} d^{-1} were achieved in MES 1 and MES 2, respectively, with current efficiencies of 83.6% and 70%. Current density increased with absolute decreasing cathode potentials, from 1.37±0.30 A m^{-2} at -0.850 V to 3.69±0.51 A m^{-2} at –0.950 V vs Ag/AgCl. During CO_2/CH_4 operation, CH_4 enrichment exceeded 80.86±4.3% and 70.69±2.48 within 24 hours and the production rates were 12.1±5.5 (2.17±0.40 A m^{-2}), 7.4±3.34 L m^{-2} d^{-1} (1.44±0.14 A m^{-2}) for MES 1 and MES 2 respectively. 16S rRNA sequencing revealed microbial community shifts over time, with enrichment of reported hydrogenotrophic methanogens and acetogens.

Notably, introduction of 1% H_2S did not impair CH_4 production rates (10.12±5.68, 6.03±3.43 L m^{-2} d^{-1} for MES 1 and MES 2). Although, both MES reactors initially showing comparable performance, the introduction of H_2S led to clear divergence. MES 2 exhibited higher SO_4^{2-} accumulation (4.9mM after 28 days) versus HS^- retention (1mM) in MES 1. We hypothesize this variation stemmed from variations in reactor anaerobicity (3.03% O_2 in MES 2 and 1.96% in MES 1), which influenced both sulfur products distribution and microbial dynamics [3]. Our system sustained biogas upgrading at methane production rates comparable to previous MES studies [2], even with 1% H_2S. These findings highlight the need for comprehensive analysis of all sulfur components and further studies on H_2S containing biogas in MES to better optimize MES performance.



References:

[1] Deutzmann JS, Kracke F, Spormann AM. Microbial electromethanogenesis powered by curtailed renewable electricity. Cell Reports Phys Sci 2023;4.

[2] Chung TH, Dhillon SK, Shin C, Pant D, Dhar BR. Microbial electrosynthesis technology for CO2 mitigation, biomethane production, and ex-situ biogas upgrading. Biotechnol Adv 2024;77:108474.

[3] Di Costanzo N, Di Capua F, Cesaro A, Carraturo F, Salamone M, Guida M, et al. Headspace micro-oxygenation as a strategy for efficient biogas desulfurization and biomethane generation in a centralized sewage sludge digestion plant. Biomass and Bioenergy 2024;183:107151.

Poster-C10: Upscaling Bioelectrochemical Systems for Biomethane Production: Insights from the Horizon Europe Biomethaverse Project

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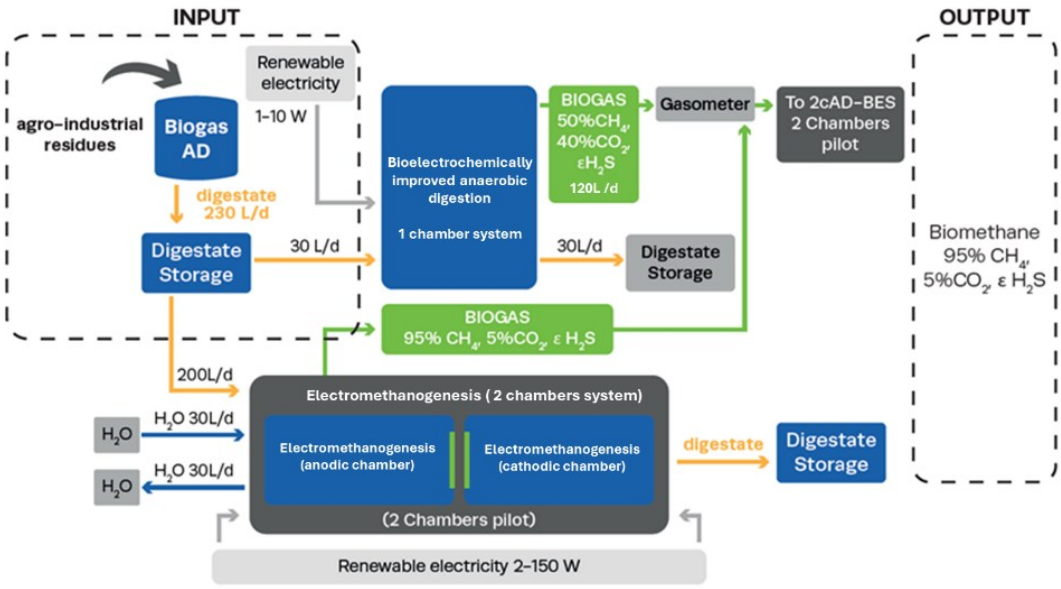
Within Biomethaverse project, five biomethane innovative production pathways are developed and tested at pilot scale. The presentation will be focusing on one of these pilots, relying on a bioelectrochemical system. The pilot is composed of two 1m³ systems: the first one relying on a 1 chamber bioelectrochemically improved anaerobic digestion for biogas production (called 1C-ADBES) and the second one being a 2 chamber electromethanogenesis reactor for biogas upgrading (called 2C-ADBES).



Progress on this project was supported by the interdisciplinary collaboration with LEITAT and DTU, who characterized performances at lab scale and provided valuable inputs for pilot engineering, TUB, who realized crucial CFD studies, guiding technological choices at the 1m³ scale and the engineering company AERIS built the pilot systems, ensuring robust designs for implementation at the ENGIE BiOZ anaerobic digestion unit in France where the pilot is tested.

Initially developed at laboratory scale, both systems demonstrated significant enhancement in terms of biogas yield and quality with production capacity up to 120L biogas/m³/day for the 1C-ADBES and gas network quality biomethane (95% CH_4) at the outlet of the 2C-ADBES. Lab results as well as upscaling process towards a 1m³ pilot scale using continuous streams from a AD unit will be detailed during the presentation. The 1 year pilot testing aims at providing meaningful results towards validating the feasibility and techno-economic benefits of these bioelectrochemical systems at a larger scale.

Acknowledgements
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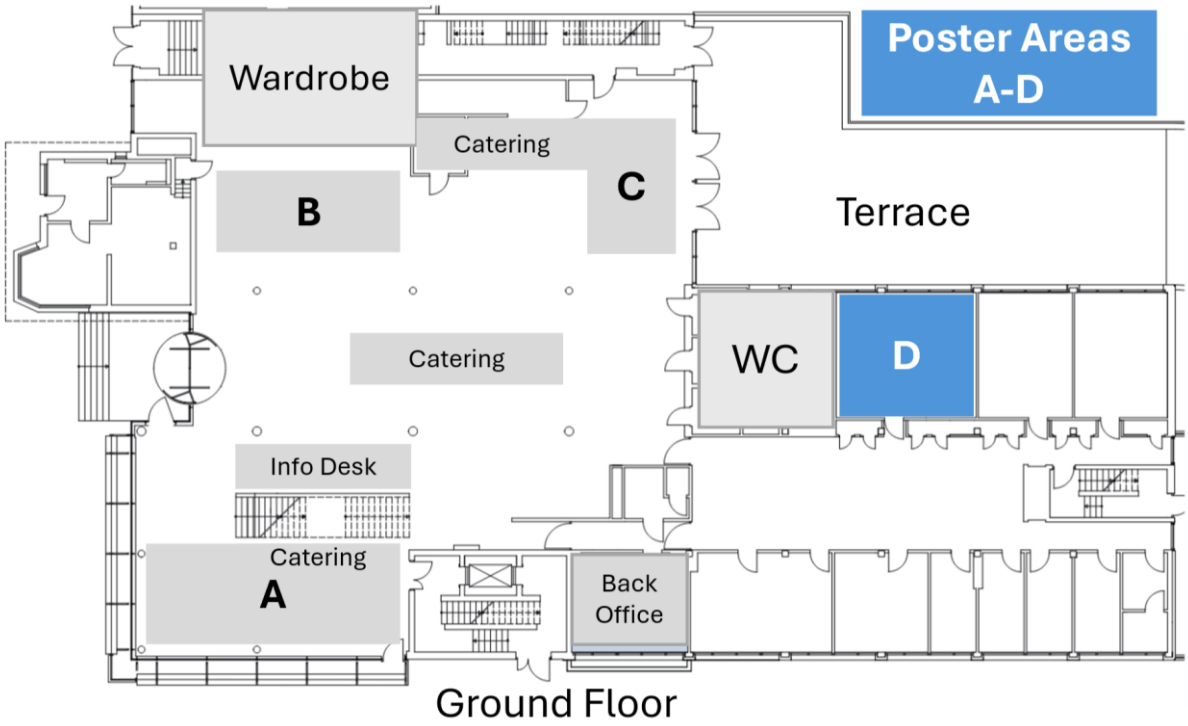
POSTER PRESENTATIONS

Microbial Fuel Cells
- Area D -



Poster-D01: Process level energy analysis of bioelectrochemical systems-integrated wastewater treatment plant

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Introduction

Treatment of wastewater is a highly energy intensive process, responsible for around 20% of the total energy consumption of municipalities in Germany.[1] Instead of viewing wastewater treatment as merely an energy sink, it can be viewed as an energy source. Particularly the stored chemical energy is considered readily usable. It is estimated that the usable chemical energy in typical municipal wastewater is around 16.1 kJ/g-COD.[2] If an average COD concentration of 600 mg/L in wastewater is assumed, this translates into an energy content of 2.68 kWh/m^3, much higher than the average energy demand of 0.43 kWh/m^3 for wastewater treatment in Germany.[3]

In recent years, bioelectrochemical systems (BES) have emerged as a promising technology platform to simultaneously treat wastewater and generate electricity. It is believed that integrating these systems into wastewater management can make them more energetically self-sufficient.

Materials and Methods

A systems level energy analysis is performed to compare a conventional wastewater treatment plant (WWTP) and BES integrated WWTP. Suitable assumptions are made about the size, capacity and energy requirements of the plant. Subsequently, energy self-sufficiency is calculated and compared.

Results

The major share of energy consumption (nearly half) in wastewater treatment is that of aeration energy needed for the conventional activated sludge process (CAS). By introducing a scaled-up Microbial Fuel Cell unit before the CAS step, considerable energy savings can be achieved. After the CAS step, anaerobic digestion is generally used to recover energy from the sludge in the form of biogas. By integrating electromethanogensis at this step, the methane yield and hence energy recovery can be improved. Overall, BES-integrated WWTPs can be more energy efficient, even if it adds to the initial capital investment. This validates the current research effort in scaling up such systems.

References

[1] M. Molinos-Senante, R. Sala-Garrido, and A. Iftimi, "Energy intensity modeling for wastewater treatment technologies," *Science of The Total Environment*, vol. 630, pp. 1565–1572, Jul. 2018, doi: 10.1016/j.scitotenv.2018.02.327.
[2] Z. Dai, E. S. Heidrich, J. Dolfing, and A. P. Jarvis, "Determination of the Relationship between the Energy Content of Municipal Wastewater and Its Chemical Oxygen Demand," *Environ Sci Technol Lett*, vol. 6, no. 7, pp. 396–400, Jul. 2019, doi: 10.1021/acs.estlett.9b00253.
[3] H. Wang *et al.*, "Comparative analysis of energy intensity and carbon emissions in wastewater treatment in USA, Germany, China and South Africa," *Appl Energy*, vol. 184, pp. 873–881, Dec. 2016, doi: 10.1016/j.apenergy.2016.07.061.

Poster-D02: Understanding the influence of external resistance on MFC performance: towards the development of a biosensor for detection of clogging in French vertical flow treatment wetlands

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French Vertical Flow Treatment Wetlands (VF TW) have become a popular technology for treating raw domestic wastewater and performing solids removal and mineralization. However, clogging, solids deposition and biofilm development in the pores of the filter media remain a critical operational challenge, requiring labor-intensive maintenance and potentially disrupting system performance. One promising solution is the use of a Microbial Fuel Cell (MFC)-based biosensor for real-time monitoring of biofilm development. Despite its potential, MFC biosensor technology still needs more in-depth research to explore the design and electrochemical performance in TW environment. This present study evaluated the electrochemical performance of MFCs operating under different external resistances, providing insights for the development of an MFC-based biosensor for clogging detection. Four air-cathode MFCs were inoculated with sludge sample from a French VF TW pilot-scale, installed at the REFLET/INRAE research platform (Craponne, France). The bacteria source was amended with phosphate buffer solution, micronutrients, and sodium acetate. During the start-up phase, the MFCs were operated with an external resistance of 1000 Ω. The system was considered stabilized when the current output remained steady. The MFC performance was then evaluated under two external resistances, tested in duplicate, 100Ω (MFC 1, MFC 2) and 1000Ω (MFC 3, MFC 4). Electrochemical characterization was performed using electrochemical impedance spectroscopy (EIS), and the anodic biofilm structure was analyzed using environmental scanning electron microscopy (E-SEM). The results showed that MFCs operating at 100Ω had the highest current output, with 0.56 mA for MFC1 and 1.11 mA for MFC2. A significant difference was observed between these duplicates ($p < 2.2.10^{-16}$). This variability may be attributed to connection problems in MFC1, which could have affected electron transfer efficiency. However, the MFCs operating at 1000Ω exhibited a lower current output (0.39 mA), which did not show a statistically significant difference between duplicates ($p = 0.8351$). These results were expected as a higher external resistance leads to reduced current generation. The current output at 1000Ω was smoother and more stable throughout the operating cycle, which is a desirable feature for long-term monitoring applications. EIS analysis indicated that MFC-1000Ω and MFC-100Ω had similar charge transfer impedance values of 36.33Ω and 33.68Ω, respectively, suggesting comparable electron transfer rates between the electrode and electrolyte. This parameter is related to response and sensitivity of the biosensor. In addition, E-SEM images demonstrated the formation of an anodic biofilm in both external resistor conditions, with a thinner biofilm observed at 1000Ω, which may help mitigate electrode fouling over time. These results highlight the influence of external resistance on the MFC operation, with direct implications for biosensor design. The higher resistance provided a more stable and sufficient signal output, making it a potential external resistor to be applied for long-term clogging monitoring in French VF TWs. Future studies should focus on optimizing electrode and design to align with the French VF TW characteristics. Furthermore, the inclusion unsaturated and intermittent feeding conditions could be interesting strategies to improve sensitivity and reliability of the biosensor.

Poster-D03: Electroactive Biofilm Characterization in Granular Activated Carbon in a Greywater Matrix

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Bioelectrochemical systems are presented as a favorable option for sustainable greywater treatment in the context of decentralization due to their easy operation and the possibility of energy recovery from waste. However, the scalability of these systems has been a challenge, considering the cost of the materials, the thermodynamic limitations associated with the design, and the operational inertia. Low-cost materials, such as granular activated carbon (GAC), have been proposed as electrodes due to their high surface area, adsorption capacities, and redox-active functional groups. This material confers the possibility of creating a three-dimensional electrode where a high population of electroactive microorganisms could develop and form a dynamic biofilm, making the system more reliable in treatment, energy recovery, and electrical response. Using this kind of system for greywater treatment in a decentralized context has its own challenges. The lack of aeration saves energy but makes a perfect environment for other microorganisms associated with gas production, such as sulfate-reducing bacteria and methanogens. The high variability of greywater could also affect the biofilm, compromising the reliability of the treatment system and the final recovered water quality. For this reason, a robust electroactive biofilm is needed, and a key step is to study the development of the electroactive biofilm in granular activated carbon in a greywater matrix media.

This work studies the interaction mechanisms between greywater contaminants and the mixed culture biofilm developed on GAC. The GAC material was previously characterized physically, chemically, and electrochemically. Packed GAC restrained with stainless steel mesh (Fig.1) were inoculated with a mixed culture of a previous microbial fuel cell (MFC) system and used as anode in a 200 mL MFC batch-fed with synthetic greywater. Cycles from one to three days were performed to avoid the growth of planktonic cells in the system, and the anode was extracted after the maximum power per cycle was stable. Biofilm from half of the GAC was extracted, and the surface of the GAC was studied in terms of its functional groups. Part of the biofilm was used for community analysis for DNA extraction, and the other part was used for quantification of extracellular polymeric substances (EPS). The other half of the GAC was used for epi-fluorescence microscopy, scanning electron microscopy, and cyclic voltammetry to identify the electrochemical response of the biofilm. The results correlate the presence of EPS components and structure with the treatment and electrical performance of the MFC. They are compared with other EPS analyses in the literature, giving a first scope of a mixed culture electroactive biofilm developed in a redox-active surface material such as GAC immersed in a greywater matrix.

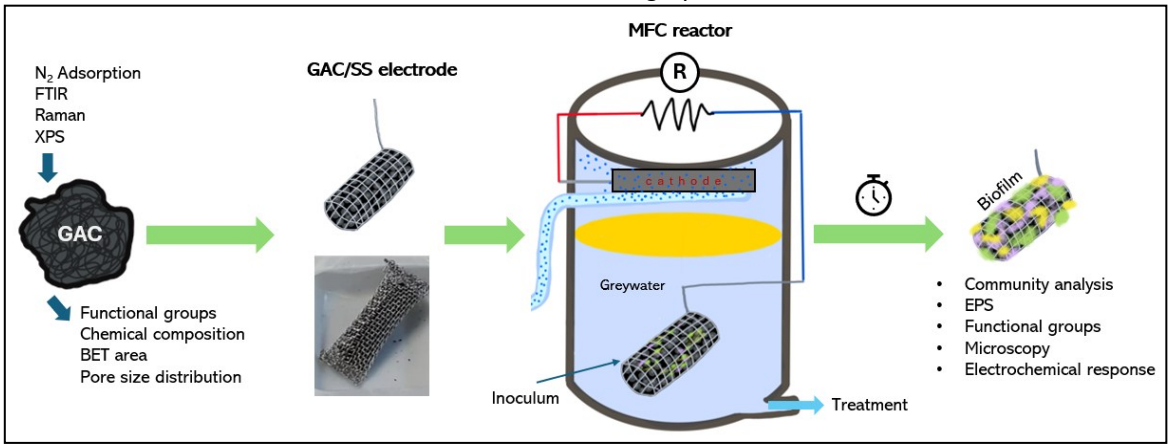

Figure 1: *Experiment schematics.*

Poster-D04: Biodesalination using a Novel Halophilic Cyanobacteria-Integrated MDC (CI-MDC) for Enhanced Desalination Efficiency

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Water scarcity is a global issue affecting about 2.3 billion people, driving demand for sustainable desalination technologies [1] . Conventional methods like Reverse Osmosis (RO) are energy-intensive and produce brine contributing to environmental degradation [1,2]. Microbial Desalination Cells (MDCs) present a promising solution by harnessing exoelectrogenic bacteria to desalinate water through ion migration. However, their scalability remains constrained by low desalination efficiency [3, 4].

To address these barriers, we are investigating a hybrid cyanobacteria-integrated MDC (CI-MDC) system that synergises bioelectrochemical desalination with phototrophic salt bioaccumulation. Halophilic cyanobacterial strains, selected for their hypersaline adaptability and ion sequestration abilities, are cultivated within a photobioreactor-augmented desalination chamber under controlled salinity gradients and optimised light regimes. This configuration aims to enhance ion migration through dual pathways: (a) electrochemical ion transport driven by exoelectrogens and (b) biological uptake via cyanobacterial ion channels.

This work aims to establish a proof-of-concept by coupling bioelectrochemical desalination with phototrophic salt bioaccumulation. The research aligns with the UN Sustainable Development Goal 6 (Clean Water and Sanitation).

References

[1] A. M. Michalak *et al.*, 'The frontiers of water and sanitation', *Nat Water*, vol. 1, no. 1, pp. 10–18, Jan. 2023, doi: 10.1038/s44221-022-00020-1.
[2] W. Rosińska, J. Jurasz, K. Przestrzelska, K. Wartalska, and B. Kaźmierczak, 'Climate change's ripple effect on water supply systems and the water-energy nexus – A review', *Water Resources and Industry*, vol. 32, p. 100266, Dec. 2024, doi: 10.1016/j.wri.2024.100266.
[3] K. Saini *et al.*, 'Microbial Desalination Cell (MDC): A Next-Generation Environmental Technology for Wastewater Treatment and Bioelectricity Generation', in *Microbiology-2.0 Update for a Sustainable Future*, J. Gupta and A. Verma, Eds., Singapore: Springer Nature, 2024, pp. 395–425. doi: 10.1007/978-981-99-9617-9_18.
[4] H. Li, S. Tong, H. Wang, and Y. Wang, 'Systematic Investigation of Microbial Desalination Cells (MDC) Performance under the Impact of Cation Migration Behavior and Multi-cation Interactions in Industrial Wastewater', *Electrochemistry*, vol. 91, no. 5, pp. 057007–057007, 2023, doi: 10.5796/electrochemistry.23-00018.

Poster-D05: Geobacter Microbial Fuel Cells for Monitoring System Service in the Public Sector: Microbial Fuel Cells in Marine, River, Wetlands and Coastal Environments.

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Keywords | GEOBACTER, ENVIRONMENT, NATURAL RESOURCES AND SUSTAINABILITY, SERVICE DESIGN FOR PUBLIC SECTOR, BIOINSPIRED DESIGN, IOT

Abstract:

The dissertation will focus on coastal areas and wetlands, especially in the area of renewable and sustainable energy sources through Microbial Fuel Cells (MFC) energy. The concept of ecosystem services appears to be fundamental and implicit in the goal of sustainable development[1]; linked to this is the respect of the biosphere by anthropogenic intervention. Such an approach results in eroding in many species of global biogeochemical cycles leading to an increasing decline of ecosystems, with the need to manage the natural capital of human society in a sustainable manner.
Currently, renewable energy, including in rural settings, remains one of the most important research topics in the public sector. Let's considering a view on Public Administration (PA), the intervention of Service Design in the public sector, land and water resources, and the potential for intervention in environmental respects: an anthropogenic intervention of sustainable and renewable energy revenue is proposed by going to work in cooperation with the ecosystem, inserting itself in a non-invasive, but beneficial way in the soil and sediments of the area. Energy supply that is reliable, efficient, and has less carbon emissions is one of the primary requirements for smart cities.
It is intended to propose a strategic bottom-up study from direct monitoring as a support to coastal sampling, working alongside the reference analysis laboratories of the prepared public bodies, with the relevant stakeholders, for the directives of the territories understood as extraregional for wetlands and coastal zones being able to ensure a capillary system and semi-permeable boundaries between states. One of the objectives of the proposal will be to search for a solution through the tools, techniques and methodologies of service design to dialogue both strategically to the objectives and operationally to the interventions that are to be developed to monitor and safeguard these environments, developing a possible technological solution. These data are collected and aimed at preventing and eliminating harm to: discharges, emissions, leaks of dangerous substances, etc. (Water Management Plan, Water Framework Directive 2000/60/CE). The Microbial Fuel Cell (MFC) technology introduced in the field of inland water quality monitoring is aimed at ensuring the protection of water environments, as opposed to the current more time-consuming and invasive methods of sampling and analysis, by implementing a sustainable, durable and regenerative process for local, off-grid energy production.
The Geobacterial Fuel Cell (GFC) experimentation directly in open environment, partly uses the methodologies of biomimicry and bio-inspired design, from the understanding that biological systems are like databases of sustainable design solutions and innovations that can be used in flood monitoring and forecasting. In this, the study of form; physical, electronic and chemical principles; and derivation of services in isolation, come in handy for the system's inclusion in complex biotopes.

[1] articulated in the 2005 Millennium Ecosystem Assessment. Board, M. E. A. (2005). Ecosystems and human well-being: synthesis of wetlands and water.

Poster-D06: Elementary Analysis of Electrodes Stirring and Shewanella Inoculation in Microbial Fuel Cells for Wastewater Treatment and Power Generation

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As promising and steadily growing areas of science due to their environmentally friendly approach in wastewater treatment and the generation of directly utilized bioelectricity, microbial fuel cells MFCs still facing limitations, such as their low electricity production rate and performance instability, which restrict their widespread use. (Obileke et al., 2021). Among the several proposed optimizations, the electrode materials, and the convenience of the application of operating conditions such as agitation or the addition of a microbial strain represent an interesting aspect to be studied further and evaluated in terms of the energy produced and the treatment result for wastewater.
Cylindrical double chamber glass reactors were used in batch operation, serving as anode and cathode chambers respectively. Substrate sampling for parameter measurements was performed by the end of the experiments. In the H-configuration, the PEM is clamped in the middle of both electrochemical cells and forms a whole system. This single design was used to examine the performance of bioelectricity generation in MFCs and the efficiency of wastewater treatment in MFCs, both under stirring and still combination conditions for cathode and anode. The first part of the experiment examined the power generation and wastewater treatment with conventional microbial community from the denitrification tank of a treatment plant whereas in the second batch the effect of inoculation of the wastewater with the bacterial strain *Shewanella oneidensis* was analyzed. The used electrodes consisted firstly of a stainless-steel mesh (SSM) carbon based composite prepared as proposed in literature (Simeon et al., 2022). The electrochemical cells were initially connected with a resistor and operated in a closed circuit until the stationary growth phase for the microorganisms was attained. Upon reaching stabilization, the external load was disconnected, and the MFCs continued to operate in an open circuit. Electrochemical measurements and chemical parameter analyses were conducted after achieving voltage curve stabilization. The procedure was then repeated, employing a smaller value resistor, accompanied by the conduction of the necessary measurements again. As a comparison in terms of the performance of the electrode materials, CF electrodes with the same size were used instead of the SSM composites, connected also to the first value resistors, but not having the subsequent analysis with a lower resistance. The first investigations revealed that all MFCs with exclusive agitation of wastewater (in the anodes) achieved the highest performance. This could be noticed in the higher values of open circuit voltage (EOC), power and current density (Pd, Jsc), and in the lower internal resistances (Rint). However, when considering the electrode material, CF electrodes delivered better results than the composite electrodes. Particularly in systems without inoculation, similar internal resistances were observed in both materials. Nevertheless, in the series with the addition of *S. oneidensis*, lower internal resistances were measured in CF MFCs. This could be attributed to the inhomogeneity for composite electrodes, related to its manual production, having lower conductivity at certain points on the surface, negatively affecting electron transport. Additionally, material removal during stirring was observed during measurement, which could have also impaired electricity generation. The cleaning efficiency examined by the determination of COD values for all systems over the cleaning period showed a clear decrease for all MFCs. However, due to the significant variation in concentration values among the systems, no concrete statement could be made about the most efficient MFC. Further studies with related conditions are required to better understand and elucidate the underlying mechanisms and to validate the presented observations.

Obileke, K., Onyeaka, H., Meyer, E. L., & Nwokolo, N. (2021). Microbial fuel cells, a renewable energy technology for bio-electricity generation: A mini-review. *Electrochemistry Communications*, *125*, 107003. https://doi.org/10.1016/j.elecom.2021.107003

Simeon, I. M., Weig, A., & Freitag, R. (2022). Optimization of soil microbial fuel cell for sustainable bio-electricity production: Combined effects of electrode material, electrode spacing, and substrate feeding frequency on power generation and microbial community diversity. *Biotechnology for Biofuels and Bioproducts*, *15*(1), 124. https://doi.org/10.1186/s13068-022-02224-9

Poster-D07: Enhancing Microbial Fuel Cell Performance: The Role of Microbial Consortia in Anodic Biofilms for Efficient Electricity Generation and Wastewater Treatment

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Microbial fuel cells (MFCs) are bioelectrochemical devices that harness microbial metabolism to convert the chemical energy stored in organic compounds into electricity. They can serve a dual purpose: producing renewable energy while efficiently treating wastewater. Their performance largely depends on the composition of the anodic biofilm, where electroactive microorganisms facilitate electron transfer to the electrode.

Typically, MFCs employ a top-down approach, allowing natural microbial communities to self-organize and adapt to the electrochemical environment. However, in this study, we adopt a bottom-up approach, where we deliberately construct a synthetic microbial consortium composed of an electroactive bacteria (*Shewanella* oneidensis) that effectively transfers electrons to the anode via extracellular electron transfer (EET) mechanisms, a filamentous fungus like *Ophiostoma* piceae with remarkable enzymatic capabilities for degrading complex organic compounds and a versatile bacteria like *Pseudomonas putida* that boosts the formation of biofilm around the anode to enhance both power output and wastewater purification efficiency.

To evaluate the efficiency of this microbial consortium in the consumption of organic waste, we analyzed the composition and structure of the anodic biofilm using flow cytometry, confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM), correlating it with the efficiency in electricity production. These techniques provided detailed insights into biofilm architecture and cell-electrode interactions, helping to assess the stability and effectiveness of the system.

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Poster-D08: Electro-bioremediation of Bisphenol A with a floating soil microbial fuel cell

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Bisphenol A (BPA) is a compound widely used in polycarbonate plastics and epoxy resins for the manufacturing of a variety of items, ranging from bottles, to kitchen utensils, electronic equipment, food coatings, beverage packaging, adhesives and paints[1]. However, the adverse effects of BPA on human health and the environment have caused concern, since the exposure to this compound has been associated with cases of cancer, obesity, diabetes, heart diseases, neurological and immunological problems and various other hormonal disorders[1]. Furthermore, its degradation is challenging due to its recalcitrant characteristics and high chemical stability[1]. Soil microbial fuel cells (SMFCs) could provide an eco-friendly and self-powered option for the bioremediation of BPA in waters and soils. SMFCs emerge as a promising technology for environmental bioremediation, allowing the depollution of contaminated soils and waters, while generating clean energy[2]. Exoelectrogenic microorganisms play a central role in this process by metabolizing organic compounds and releasing electrons that are transferred to the anode and, finally, to the cathode via an external circuit thus producing electricity[2].

In this study, we explore the ability of a floating SMFC to biodegrade BPA. The SMFC consists of a simple flat plate geometry with the anode submerged in the soil-water slurry and the cathode exposed to air. No membrane is adopted thus resulting in a simple and cost-effective system. The SMFCs are exposed to increasing levels of BPA ranging from 1.0×10^{-3} mg L^{-1} to 10.0 mg L^{-1} and electrochemical tests and fuel cell voltage monitoring over time, are complemented by HPLC-MS measurements to monitor the kinetics of BPA biodegradation over time, as well as the formation of by-products resulting from this degradation. Results are compared with a control system with no SMFC, to confirm the relevance of the electroremediation process in BPA degradation. Experiments are ongoing, nonetheless so far our results show that BPA has a positive effect on the voltage output, thus suggesting that it may be used as a carbon source for the electroactive bacteria. In particular, the voltage output increased by 1.3 times in the presence of BPA, with a value of of 183.7 ± 11.0 and 142.8 ± 7.3 V in the presence and absence of BPA, respectively. A maximum power density of 22.8 ± 3.4 mW m^{-2} and 32.1 ± 1.8 mW m^{-2} was obtained in the MFCs in the absence and presence of BPA, respectively, with an internal resistance (Rint) of changing from 2145.8 Ω (at the start of the tests) to 973.9 Ω and 739.4 Ω in the absence and presence of BPA respectively. Accordingly, the charge transfer increased from 758.2 mC (at the start of the tests) to 1651.5 mC (absence of BPA) and 2878.3 mC (presence of BPA). HPLC-MS tests are ongoing to assess the ability of the SMFCs to electro-bioremediate BPA.

Acknowledgements
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References
[1]Cunha, A. C. *et al*. Int. J. Environ. Sci. Technol. (2025). https://doi.org/10.1007/s13762-025-06351-2.
[2]Dziegielowski, J.; Metcalfe, B.; Di Lorenzo, M. J. Power Sources, v. 515, 230591, 2021.

Poster-D09: Enhancing Anaerobic Biodegradation of plastics (PET) for Energy Recovery in Microbial Fuel Cells

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Microplastics pose a significant threat to marine ecosystems, affecting air and water quality, food resources, and broader ecological processes. The detection of plastics in the human placenta and their potential link to cardiovascular diseases further intensifies concerns. To address this issue, bacteria could be exploited to degrade plastics while simultaneously upcycling the by-products into value-added products such as bioenergy. However, anaerobic biodegradation of plastics—critical for waste management systems like wastewater treatment and landfills—remains poorly understood. This study explores how various pretreatment techniques enhance the anaerobic biodegradation of polyethylene terephthalate (PET), one of the most used types of plastic, and evaluates its potential for conversion into electricity using microbial fuel cells (MFCs), thus integrating waste management with renewable energy production.

The impact of different pretreatment methods—UV, thermal, and chemical—on PET biodegradation in MFC-oriented anaerobic environments was investigated. PET surface modifications were analysed using Fourier transform infrared spectroscopy (FTIR), while degradation efficiency was assessed via weight loss studies and analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS).

Among the tested pretreatment methods, UV-treated PET exhibited the highest biodegradation efficiency, with a weight loss of 29.2% over 40 days. Comparatively, heat-treated PET, chemically treated PET, non-treated PET with microorganisms, and non-treated PET without microorganisms exhibited degradation efficiencies of 21.4%, 20.5%, 11.7%, and 1.3%, respectively. FTIR analysis revealed a reduction in the ester bond, with a notable peak at 1714 cm^{-1} (C=O stretching of the ester group), suggesting the formation of carbonyl-containing oxidation products in UV-treated PET after 40 days. Additional changes in crystallinity and ester hydrolysis were indicated by peak intensity changes at 1240 cm^{-1}. Ethanol and other degradation products were detected through GC-MS and HPLC. MFC performance analysis demonstrated that electricity generation coincided with PET biodegradation. The MFCs fed with UV-treated PET achieved a closed-circuit voltage ranging from 25 mV to 186 mV across a 2200-ohm external resistor, with an anode electrode size of 25 cm^2. The highest power density (1.14 mW m^{-2}) and current density (5.7 mAm^{-2}) were recorded for UV-treated PET. In comparison, chemically treated PET, heat-treated PET, non-treated PET with microorganisms, and non-treated PET without microorganisms exhibited maximum power densities of 0.28 mWm^{-2}, 0.55 mWm^{-2}, 0.20 mWm^{-2}, and 0.13 mWm^{-2}, respectively.

This study provides valuable insights into the potential for upcycling synthetic polymers into useful products, demonstrating that UV pretreatment enhances PET biodegradation and energy (electricity) recovery under anaerobic conditions. These findings have implications for a more sustainable, circular economy by integrating plastic waste management with bioenergy production.

Poster-D10: Anodic Performance of Screen-Printed Electrodes Modified with Polyaniline-Based Hybrid Materials in Biofuel Cells

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In this work, we explore the influence of modifying screen-printed electrodes (SPE) with polyaniline (PAni) and its hybrid with reduced graphene oxide (rGO)[1] on enhancing the bioelectric performance of *Saccharomyces cerevisiae* biofilms. PAni was synthesized via *in-situ* chemical polymerization, and rGO was produced using the modified Hummers method. These materials were characterized and used to modify the graphite-based ink, resulting in batch-modified SPEs. The modified electrodes were then used to investigate the anodic performance in both a single-chamber setup and a compartmentalized biofuel cell (BFC) system. Electrochemical characterization was carried out using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and polarization curves. The results show that modifying the electrodes significantly improved the electron transfer properties of the yeast biofilm. Both PAni and the PAni-rGO hybrid enhanced the formation and stability of the *S. cerevisiae* biofilm on the electrodes. These modifications promoted proton-coupled electron transfer (PCET) dynamics, which are crucial for improving the bioelectric performance of the biofilm. Compared to unmodified SPEs, the PAni and PAni-rGO modified electrodes exhibited higher current densities, demonstrating their potential for optimizing bioelectrochemical systems, especially in BFCs. The extracellular polymeric substances (EPS) produced by *S. cerevisiae* contribute to the enhanced bioelectric performance observed in this study. The EPS not only provide structural stability to the biofilm but also create an environment that facilitates efficient electron transfer. By interacting with the conductive polymer-modified electrodes, the EPS help optimize the proton-coupled electron transfer (PCET) dynamics, which are crucial for improved bioelectric performance. EPS are vital in microbial extracellular electron transfer (EET), as they help establish an electrochemically active environment that supports these processes [2]. This synergy between the conductive polymers and EPS plays a key role in boosting the bioelectric output of the biofilm, demonstrating the potential of PAni and PAni-rGO modified electrodes for enhancing microbial fuel cell applications.

Key-words: Biofuel cells (BFCs), screen-printed electrodes (SPE), polyaniline (PAni), reduced graphene oxide (rGO), *Saccharomyces cerevisiae* biofilm.

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References
[1] ARAÚJO, G. M; CARDOSO, M. A.; CODOGNOTO, L.; BRETT, C. M.A.; SIMÕES, F. R.. Screen-Printed Electrodes based on conductive inks of polyaniline/graphene hybrids and their application to progesterone detection. Ecs Advances, [S.L.], v. 2, n. 1, p. 016504, 1 mar. 2023. The Electrochemical Society.
[2] XIAO, Yong; ZHANG, Enhua; ZHANG, Jingdong; DAI, Youfen; YANG, Zhaohui; CHRISTENSEN, Hans E. M.; ULSTRUP, Jens; ZHAO, Feng. Extracellular polymeric substances are transient media for microbial extracellular electron transfer. Science Advances, [S.L.], v. 3, n. 7, 7 jul. 2017. American Association for the Advancement of Science (AAAS).

Poster-D11: Developing a microbial fuel cell-based sensor for soil quality

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Having healthy soils is key to maximising agricultural productivity, and maximising the productivity of existing agricultural land is vital to addressing two of the challenges we currently face: feeding a growing human population and minimising the amount of natural habitat that is destroyed to create new cropland. Intensive agricultural practices that have traditionally been used to increase the productivity of cropland, such as high applications of synthetic fertilisers, have damaged the natural environment, can degrade agricultural soils and are unsustainable. It is therefore important to develop tools that enable the monitoring of key parameters in agricultural soil to tailor interventions and reduce/prevent uncontrolled use of fertilisers. The soil microbial fuel cell (SMFC) is an emerging technology that harvests energy directly from organic matter in soil, taking advantage of a soil's endogenous electroactive microorganisms. Few studies provide encouraging results on the use of SMFCs as sensors for soil quality monitoring. For SMFC technology to be effectively used as soil sensor, the electrochemical response to different soil conditions, such as content of organic matter, carbon/nitrogen ratio, pH, conductivity, must be properly assessed. We will present our preliminary results on the use of a flat-plate, air-cathode and membrane-less SMFC system as a sensor for soil quality monitoring, First, we investigate the impact that water content, from a water-saturated soil condition to a slurry, has on the SMFC performance. Subsequently, with a design of experiment approach, we vary the organic carbon to nitrogen ratio in soil and investigate how the SMFC with increasing water content can be used as a reliable sensor. In our study, electrochemical measurements are coupled with biological, optical and metabolomic techniques to provide an holistic overview that can support our understanding on the processes involved and inform our sensor development design.

Poster-D12: Maximum Power Point Tracking operation of a Microbial Fuel Cell with Automatic Resistance Switching Device

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Introduction

In microbial fuel cells, the growth conditions of anode microorganisms during the start-up phase are thought to influence the power generation performance of stable phase. However, it has not been investigated adequately. The authors thought Maximum Power Point Tracking (MPPT) Operation might be one possible answer of the optimum growth condition. In this study, we created a device that automatically changes the external resistance so that the microbial fuel cell always generates nearly maximum power. Then operated an MFC from start-up phase to investigete the effect on the power generation capacity after stable phase.

Materials and methods

Single-chamber Microbial fuel cells, with the anode of the previously reported one (ichihashi and Hirooka, 2013) changed from carbon felt to carbon paper, were operated using artificial wastewater with acetate as the substrate. An automatic external resistance switching device was created using a relay and a microcontroller (Figure 1). MPPT operation was performed by measuring and comparing the power of the three resistors, present one, +10% and -10%, once every 30 minutes, and changing to the one with the highest power, and initial resistance was 10kΩ. Power was calculated from the cell voltage and current measured using the 4-terminal method. The control MFC was started with an external resistance of 100 Ω, changed to 10 Ω when the voltage exceeded 500 mV, and remained unchanged thereafter.

Results and Discussion

External resistance of the MPPT-MFC dropped significantly during the initial 25 days, then gradually decreased through the 50th day, and after that, it was mostly stable (data not shown). Figure 2 shows the change over time of the power density of both MFCs. Although there was no clear difference between the current densities of the two MFCs (data not shown), the power density of the MPPT-MFC was higher than that of the control during the period up to about 50 days. The time required for the power density to stop increasing (start-up period) was about 50 days for the control, while it took about 25 days for the MPPT-MFC, about half the time required for the control. These results suggest that MTTP operation will increase the power production during the start-up phase of MFCs, resulting in a shorter start-up period.

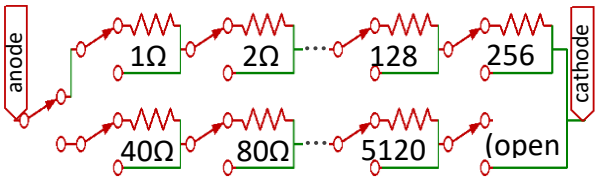

Figure 1 Overview of auto resistor switching system

The upper circuit allows to select from 1 to 511 Ω (1 Ω each), and lower circuit allows open circuit or 40 to 10,200Ω (40Ω each). Switching of the upper and lower circuits and ON/OFF of each resistor was automatically done by relays and a microcontroller.

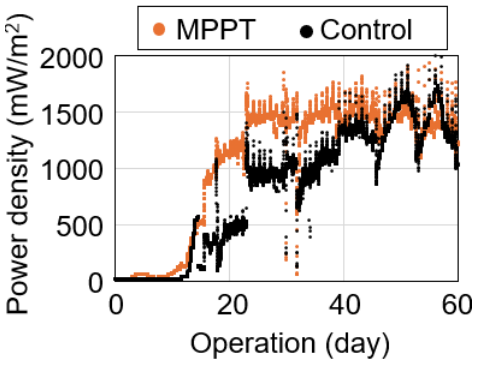

Figure 2 Power density during operation

References

(1) Ichihashi O. and Hirooka K., *Journal of Microbial & Biochemical Technology*, S6, 2013.

Poster-D13: Addressing the Challenges of Scaling Up Microbial Fuel Cells From millilitre to kilolitre for the Treatment of Municipal Wastewaters

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Conventional treatment processes used to treat municipal wastewaters, such as activated sludge treatment are facing a significant number of challenges as it enters the second century of successful deployment. The activated sludge process challenges are it requires a significant amount of energy for aeration, the municipal wastewaters for treatment are becoming more variable in organic load and composition and the regulatory challenges are increasing which require tighter controlled costs whilst achieving reduced greenhouse gas emissions, improved treatment efficiency and the removal of increasing range of emerging pollutants. Microbial fuel cells (MFCs) have been proposed as alternative treatment process technology as they can potentially produce energy thus reducing process energy input, have improved robustness as they are a immobilized biomass process and their unique microbial metabolism can be manipulated to remove a wider range of pollutants. However the scale up and industrial deployment of microbial fuel cells also faces a number of challenges, these include cost reduction for anodes, cathodes and membrane separator, monitoring and control regimes for multiple cathode and anode arrays and maintaining reactor integrity as water head pressure increases as microbial fuel cell volume is extended.

To address these issues we have undertaken a number of design reviews informed by over 12 months of MFC prototype operation and data collection on municipal wastewater sites to develop a number of integrated solutions to develop a scalable lower cost MFC for the treatment of municipal wastewater.

We identified several challenges from the operation on municipal wastewater sites. The municipal wastewater was found to be very variable in strength and composition. The organic load was highly variable and could be as low as 25 mgL Chemical Oxygen Demand (COD) but as high as 700 mgL COD at other times. The municipal wastewater was found to rapidly degrade the performance of a variety of membranes used as separators, even those previously successfully used on synthetic wastewater for over 12 months, failed to maintain performance over a 3-month period on raw sewage. Our conclusion was to develop a membrane less MFC design, with the main target of COD removal but accepting a lower columbic efficiency for the process. To further reduce the cost and increase microbial activity the new MFC design utilized a biocathode as well as bioanode. The bioelectrode width was successfully increased from 100mm to 700 mm in arrays of up to 40 bioelectrodes. The spatial arrangement of the bioanodes and biocathodes was able to eliminate or minimize the impact of water head pressure without the excessive use of support materials. The reactor working volume design was increased from 2 liters to 400 liters whilst maintaining MFC activity. To support the control of the anode and cathode arrays a comprehensive PMS was designed and tested to effectively track the Maximum Power Point (MPP) and recover energy from up to five individual MFCs reactors per control board. The energy obtained from these MFCs was used to provide power to the PCB, with any excess energy being directed towards illuminating an LED. This Power Management System (PMS) provided distinct advantages compared to previously reported systems such as individual and rapid MPPT for up to 5 MFCs per control board with energy harvesting capabilities with a voltage up to 3.3 V at 5.8 mW, a high efficiency of 87%.

In conclusion we have developed a scalable multiarray MFC system design targeted at the treatment of municipal wastewater. We have increased the working volume from 2 liters to 400 liters with the 2 liter unit capable of 70% COD removal at a 24 hour HRT. The 400 liter reactor is currently undergoing operational testing to determine the performance envelope and structural resilience.

Poster-D14: Remediating Organics from Recirculating Aquaculture Systems using Microbial Fuel Cells

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Developing sustainable aquaculture practices is necessary to meet the demands of a growing human population's need for marine protein. Moving fish rearing operations from natural waterways onto land can prevent many of the environmental impacts associated with open pen systems (e.g., eutrophication, habitat destruction, disease). Recirculating aquaculture systems (RAS) raise fish in higher densities while filtering and circulating water in a closed loop, thus they require efficient water purification systems. Inefficient removal of toxic by-products can accumulate over time to unsafe levels that harm or lower the quality of the fish. This results in a lower quality product and limits profitability of RAS. This research seeks to investigate Microbial fuel cells (MFCs) as for treating contaminated affluent in RAS.

MFCs exploit some microbe's ability to access reactants outside the cell - a mechanism that has been suggested by which MFCs could remediate contaminants in aquaculture. Compared to conventional RAS water treatment, MFCs have several potential benefits; they accelerate the decomposition of organic matter in wastewater while simultaneously producing power (Ishii et al., 2013). MFCs do not rely on aeration - an expensive and energy intensive component of the water treatment process (Rozendal et al., 2008). It has been previously demonstrated that SMFCs can accelerate the remediation of sediments contaminated by open pen aquaculture (Algar et al., 2020). We hypothesize that MFCs could also be used to treat the accumulation of organics in RAS.

To test this hypothesis, we will conduct a laboratory-scale proof-of-concept bench top experiment. We will conduct a series of experiments where MFCs are inoculated with fish waste from a local RAS company (Sustainable Blue). MFCs are monitored and compared to open-circuit MFC controls. Organics, biological oxygen demand (BOD), nutrients, and 16S rRNA samples will be taken throughout the duration of the experiment to assess the effectiveness of the MFC. By comparing MFCs and open circuit controls we will test the effectiveness of MFCs for treating commercial RAS affluent. If confirmed, our hypothesis suggests MFCs could reduce the operation costs of remediating and recycling RAS water, making the practice more competitive and sustainable.

Algar C. K., Howard A., Ward C. and Wanger G. (2020) Sediment microbial fuel cells as a barrier to sulfide accumulation and their potential for sediment remediation beneath aquaculture pens. *Sci. Rep.* **10**, 1–12. Available at: https://doi.org/10.1038/s41598-020-70002-4.

Ishii S., Suzuki S., Norden-Krichmar T. M., Wu A., Yamanaka Y., Nealson K. H. and Bretschger O. (2013) Identifying the microbial communities and operational conditions for optimized wastewater treatment in microbial fuel cells. *Water Res.* **47**, 7120–7130..

Rozendal R. A., Hamelers H. V. M., Rabaey K., Keller J. and Buisman C. J. N. (2008) Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol.* **26**, 450–459.

Poster-D15: DREAMing of electrons – Assessing bacterial electron transfer activity in response to oxygen shifts at the cathode of a microbial fuel cell

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Oxygen availability plays a crucial role in the performance of a microbial fuel cell (MFC), exerting distinct influences at the anode and cathode[1]. Owing to its ubiquity and high redox potential, oxygen serves as an effective terminal electron acceptor at the cathode, facilitating the completion of the cathodic reaction. Considerable efforts have been made to develop low-cost catalysts to overcome the sluggish kinetics of the oxygen reduction reaction at the cathode[2]. A strong correlation observed between cathodic oxygen availability and current density in our earlier experiments[3] prompted this study on the dynamics of bacterial electron transfer activity. Bacterial electron transfer activity was assessed using the dye reduction-based electron-transfer activity monitoring (DREAM) assay developed by our group, which measures methylene blue reduction colorimetrically under controlled conditions[4]. A two-chambered MFC was constructed using custom glass chambers (Bright Glassworks, India), carbon cloth electrodes, and a cation exchange membrane (Membranes International, USA). Oxygen availability at the cathode was modulated by alternately switching aeration on and off at regular intervals, and dissolved oxygen levels were monitored using a non-invasive oxygen sensor (Fluorometrix Corp., USA). At the end of each aeration interval, 3 ml samples were drawn from the anode chamber to perform the DREAM assay. Absence of aeration in the anode chamber ensured that the electrons from the bacteria in the samples are available to be transferred to the dye during the assay. Voltage was recorded continuously using a data acquisition module (LabJack U12, USA), and coulombic efficiency was calculated using standard methods. Preliminary experiments using one-hour aeration cycles revealed an increase in the lag time of bacterial electron transfer activity during oxygen depletion at the cathode. Further studies will explore variations in the duration of aeration intervals and correlate numerical parameters from the DREAM assay with cathodic oxygen levels, current density, and coulombic efficiency of the MFC. The insights gained from this study can inform real-time monitoring strategies for bacterial electron transfer activity in MFCs. Quantitative correlations between DREAM assay parameters and cathodic oxygen availability may also aid in optimizing the performance of microbial electrochemical systems under fluctuating environmental conditions.

References

1. Vishwanathan, A. S., Siva Sankara Sai, S. & Rao, G. The oxygen paradox in microbial fuel cells. *Curr. Sci.* **109**, 1390–1391 (2015).
2. Vishwanathan, A. S. Microbial fuel cells: a comprehensive review for beginners. *3 Biotech* **11**, 1–14 (2021).
3. Vishwanathan, A. S., Rao, G. & Siva Sankara Sai, S. A novel minimally invasive method for monitoring oxygen in microbial fuel cells. *Biotechnol. Lett.* **35**, 553–558 (2013).
4. Vishwanathan, A. S., Devkota, R., Siva Sankara Sai, S. & Rao, G. DREAM Assay for Studying Microbial Electron Transfer. *Appl. Biochem. Biotechnol.* **177**, 1767–1775 (2015).

Poster-D16: Bonechar Roll-pressed Air-Cathode for Microbial Fuel Cells

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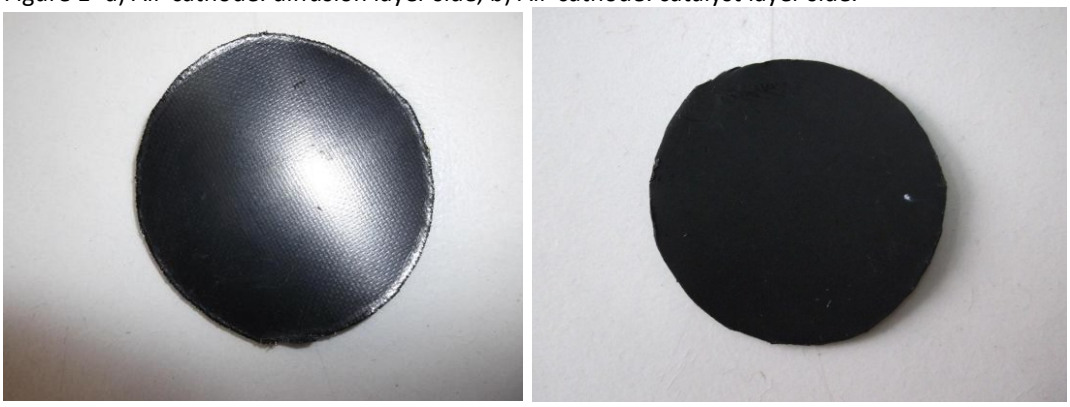
Microbial Fuel Cell (MFC) is a promising technology that can generate electricity and at the same time treat wastewater. Air cathodes are used in MFCs to produce high power from readily available oxygen in the air without the need for wastewater aeration. Catalysts are needed to reduce the overpotential for oxygen reduction, and Pt is commonly used in lab-scale reactors. However, Pt is a very expensive and precious metal, and its catalytic performance can significantly decrease over time due to chemical and biological fouling. Even though many studies were made to lower MFC cost and catalysts, there are still materials that can further reduce cathode costs to push the technology to market. Different materials were tested for new catalysts in MFC, and activated carbon showed a lower price and similar performance compared to Pt cathodes. Since activated carbon can come from different carbon sources, the goal of this study is to analyze the construction of an roll-pressed Bonechar air-cathode considering the cost of bonechar is $2/kg while Pt/C cost $1,130/kg, reducing cathode price 283 times. The rolling press bonechar air-cathod construction process involves an stainless steel mesh, bonechar, carbon black, ethanol, and a 60% PTFE emulsion. The cathode consists of two layers, a catalytic layer and a diffusion layer, which are applied to the steel mesh using a roller, as can be seen in Figure 1. This process aims not only to meet the growing demand for more sustainable energy solutions but also to explore the economic feasibility of this innovative technology on an industrial scale. The use of bovine bone waste as a source of bonechar exemplifies a practical application of the circular economy, utilizing resources sustainably.

Figure 1- a) Air-cathode: diffusion layer side, b) Air-cathode: catalyst layer side.



Poster-D17: Steel industry effluent treatment with Microbial Fuel Cells (MFCs)

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Microbial Fuel Cells (MFCs) represent an innovative solution for concurrent wastewater treatment and electricity generation, addressing energy and water security challenges. This study evaluated two MFCs (MFC-GPT and MFC-HF) treating distinct industrial effluents. Following 30 days of acetate feeding yielding ~500 mV, the cells were transitioned to their respective industrial wastewaters at 20% concentration: GTP Coal Water produced ~45 mV while WTT High Furnace generated ~400 mV (Figure 1).

The effluents differed significantly in composition: HF contained moderate pollutant levels (212.99 mg/L ammonia, 8 mg/L cyanide, 700 mg/L COD), while GTP presented substantially higher concentrations (10,000 mg/L ammonia, 10,000 mg/L COD, 1,000 mg/L phenol). This compositional variance significantly impacted performance, with MFC-HF achieving 81.70% COD removal versus 34.20% for MFC-GPT.

Electrochemical characterization confirmed MFC-HF's superior performance (1.66 A/m² peak current, 649 mV peak voltage, 248.64 mW/m² power density) compared to MFC-GPT (0.18 A/m², 191 mV, 11.62 mW/m²) (Figure 2). Notably, electroactive bacteria in MFC-GPT failed to survive at 40% effluent concentration, highlighting the inhibitory nature of the GTP wastewater on microbial communities.

Figure 1 - Voltage output for (a) MFC-GTP and (b) MFC-HF

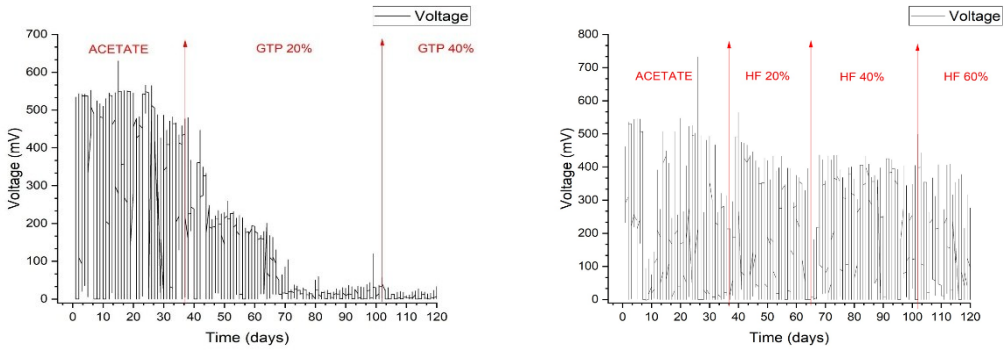
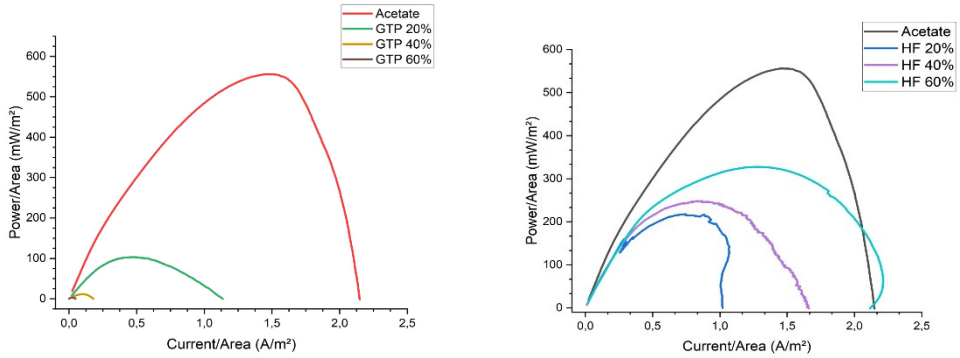


Figure 2 – Power density curves for (a) MFC-GTP and (b) MFC-HF



Poster-D18: Investigation of Ethanol Supplementation on Rice-Washing Wastewater Treatment using a Microbial Fuel Cell

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Rice-washing wastewater (RWW) represents a promising feedstock for renewable electricity generation via microbial fuel cells (MFCs). However, its high chemical oxygen demand (COD, 2000–13000 mg L^{-1}) can inhibit exoelectrogen growth and constrain MFC performance (Lóránt, 2021). Previous reports have shown ethanol supplementation can enhance exoelectrogenic activity (Zhou et al., 2024). This study investigated the effects of ethanol concentration and COD on RWW treatment in single-chamber aircathode (SCAC)-MFCs. RWW sourced from an awamori distillery in Okinawa, Japan, was treated in labscale SCAC-MFCs (230 mL, carbon-brush anode) over 34 d at 25 ° C under 1000 Ω. Six fed-batch treatments (R1–R6) were performed: R1–R3 at 2900 mg L^{-1} COD with ethanol concentra☒ons of 0%, 0.07%, and 0.15% (v/v), and R4–R6 at 8000 mg L^{-1} COD with the same ethanol levels of R1-R3. Prior to treatment, each SCACMFC was incubated with 100 mL anaerobic sludge from commercial MFCs and 100 mL RWW at 25 ° C for 30 d. Samples were collected every 2–3 d for COD and volatile acid measurements, voltage was monitored with a midi LOGGER GL240, methane gas was measured with a Biogas 500, and microbial community structure was analyzed via 16S rRNA gene sequencing. Over 34 d, R2 (2900 mg L^{-1} COD, 0.07% ethanol) achieved the highest COD removal (89% ± 8%), followed by R5 (80% ± 5%), R4 (78% ± 3%), R6 (75% ± 2%),R1 (70% ± 1%), and R3 (63% ± 2%) (Fig. 1a, b) , while the highest average power densities were observed in R5 (576.5 ± 127.0 mW m^{-2}), R6 (482.4 ± 104.9 mW m^{-2}), R1 (446.1 ± 170.2 mW m^{-2}), R2 (400.0 ± 276.6 mW m^{-2}), R4 (254.7 ± 151.2 mW m^{-2}), and R3 (111.4 ± 254.7 mW m^{-2}) (Fig. 2). These results indicate that 0.07% ethanol effectively enhances organic removal, in agreement with Pan et al. (2017), whereas higher ethanol (0.15%) reduces efficiency due to microbial toxicity (Kim et al., 2007; Paz-Mireles et al., 2019). Higher COD concentrations led to lower organic removal but increased power output, consistent with Quintero-Diaz and Gil-Posada (2024). Compared to R2, R5 showed faster ethanol depletion (177.8 ± 8.9 mgCOD L^{-1} d^{-1}) (Fig. 5), suggesting that continuous ethanol supply at 0.07% is necessary under high COD conditions. Additionally, R5 showed higher methane yield (6.0 mgCODmethane gCODremoved^{-1}) (Fig. 3) and a 2.3-fold increase in Methanosaeta (Fig. 4), indicating methanogen proliferation may contribute to accelerated ethanol degradation. Overall, these findings highlight that controlled ethanol addition at 0.07% (v/v) can enhance RWW treatment in SCAC-MFCs, and suggest that incorporating ethanol fermentation as a pretreatment could further improve performance under higher COD conditions.

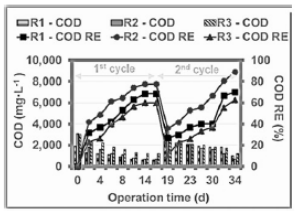

Fig. 1a COD removal (R1-R3)

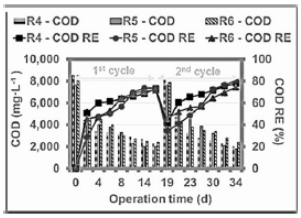

Fig. 1b COD removal (R4-R6)

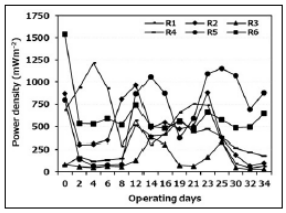

Fig. 2 Power density (R1-R6)

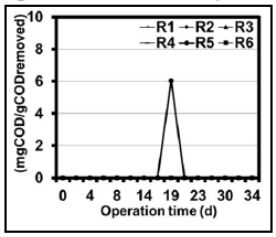

Fig. 3 Methane gas (R1-R6)

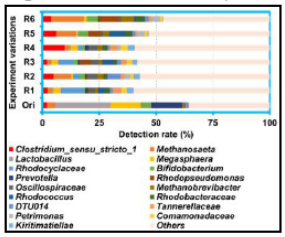

Fig. 4 Microbial community

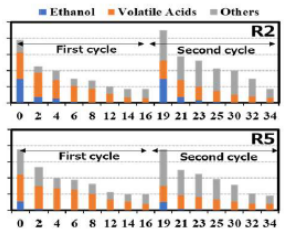

Fig. 5 COD balance (R2, R5)

Poster-D19: Complete treatment of domestic wastewater and energy generation in a microbial fuel cell coupled with nitrifying and denitrifying biocathodes

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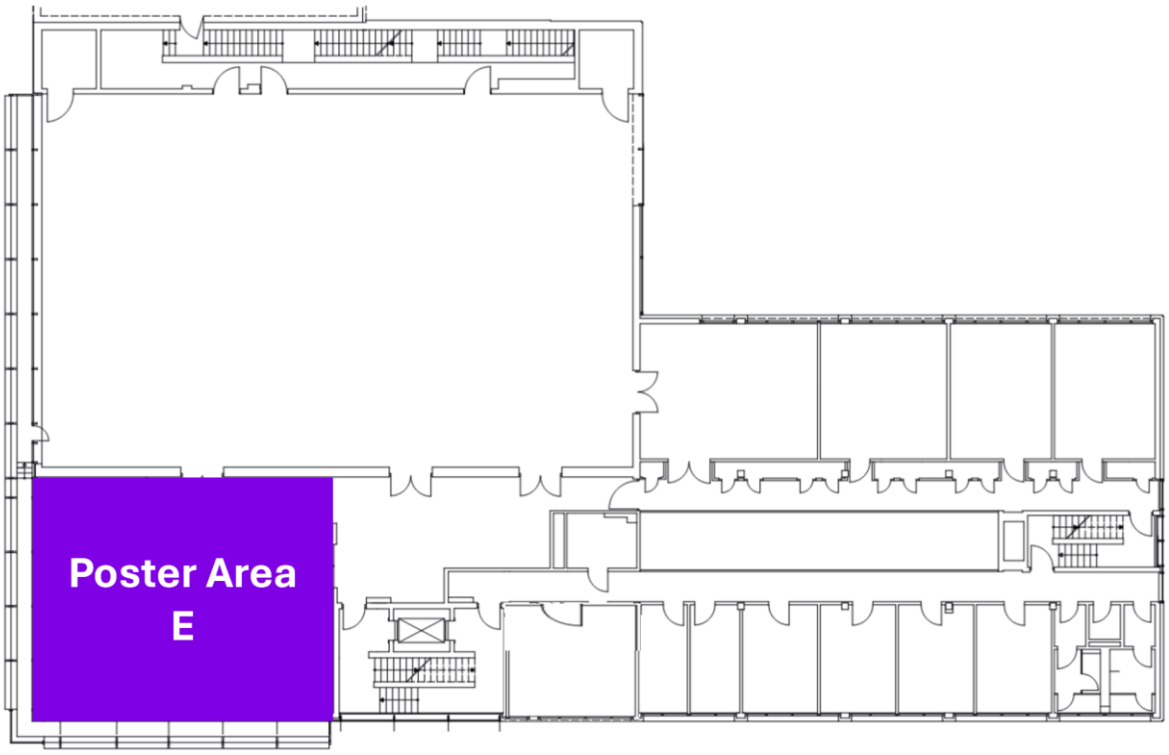
Microbial metabolism on biocathodes can be used to produce useful compounds or remove unwanted products such as ammonia and nitrate from wastewater [1]. In this research, a coupled dual MFC system consisting successively of an aerobic nitrifying biocathode MFC (N-MFC) and an anaerobic denitrifying biocathode MFC (D-MFC) was used for the simultaneous removal of carbon and nitrogen from domestic wastewater. Each dual-chamber MFC unit consisted of a niobium-modified granular activated carbon (GACNb) anode and an unmodified raw granular activated carbon (GAC) cathode, separated by a proton exchange membrane. The anode and cathode chambers of the same volume (120 ml) were inoculated with raw domestic wastewater supplemented with nutrients, micronutrients, and sodium acetate to normalize 1 gCOD.L-1. The experiment was conducted in 2 phases. The first phase consisted of separate individual operation of the 2 MFCs for inoculation and adaptation of specific microbial communities. After 8 weeks of independent operation, the N-MFC and D-MFC moved to the second phase, electrically connecting one anode to the other and one cathode to the other in parallel. From a hydraulic point of view, the D-MFC anode was fed with domestic wastewater and its effluent was directed to the N-MFC anode to complete the oxidation of the more complex and less biodegradable organic matter. This effluent was then recirculated to the aerated cathode chamber of the N-MFC to be used as an electrolyte contributing to oxygen reduction. The objective was also to develop nitrifying bacteria to oxidize ammoniacal nitrogen into nitrite and nitrate. The effluent from the nitrifying cathode (N-MFC) was directed to the denitrifying cathode (D-MFC). During all phases, the MFCs were operated in continuous flow. The efficiency of the twin MFC system was evaluated using physicochemical and electrochemical methods, such as: COD, TN (total nitrogen), IC (ion chromatography), pH, conductivity, DO (dissolved oxygen), potential, EIS and polarization curve. The electrodes were characterized by XRD, XRF, Raman and MIP, in addition to microbiological characterization.
The XRD, XRF and RAMAN of the electrodes showed characteristics of mesoporous and/or amorphous materials. The XRD indicates that niobium is in its amorphous state or in uniform dispersion in the GAC. In addition, the XRF shows that the proposed percentages of niobium (w/w) were obtained and indicates that the methodology used was adequate. In the Raman, a band was attributed to the stretching vibration of the Nb=O double bond. The analyses indicated that the synthesis process combined the GAC and Nb materials instead of replacing them. The MIP suggested that increasing Nb resulted in a decrease in porosity, total pore area, and total intrusion volume. On the other hand, increasing Nb increased the average pore diameter. In terms of performance, the maximum power densities were 34.7 $W.m^{-3}$ for N-MFC and 11.2 $W.m^{-3}$ for D-MFC. N-MFC showed a coulombic efficiency of 17%, while D-MFC was 4.5%. The average COD removal rates reached 80% at the outlet for both. For total nitrogen removal, including anodic and cathodic processes, the average was 38% for N-MFC and 32% for D-MFC. According to the extracted DNA analysis, *Proteobacteria* and *Firmicutes* were the predominant bacterial phyla in the anodic chamber. In the biocathodes of the N-MFC, the genus with the highest relative abundance was *Nitrosomonas*, whereas in the D-MFC, *Dokdonella* accounted for 30% of the bacterial community.
The coupling phase of the N-MFC and D-MFC is currently in progress, with data being collected and analyzed. The results are being processed and will be presented at the conference.

[1] Edson Baltazar Estrada-Arriaga, Jesús Hernández-Romano, Liliana García-Sánchez, Rosa Angélica Guillén Garcés, Erick Obed Bahena-Bahena, Oscar Guadarrama-Pérez, Gabriela Eleonora Moeller Chavez,
Domestic wastewater treatment and power generation in continuous flow air-cathode stacked microbial fuel cell: Effect of series and parallel configuration, Journal of Environmental Management, Volume 214, 2018, Pages 232-241, ISSN 0301-4797, https://doi.org/10.1016/j.jenvman.2018.03.007.

POSTER PRESENTATIONS

Environmental BES - Bioremediation and Soil Systems
- Area E -



2nd Floor

Poster-E01: Synergistic Microplastic Degradation In Wastewater: How Can Electrochemical Oxidation Enhance Biodegradation?

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Microplastics (MPs) accumulation is a pervasive environmental issue that poses risks to human health, wildlife, and ecosystems. WWTP effluent is a major pathway for MPs to infiltrate ecosystems, contributing 37% of MPs entering the world's oceans[1], despite the ability of WWTPs to remove a substantial fraction of these particles. Removal strategies in WWTPs rely in physical separation of MPs into sludge rather than their degradation, which is problematic since sludge can be used as a fertilizer.

This study presents a bioelectrochemical platform targeting the degradation of polyethylene (PE) and polypropylene (PP), two widely used thermoplastic polymers known for their high resistance to biodegradation. The platform currently consists of two separate steps: an Electro-Fenton process to pre-oxidize plastics, enhancing biodegradability by modifying their surface[2]; followed by a bioreactor for their biodegradation. First, Electro-Fenton is performed in a single-cell reactor with a stainless-steel cathode and titanium metal mixed oxide anode, operating at 0.5 A for 5 hours with an iron concentration of 10 mg/L. Changes in the biodegradability of plastic samples were subsequently tested using an aerobic enriched culture for PE and PP degraders. Plastic modification and degradation were assessed through weight loss, particle distribution, and FT-IR, while biodegradability was evaluated by monitoring oxygen consumption with plastic as the sole carbon source over 21 days.

Tests were performed with granular pure PE (200–500 µm) and naturally weathered plastics from an agricultural site (PE and PP, 1 cm^2 pieces). None of the plastics tested showed significant weight loss after 5 hours of Electro-Fenton treatment and in 21 days of biological degradation. However, in granular pure PE, particle distribution analysis showed disaggregation into smaller particles. Similarly, FT-IR analysis revealed that Electro-Fenton induced significant structural changes and introduced functional groups (e.g., ester, ketone, acid, vinyl) in the polymer structure in tested plastics. Biodegradation tests (BOD measurements in 21 days, Figure 1) suggested Electro-Fenton pretreatment enhanced plastic biodegradability, increasing the BOD threefold the in pure PE, twofold in real PE, and fourfold in real PP. Our findings suggest Electro-Fenton can be a promising methodology to enhance plastic biodegradability by accelerating the weathering of highly recalcitrant polymers.

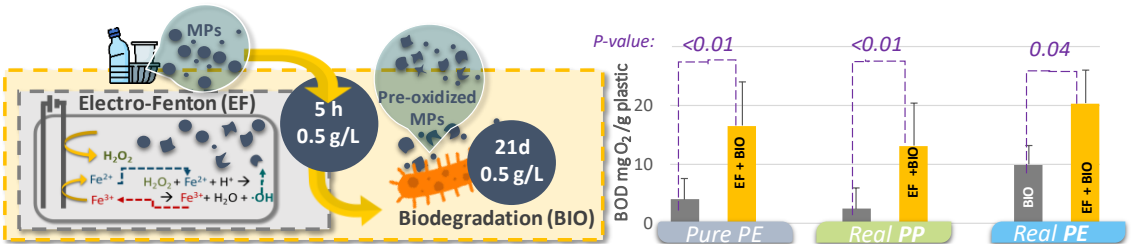

Figure 1: (Left) Summary of the overall treatment. (Right) Main results of plastic biodegradation without pretreatment (gray bar) and after electro-Fenton (EF) pretreatment (yellow bar).

References:

1. Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. 43pp. https://doi.org/10.2305/IUCN.CH.2017.01.en

2. Zhang, Y., Pang, X., Yang, Y., & Lv, Y. (2022). Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery. J. Clean. Prod., 350, 131508. https://doi.org/10.1016/j.jclepro.2022.131508

Acknowledgments: This work was funded by the EU's Horizon 2020 (NYMPHE project, No. 10106625). S.P. is a Serra Hunter Fellow (UdG-AG-575) and acknowledges the ICREA Academia award. LEQUiA [2021-SGR-01352] and Ecoaqua [2021-SGR-1142] are consolidated research groups recognized by the Catalan Government.

Poster-E02: Electro-bioremediation for the removal of Total Petroleum Hydrocarbons from groundwater (300 mL to 3L scale)

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Groundwater contamination by hydrocarbons in industrial areas constitutes a major environmental issue, threatening the health and integrity of aquifers and surface waters. Based on bioelectrochemical systems (BES), electro-bioremediation is an interesting approach for removing pollutants from contaminated waters, combining both biological and electrochemical processes. BES take advantage of electroactive microorganisms, attached to the electrode surface, that can exchange electrons with solid electrodes. Catalyzed by these microorganisms, oxidative reactions occur at the anode and reductive reactions at the cathode, also stimulating naturally occurring microbial degradation processes.

The use of BES for the removal of total petroleum hydrocarbons (TPH) in groundwater has been recently explored within the BIOSYSMO project. In the initial phase, single-chamber BES reactors (VidraFoc, Spain) were adopted, with a carbon fibers brush as anode material (9.7 m^2 anode/m^3 reactor) and unidirectional carbon fibers (UDCFs, SGL, Germany) with an oxygen reduction reaction catalyst (Pajarito powder) as cathode. These were operated in batch mode, using 300 mL of groundwater as feed. The anode potential was poised at +0.6 V vs Ag/AgCl, and two different cathode configurations were tested: air-diffusion cathode and liquid-cathode (no air diffusion), with duplicates for each. The reactors achieved removals of over 90% BTEX and ~35% of TPHs under both configurations. Negative average removals of C_5-C_{10} TPHs in all cases proved the breakdown of large chain TPHs into smaller molecules. However, the liquid cathode reactor was chosen for the next experiments, due to better performance in C_{10}-C_{40} TPHs elimination (73 % vs. 57% removal in the air-diffusion cathode reactor).

In the second phase, a scale-up experiment was performed. Three flat plate BES reactors were constructed (2 electro-stimulated, one control), using once again carbon fiber brushes as the anode (64.6 m^2 anode/ m^3 reactor) and a liquid-cathode of UDCFs + catalyst. This change in cell architecture, from H-type to flat plate cells, was performed for enhancing biomass growth stability. Each BES cell is connected to a 3L buffer tank containing polluted groundwater. The initial experiments were conducted in batches with a duration of 7 days. Total and C_{10}-C_{40} TPHs removals increased to 69% and 93% respectively, while BTEX was almost eliminated (99% removal). Notably, a significant increase in C_5-C_{10} TPH removal (42 %) was also observed, hinting at possible mineralization of some of the TPHs. An average current density of 0.017 A/m^2 was obtained in this phase, which was higher than in the previous one. Currently, operational conditions are being optimized in terms of operation time and electrochemical operation. Next steps of the project involve further study of reactions involved within the BES, and installing a BES reactor at pilot-plant scale.

Overall, this study demonstrates that electro-bioremediation can be effective in treating hydrocarbon-contaminated groundwaters from industrial areas. In addition, the proven advantages of using a liquid-cathode configuration in terms of higher degradation rate and lower energy consumption highlight the potential use of the proposed technology for *in-situ* treatments.

Acknowledgements: Financial support by H2020 project BIOSYSMO contract No. 101060211 is greatly appreciated.

Poster-E03: Integration of Biochar and Bioelectrochemical Systems for the Remediation of TCE-Contaminated Groundwater

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Groundwater contamination by chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethylene (TCE), poses a critical challenge for environmental remediation due to their persistence, toxicity, and mobility. In particular, the presence of separate phases and secondary sources complicates the application of single-strategy approaches. Bioelectrochemical systems (BESs) and bio-based materials such as biochar are emerging as promising solutions for developing sustainable permeable reactive barriers.
In this study, we propose a combined BES-biochar system in which biochar (pinewood pyrolysis at 1040 °C) acts as an adsorbent material, capable of containing contaminant plumes, while simultaneously, the BES provides a continuous supply of electron donors, supporting microbial growth and metabolic activity. This synergy enables the degradation of contaminants adsorbed onto the biochar surface, thus regenerating its active sites and preventing saturation, reducing the risk of breakthrough and extending the functional lifespan of biochar. The experimental setup includes three identical BES reactors (each 380 mL) (Figure 1-a): each BES (Figure 1-a) filled with sand (grain size 0.4–0.8 mm); a second control with sand and biochar (4% w/w) operated under open-circuit condition; and a test column filled with sand and biochar in a polarized BES. The BES employs a concentric three-electrode configuration, with a central graphite rod anode wrapped in graphite felt and enclosed in a filter tube to prevent short circuits, and an external cathode also made of graphite felt (Figure 1-b, 1-c). All columns have been inoculated with a dechlorinating microbial culture and hydraulically characterized. BES operation has been performed with a TCE-contaminated synthetic medium, monitoring the removal of TCE and its degradation products and microbial community dynamics. This integrated approach aims to improve the durability and performance of adsorbent materials, minimize breakthrough phenomena, and offer a sustainable and effective solution for in situ remediation of chlorinated solvent-contaminated groundwater.

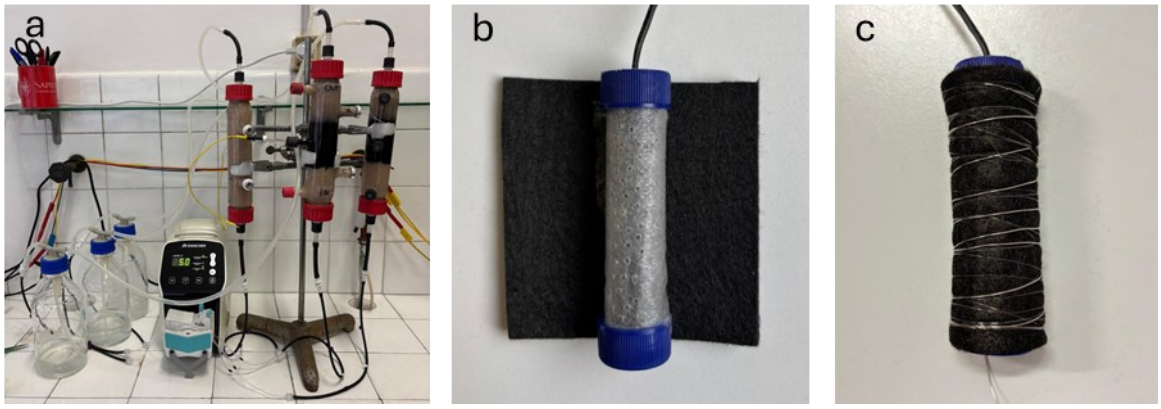

Figure 1: Experimental platform setup (a), counterelectrode (b) and working electrode configuration (c)

Acknowledgements: This work has been conducted as part of the RETURN (Multi-risk science for resilient communities under a changing climate) project (PE00000005), funded under the framework of the National Recovery and Resilience Plan (PNRR), Mission 4 "Education and Research" - Component C2, Investment 1.3 NextGenerationEU.

Poster-E04: Electroassisted Bioleaching for Cobalt Recovery from Mine Tailings in a Single Chamber Reactor

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Mining plays a crucial role in the economic development of low- and middle-income countries, contributing 13.7% to Chile's GDP in 2022. However, the sector faces significant challenges, including declining ore grades, rising energy costs, and environmental risks associated with tailings. Chile alone has over 700 tailings deposits, representing approximately 2,263 million tons of material, including an estimated 17.3 kT of unexploited cobalt reserves in the Antofagasta Region, valued at around USD 459 million. Given that global demand for cobalt is expected to double by 2040, and that 73% of current supply originates from the Democratic Republic of Congo, diversifying cobalt sources is a critical priority. Bioleaching is considered an environmentally friendly and economically attractive alternative for metal extraction from low-grade ores. It relies on the metabolic activity of specialized microorganisms capable of oxidizing reduced compounds in mineral matrices, enabling the solubilization of valuable metals. These microbes thrive in highly acidic environments (pH < 3.0), and among the most studied are iron- and sulfur-oxidizing bacteria such as Acidithiobacillus ferrooxidans. This bacterium typically uses the aerobic oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), or the oxidation of reduced sulfur compounds, as its primary energy source. However, A. ferrooxidans is metabolically versatile and has been classified as electroactive due to its capacity to grow by directly utilizing electrons from a polarized electrode, potentially coupled to either oxygen or ferric iron (Fe^{3+}) reduction.
The objective of this study was to investigate cobalt recovery from mine tailings through oxygen-free electro-assisted bioleaching in a single-chamber reactor. To achieve this, bioleaching experiments were conducted using a pure culture of A. ferrooxidans over 30 days under three conditions: bioelectrochemical systems (BES), aerobic, and anoxic. BES reactors were equipped with 2.5 × 2.5 cm^2 carbon plates as both anode and cathode, connected to a power source set at Δ0.4 V. Reactors had a working volume of 600 mL, were inoculated with 15 mL of pre-cultivated strain, and operated in 9K medium supplemented with 5% (w/w) tailings.
Cobalt recovery in BES reached 187.7 ± 65.7 mg/L by the end of the experiment—77% higher than under anoxic conditions (44.0 ± 38.2 mg/L) and 55% lower than under aerobic conditions (425.0 ± 35.3 mg/L). Regarding total iron, BES maintained 75.0 ± 4.1% of the initial iron in solution, with a Fe^{3+}/Fe^{2+} ratio of 0.86 ± 0.25. In contrast, under aerobic and anoxic conditions, total dissolved iron decreased steadily to 26.7 ± 6.8% and 3.1 ± 2.0%, respectively. These differences reflect a combination of microbial activity and salt precipitation influenced by the physicochemical conditions. However, it can be hypothesized that polarized electrodes help maintain iron in solution through constant redox cycling, enhancing its availability for microbial metabolism. These findings are preliminary, and additional experiments are currently being completed to provide further clarity and validation of the observed trends.

References

1. F.F. Roberto, A. Schippers, Progress in bioleaching: part B, applications of microbial processes by the minerals industries, Appl Microbiol Biotechnol 106 (2022) 5913–5928. https://doi.org/10.1007/s00253-022-12085-9.
2. J. Mäkinen, M. Salo, M. Khoshkhoo, J.E. Sundkvist, P. Kinnunen, Bioleaching of cobalt from sulfide mining tailings; a mini-pilot study, Hydrometallurgy 196 (2020). https://doi.org/10.1016/j.hydromet.2020.105418.
3. T. De Campos Rodrigues, M.A. Rosenbaum, Microbial Electroreduction: Screening for New Cathodic Biocatalysts, ChemElectroChem 1 (2014) 1916–1922. https://doi.org/10.1002/celc.201402239.

Poster-E05: Cobalt Complexes Enable Electron Transfer Between the Organohalide Respiratory Complex of *Dehalococcoides mccartyi* and an Electrode

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Dehalococcoides mccartyi strain CBDB1, a model organism for energy conservation *via* organohalide respiration (OHR), couples extracellular electron transfer (EET) to proton translocation across the membrane, enabling ATP generation *via* ATPase to support metabolism and growth. EET, occurring either through direct interaction between the microorganism and the external material or *via* redox-active mediators, allows microbial respiration to be coupled to external electron acceptors, such as electrodes, making bioelectrochemical cultivation possible (Kato, 2015). In *D. mccartyi*, EET/respiration is driven by the membrane-bound OHR complex consisting of three functional modules, where the reductive dehalogenation of halogenated compounds by RdhA is coupled to hydrogen oxidation by the hydrogen-uptake hydrogenase HupL. Electron transfer between RdhA and HupL is facilitated by metallocofactors of the OHR complex forming a conductive 'wire' (Kublik et al., 2016; Seidel et al., 2018).
To identify suitable anode mediators for the OHR complex, we tested various classes of mediators and found that cobalt complexes were readily reduced in an *in vitro* methyl viologen-based activity assay. To verify that this reduction was catalyzed by the OHR complex, we conducted activity assays using molecular hydrogen as the electron donor, showing that cobalt complexes can serve as alternative electron acceptors in the OHR process, replacing halogenated compounds. Cyclic voltammetry using gold and indium tin oxide (ITO) working electrodes showed that these chelates are capable of exchanging electrons with electrodes. Notably, methyl cobaloxime(III) and methyl cob(III)alamin showed preferential redox activity on gold electrodes, undergoing both reduction and oxidation. Moreover, bioinformatic analyses predicted that methyl cobaloxime(III) binding to the active site of RdhA is thermodynamically favorable, with a binding energy of –28 kJ mol^{-1} (Eberwein et al., 2024).
In summary, our findings demonstrate that cobalt chelates can be reduced by the OHR complex, most likely *via* RdhA, as supported by experimental data and bioinformatic predictions. These results highlight the potential of cobalt chelates as electron shuttles for the bioelectrochemical cultivation of *D. mccartyi*.

References:

Eberwein, M., Hellmold, N., Frank, R., Deobald, D., Adrian, L. (2024). Reductive dehalogenase of *Dehalococcoides mccartyi* strain CBDB1 reduces cobalt-containing metal complexes enabling anodic respiration *Front. Microbiol.* 15, art. 1457014 10.3389/fmicb.2024.1457014

Kato, S. (2015). Biotechnological aspects of microbial extracellular electron transfer. *Microbes. Environ.* 30, 133–139. doi: 10.1264/jsme2.ME15028

Kublik, A., Deobald, D., Hartwig, S., Schiffmann, C. L., Andrades, A., Bergen, M., et al. (2016). Identification of a multi-protein reductive dehalogenase complex in *Dehalococcoides mccartyi* strain CBDB1 suggests a protein-dependent respiratory electron transport chain obviating quinone involvement. *Environ. Microbiol.* 18, 3044–3056. doi: 10.1111/1462-2920.13200

Seidel, K., Kühnert, J., and Adrian, L. (2018). The complexome of *Dehalococcoides mccartyi* reveals its organohalide respiration-complex is modular. *Front. Microbiol.* 9:1130. doi: 10.3389/fmicb.2018.01130

Poster-E06: Electrochemical Disinfection of Free-Swimming Parasites; Free Chlorine and Electric Field as Potent Disinfection Agents for *Schistosoma mansoni* Cercariae

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Electrochemical disinfection is emerging as a key water treatment method for isolated communities, where cost, transport, storage and handling of chemicals can be a hurdle. Although electrochemical disinfection has been proven effective against feacal bacteria and viruses[1], no reports on electrochemical disinfection of free-swimming parasites exists at present. Such parasites are *Schistosome* cercariae, agents of the parasitic disease schistosomiasis, known also as bilharzia. Second only to malaria in prevalence, schistosomiasis affects more than 240 million people, the strike majority of which live in rural communities in sub-Saharan Africa[2]. Control of schistosomiasis in endemic areas mainly relies on chemotherapy, but WHO guidelines emphasize the importance of access to safe water and adequate sanitation to break the reinfection cycle[3].
Braun et al. 2020[3], the only recent study on water treatment against cercariae, suggested chlorination with a minimum 1 mg/L residual chlorine and 30 mg·min/L contact time (CT) for cercaria inactivation. Inactivation was judged as loss of cercariae motility [3]. Cercariae are consisted of a head and a tail (Figure 1), with the latter aiding cercariae motility required to penetrate the human skin and cause infection.

Using this CT value as design guideline, we have set experiments with three variable parameters: 1) initial Cl^- concentration (78, 140, and 350 mg/L), 2) applied current (10, 50, 100 mA) and 3) temperature (20°C vs. 28°C). Additionally, we have tested two different electrodes: 1) Ru MMO, the best catalyst for chlorine evolution and 2) graphite, suitable for low-cost applications. Disinfection experiments were performed in a 250 mL non-divided electrochemical reactor connected to a DC power supply. The electrolyte was synthetic lake water with and without 10^3 freshly shed cercariae. Cercariae viability was evaluated under a stereoscope.

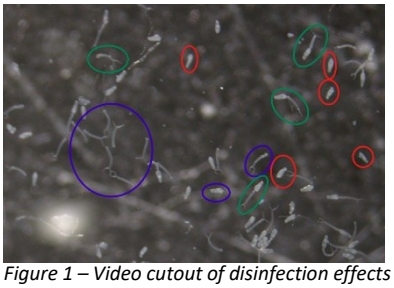


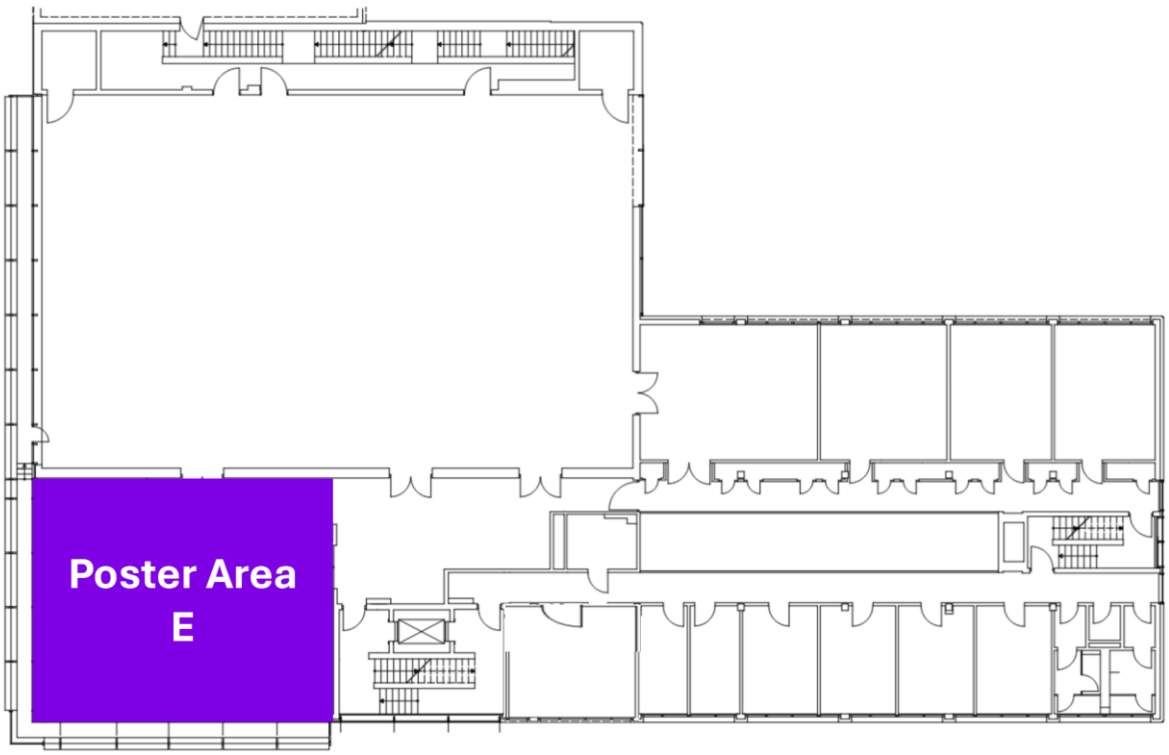
Figure 1 – Video cutout of disinfection effects after 60 min electrolysis with Ru MMO electrodes at 50mA. The motility and loss of thereof was observed and video recorded at 4× magnification under a stereoscope. Intact (but non-motile) cercariae are circled in green, the separated tails with blue and the separated heads with red.

Working with two Ru MMO electrodes for 30 minutes at 50 mA, 28 ℃ and 78 mg/L initial Cl^- concentration, we obtained 1.7 (±0.5) mg/L free chlorine. Under these conditions, we observed complete loss of cercariae motility. After extending the electrolysis time to 60 min, we observed definite inactivation indicated by increased numbers of single heads and tails (Figure 1). Furthermore, we noticed an intermediate effect of the electric field alone on cercariae motility. Specifically, when applying a current of 10 mA for 30 minutes, or when using graphite electrodes at 50 mA for 30 min, the cercariae exhibited sluggish movement, even in the absence of detectable free chlorine. We are currently extending our tests to investigate this phenomenon further and provide a reliable data source pertaining to water interventions targeting parasitic diseases and specifically, cercariae inactivation through electrolysis.

1. Atrashkevich A et al. *Water Res*. 2024;250(November 2023). doi:10.1016/j.watres.2023.121014
2. Anyolitho MK et al.of western Uganda. *PLoS Negl Trop Dis*. 2022;16(2):1-21. doi:10.1371/journal.pntd.0010190
3. Braun L et al. *PLoS Negl Trop Dis*. 2020;14(8):1-16. doi:10.1371/journal.pntd.0008665

POSTER PRESENTATIONS

Environmental BES - Nitrogen Cycling
- Area E -



2nd Floor

Poster-E07: Assessment of Biocathode Denitrification and the Key Governing Factors: A Systematic Review

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Bioelectrochemical reactors can be a robust wastewater treatment solution with simultaneous organic and NOx removal from wastewaters if it is coupled with an efficient denitrifying biocathode. During the last 10 years, a significant amount of research on denitrifying biocathodes was published, demonstrating the increasing interest in this research field. However, the nitrate removal rates in the available literature show large variations from 0.03 to 23.52 g-N.d^{-1}.m^{-2} [1,2]. When analyzing biocathode denitrification efficiencies from the literature, a large distribution of performances is also observed with mean nitrate removal of 63.9±30.5%. So, what could explain such a drastic difference in biocathode denitrification performance?

This presentation aims to provide a systematic literature review (as per the methodology adopted by de Fouchécour et al., 2022) [3], based on a homogenized database that would reflect on the key parameters affecting the biocathode denitrification performance.

It is noteworthy that there are no standard indices to report the biocathode denitrification performance (e.g. reported as per unit area or unit volume of electrode or per unit reactor effective volume); which increases the complexity of result analysis. For a better documentation of the key factors governing cathode efficiency, a systematic literature review is conducted through three major steps: (i) collection of enough data, (ii) homogenization of raw data, and (iii) careful statistical investigation of the key governing factors.

Thus, this presentation aims to provide a systematic review based (Fig. 1) on a rectified and homogenized database to reflect on the impacts of influent characteristics (influent type, nitrate/nitrite concentration, pH), reactor configurations (reactor volume, types of electrodes and membrane, inoculum), and operational conditions (cathode potential, temperature, HRT, effluent feeding) on the bioelectrochemical NOx removal performance. This approach aims at constituting a consistent database to perform statistical analysis to identify the key governing parameters for biocathode denitrification.

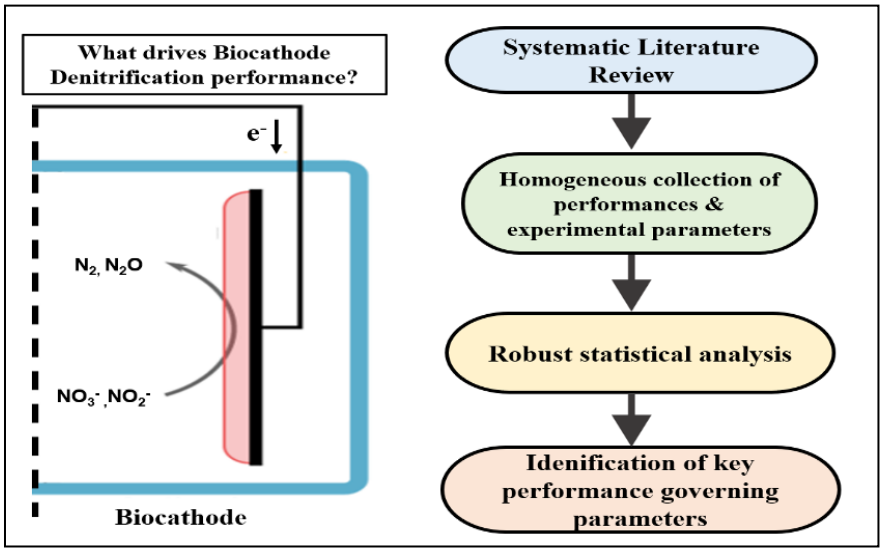

Fig. 1 Process diagram for identification of key factors governing biocathode denitrification

Keywords: autotrophic denitrification, denitrifying biocathodes, systematic literature review.

References:

1. Zhang, Y. and Angelidaki, I., 2013. A new method for in situ nitrate removal from groundwater using submerged microbial desalination–denitrification cell (SMDDC). Water research, 47(5), pp.1827-1836.
2. Kondaveeti, S., Kang, E., Liu, H. and Min, B., 2019. Continuous autotrophic denitrification process for treating ammonium-rich leachate wastewater in bioelectrochemical denitrification system (BEDS). Bioelectrochemistry, 130, p.107340.
3. de Fouchécour, F., Larzillière, V., Bouchez, T. and Moscoviz, R., 2022. Systematic and quantitative analysis of two decades of anodic wastewater treatment in bioelectrochemical reactors. Water Research, 214, p.118142.

Poster-E08: Efficient Ammonium Removal from Coastal Sediments at Low Temperatures via Sediment Microbial Fuel Cells

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Over the past few decades, the influx of inorganic nitrogen into coastal ecosystems has more than doubled due to increasing human activities, posing serious ecological risks, including eutrophication, hypoxia, and biodiversity loss. Various strategies have been developed for sediment remediation, such as oxygenation, phytoremediation and dredging (Zhang et al., 2020). However, these methods are often expensive, act only for a short period, or may lead to secondary pollution. In contrast, sediment microbial fuel cells (SMFC) offer a promising, sustainable alternative for treatment. Previous work demonstrated that SMFC are efficient in preventing release of H_2S, nitrogen and phosphorus from harbor sediments at temperate temperatures (Brock et al., 2023). However, the Baltic Sea suffers from cold bottom temperatures in winter, so SMFCs still need to be demonstrated as a technology resilient to seasonality in cold regions.

Thus, two SMFC were set at 10°C using stainless steel or charcoal as anodes, both using stainless steel cathodes. Four reactors were filled with approximately 5 L of sediment and 15 L of artificial seawater each. Foam panels were used as barriers preventing oxygen penetration in the sediments yet allowing water exchange along the column. Anodes were buried 3 cm below the sediment surface, while the cathode was positioned at the column top. The electrodes were connected by isolated cables to a 1000 Ω resistor. Controls used the same set-up but with open circuits.

During the first 74 days, the bottom dissolved oxygen (DO) was kept above 1 mg/L, and for the final 78 days, it was kept below 1 mg/L. Voltage and physicochemical parameters were monitored periodically throughout the study, while microbial diversity was analyzed at start and end of the experiment.

The results indicated that both SMFCs removed about 75% of the ammonium released to the water by the controls, with the stainless-steel electrode showing faster removal. Notably, N_2O emissions remained negligible. Nitrate accumulated below the control ammonium levels, suggesting overall N removal. Additionally, when bottom water DO dropped below 1 mg/L, SMFC with charcoal electrodes promoted higher releases of methane and hydrogen sulfide from the sediments. Overall, the SMFC with stainless steel electrodes performed superior in terms of sediment remediation efficiency.

Brock, A.L., Kostadinova, K., Mørk-Pedersen, E., Hensel, F., Zhang, Y., Valverde-Pérez, B., Stedmon, C., Trapp, S., 2023. Remediation of marine dead zones by enhancing microbial sulfide oxidation using electrodes. Marine Pollution Bulletin, 193, 115142.

Zhang, Y., Luo, P., Zhao, S., Kang, S., Wang, P., Zhou, M., Lyu, J., 2020. Control and remediation methods for eutrophic lakes in the past 30 years. Water Sci. Technol. 81, 1099–1113.

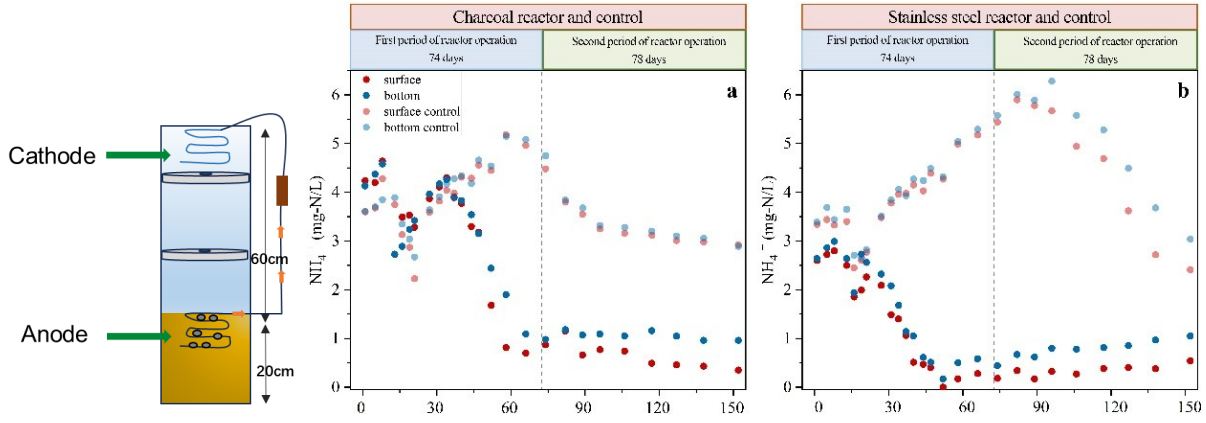

Fig. 1 Sediment batch incubation diagram. NH_4^+ concentrations in surface and bottom water.

Poster-E09: Enhanced Bioelectrochemical Removal of Nitrate from Contaminated Groundwater using Conductive Graphite Particles

Daniele Cabras[1], Seyedmehdi Hosseini[2], Matteo Tucci[3], Federico Aulenta[3], Stefano Milia[1]
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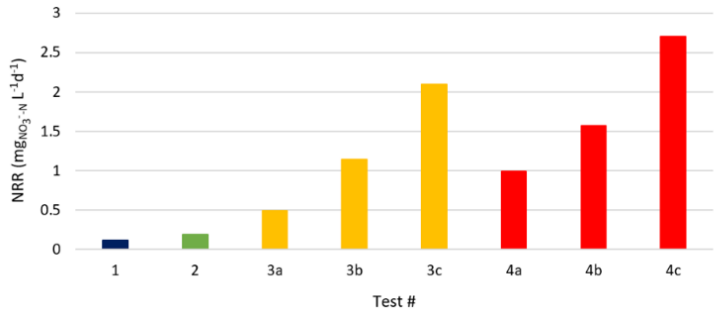
Bioelectrochemical systems (BES) have been proven to be a valid alternative for the remediation of nitrate-contaminated groundwater (Ceballos-Escalera et al., 2024; Puggioni et al., 2022). However, the *in-situ* application of electro-bioremediation is constrained by the limited radius of influence of the electrodes (Aulenta et al., 2025). In this study, the possibility of adding micronized graphite to increase the electroactive zone in a sandy aquifer was investigated, using a two-chamber BES fed with real nitrate-contaminated groundwater. The cathodic and anodic chambers were separated by a cation exchange membrane. Both chambers had an empty volume of 128 mL, and they were filled with 85 mL of commercial sand (d <2mm). Micronized graphite (particle size <20 µm) was added to sand at a 1% ratio (w/w). Graphite rods (d=0.5 cm) were used as bio-cathode and anode and were connected to a potentiostat. The bio-cathode was poised at -500 mV vs Ag/AgCl. The cell was operated in batch mode with intense recirculation. The cathodic and anodic chambers were fed with real groundwater (NO_3^--N, 25±2 mg L^{-1}) and tap water, respectively. Activated sludge was used as the inoculum in tests #3a and #4a. Groundwater and tap water were replaced in tests #3b,c and #4b,c. As shown in Figure 1, the presence of micronized graphite enhanced nitrate removal rates (NRR), which progressively increased up to 2.70 $mg_{NO_3^-N}$ $L^{-1}d^{-1}$, compared to sand alone (up to 2.10 $mg_{NO_3^-N}$ $L^{-1}d^{-1}$).



Test #	Duration (d)	NRR	Circuit	Inoculum	Graphite
1	20	0.12	Open	No	Yes
2	20	0.19	Closed	No	Yes
3a	20	0.49			
3b	20	1.14	Closed	Yes	No
3c	12	2.10			
4a	20	0.99			
4b	16	1.57	Closed	Yes	Yes
4c	10	2.70			

Figure 1. Nitrate removal rates in batch tests with different operating conditions

The presence of NO_2^--N, NH_4^+-N, and N_2O was not detected, suggesting complete conversion of nitrate into dinitrogen gas. The observed coulombic efficiencies were above 100%, likely due to the contribution of non-nitrate electron acceptors and/or the occurrence of side reactions at the bio-cathode. Abiotic tests with micronized graphite showed negligible nitrate removal, compared to the biotic ones. These results are promising and encourage the addition of conductive particles in soils to improve *in-situ* autotrophic denitrification using BES. Further investigation is needed to assess the long-term performance of the process under varying operating and hydraulic conditions.

Acknowledgements. DC, SM, MT, FA acknowledge financial support under the National Recovery and Resilience Plan (NRRP), M.4,C.2,I.1.1, Call for tender No. 104 published on Feb. 2, 2022 by the Italian Ministry of University and Research (MUR), funded by the EU – NextGenerationEU – Project Title: SteeRing GroundwatEr Electro-bioremediAtion with ConducTIVe ParticlEs (REACTIVE) – CUP: B53D23018110006 - Grant Assignment Decree No. 1048 adopted on Jul. 14, 2023 by MUR.

References

Aulenta et al., 2025. Environ. Sci. Ecotech., Doi: 10.1016/j.ese.2024.100516.
Ceballos-Escalera et al., 2024. Chemosphere, 352, 141370. Doi: 10.1016/j.chemosphere.2024.141370.
Puggioni et al., 2022. Sci. Total Environ., 845, 157236. Doi: 10.1016/j.scitotenv.2022.157236.

Poster-E10: Seasonal effects on denitrifying bioelectrochemical systems: Impact of dynamic flows and reactor reactivation after prolonged starvation

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Introduction

Denitrifying bioelectrochemical systems (d-BES) have been proven to efficiently reduce harmful nitrate (NO_3) to harmless dinitrogen gas. Hydrogen (H_2) is produced in-situ through water electrolysis and utilized by cathodic biofilms as electron donor (M. Xu et al., 2025). d-BES can be utilized to enhance the performance of engineered systems, such as constructed wetlands, treating NO_3 contaminated wastewater with low C/N ratios (D. Xu et al., 2017). These systems, namely bioelectrochemically assisted constructed wetlands, are exposed to dynamic fluctuations in hydraulic retention times (HRTs) due to rainfall variation during winter season, leading to accumulation of denitrification intermediates, and dry periods during summer season, with potential emissions of greenhouse gases due bacterial degradation. The main objectives of the study are to (i) evaluate the response of d-BES biofilms to prolonged no flow periods, simulating shut-down of reactors during summer periods, (ii) assess the reactivation of the reactors after no flow periods, and (iii) evaluate effect of variable HRTs during winter rainfall season.

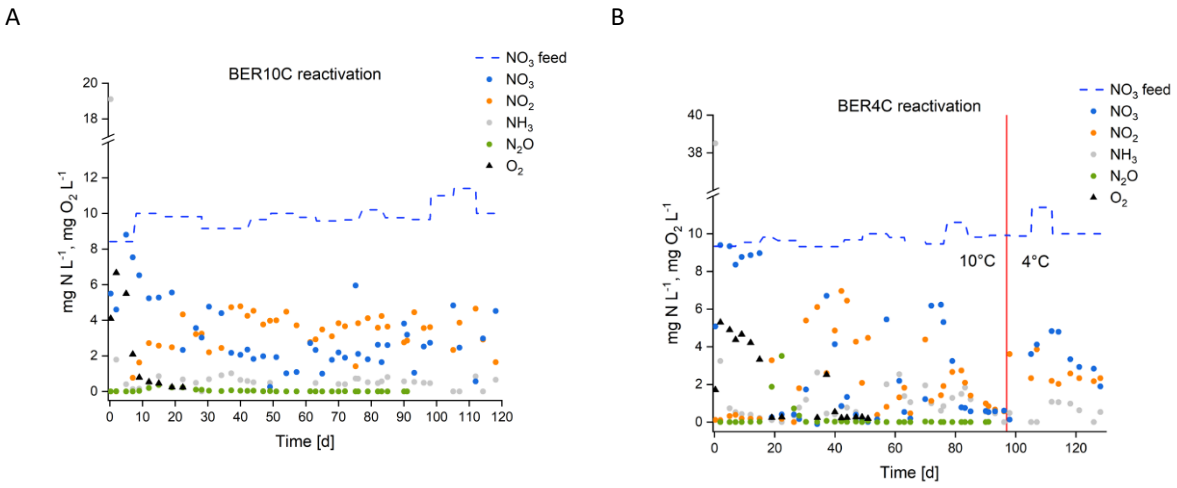

Figure 1: BER10C (A) and BER4C (B) performance during reactivation period.

References

Xu, D., Xiao, E., Xu, P., Zhou, Y., He, F., Zhou, Q., Xu, D., & Wu, Z. (2017). Performance and microbial communities of completely autotrophic denitrification in a bioelectrochemically-assisted constructed wetland system for nitrate removal. *Bioresource Technology*, *228*, 39–46. https://doi.org/10.1016/j.biortech.2016.12.065
Xu, M., Savio, F., Kjærgaard, C., Jensen, M. M., Kovalovszki, A., Smets, B. F., Valverde-Pérez, B., & Zhang, Y. (2025). Inorganic bioelectric system for nitrate removal with low N2O production at cold temperatures of 4 and 10 °C. *Water Research*, *274*, 123061. https://doi.org/10.1016/j.watres.2024.123061

Poster-E11: Nitrogen recovery from digestate using a Microbial Electrolysis Cell and hydrophobic membrane pilot plant

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Anaerobic digestion (AD) is a well-established process for the effective management of livestock manure, combining organic matter degradation with energy recovery through biogas production. The resulting effluent, known as digestate, retains valuable nutrients such as nitrogen, representing an important resource for plant fertilization. However, direct application of digestate can lead to nitrogen losses and environmental pollution. Therefore, efficient nutrient recovery techniques are essential to enhance its fertilizing potential and reduce its environmental footprint. Bioelectrochemical systems (BES) represent a promising technology to recover nutrients from AD digestate [1].

This study investigates the performance of a laboratory-scale three-compartment MEC and a pilot plant with three MEC modules designed to recover ammonium from digestate. The lab-scale MEC configuration, of 600 mL volume per compartment, includes a carbon felt anode, a stainless-steel mesh cathode, and a cation-exchange membrane (CEM - Ultrex CMI-7000, surface of 20 cm^2) to facilitate selective ammonium migration from the digestate to the catholyte. The catholyte consists of a NaCl (0.1 g/L) solution where ammonium is accumulated. To prevent ammonium volatilization due to pH increase, the ammonium is subsequently transferred from the NaCl solution to an acidic solution (H_2SO_4 10% v/v) using a flat hydrophobic membrane.

A preliminary test was conducted with the lab-scale MEC fed with digestate from an AD reactor operating at thermophilic condition. The substrate of the reactor was a mix of pig slurry with vinasse. The MEC achieved a nitrogen removal rate of 31% from the digestate, achieving a maximum flux across the hydrophobic membrane of 61.4 g $N/m^2/d$.

Building upon these results, a pilot-scale system has been developed, consisting of three double-compartment MEC units of 1 L per compartment, electrically connected in parallel, with a feeding rate of 1.5 L/d of digestate. An initial current of 35 mA was applied to the MEC system by an external power source. The catholyte solution is circulated continuously to promote efficient ammonium capture. When the pH of the catholyte increases over a value of 8, due to ammonium migration from the digestate, the catholyte is automatically recirculated through a hydrophobic membrane contactor to allow the migration of ammonia into the acidic solution and the immobilization of nitrogen as ammonium. The pilot system aims to evaluate the scalability of this technology and improve ammonium recovery efficiency for potential fertilizer production.
These findings contribute to the optimization of BES technology for digestate treatment and nutrient recovery, highlighting its potential applicability in sustainable agricultural practices.

ACKNOWLEDGEMENTS:
This project has received funding from the European Union's Horizon Europe Research and Innovation programme under Grant Agreement No. 101112708. The support of the CERCA Program and of the Consolidated Research Group of Sustainability in Biosystems (ref. 2021 SGR 01568), both from the Generalitat de Catalunya, is also acknowledged.

REFERENCES:
[1] Cerrillo, M., Riau, V., & Bonmatí, A. (2023). Recent advances in bioelectrochemical systems for nitrogen and phosphorus recovery using membranes. *Membranes*, *13*(2), 186.

Poster-E12: Ammonia recovery using a 65-litre microbial electrolysis cell with sludge return liquor

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During thermal hydrolysis-anaerobic digestion (THP-AD), proteins are hydrolyzed, which releases large quantities of ammonium into the aqueous phase. Dewatering digested sludge generates a high strength side-stream (return liquor) that has total ammonia nitrogen (TAN) concentrations between 0.5 – 2.5 g L^{-1} [1]. These side-streams increase TAN loading onto the activated sludge process, leading to greater energy consumption and potentially poorer final effluent quality. Integrating ammonia recovery technologies, such as microbial electrolysis cells, into these streams can extract valuable reactive nitrogen as fertilizers and lower anthropogenic nitrous oxide emissions. Previous research has used synthetic and real liquor as a substrate for TAN recovery with lab-scale microbial electrolysis cells. It has been reported that these liquors lack biodegradability for exoelectrogens to generate high currents to remove ammonium [2]. However, it is not clear whether the liquors used in these studies were derived from THP-AD sludges, which could make a difference since THP increases COD solubilization [1] which is more favorable for the exoelectrogens to yield higher currents.

This research assessed the performance of a 65-litre microbial electrolysis cell for TAN recovery at a wastewater treatment facility over a 5-month period using a mixed return liquor (soluble COD = 1001 ± 252 mg L^{-1}, TAN = 246 ± 31 mg L^{-1}, which was partly derived from THP-AD digested sludge centrate, raw centrate and thickening filtrate. The system contained 3 flat-plate electrode cassettes [3] which comprised carbon felt anodes (0.0728 m^2 $cassette^{-1}$) and stainless steel wool cathodes (9.9 ± 0.7 g $cassette^{-1}$). Phase 1 of the experiment involved sampling the mixed return liquor over a 3-week period (n = 6) for volatile fatty acids, TAN, cations and COD to determine the theoretical ammonium recovery potential based on electron equivalents. Phase 2 evaluated TAN recovery rates, catholyte pH, ion transport, COD removal and Coulombic efficiencies at E_{ap} between 0.55 to 1 V and open-circuit (control) conditions. Each condition was applied over a 1-week period with daily sampling of the anolyte influent/effluent and catholyte. The system was operated under continuous conditions with a 1-day hydraulic retention time (0.065 m^3 d^{-1}).

Based on acetic acid content, the average ammonium recovery potential was 1.0 ± 0.2, indicating the liquor had enough electrons contained in the acetic acid to recover all the TAN present (assuming 100% Coulombic efficiency and 100% COD removal). The maximum TAN recovery rate was 1.7 ± 0.2 g m^{-2} d^{-1} at E_{ap} = 1 V and current densities between 0.09 to 0.15 A m^{-2} (total current = 6.6 to 14.6 mA) yielded alkaline catholytes that were suitable for extracting ammonia. Similar COD removal efficiencies were observed between the closed-circuit conditions (7.5 – 26.5 %) and open-circuit conditions (10.7 %) which suggested that there was significant competition for organic substrate between exoelectrogens and other metabolic processes, e.g., methanogenesis. As a result, the produced current densities were ineffective for transporting ammonium across the cation exchange membrane (load ratio = 0.03).

1. Devos P, Filali A, Grau P, Gillot S. Sidestream characteristics in water resource recovery facilities: A critical review. Water Research 2023;232. doi:https://doi.org/10.1016/j.watres.2023.119620.
2. Barua S, Zakaria BS, Chung T, Hai FI, Haile T, Al-Mamun A, et al. Microbial electrolysis followed by chemical precipitation for effective nutrients recovery from digested sludge centrate in WWTPs. Chemical Engineering Journal. 2019;361:256-65. doi:https://doi.org/10.1016/j.cej.2018.12.067.
3. Heidrich ES, Edwards SR, Dolfing J, Cotterill SE, Curtis TP. Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12-month period. Bioresource Technology. 2014;173:87-95. doi:https://doi.org/10.1016/j.biortech.2014.09.083.

Poster-E13: Understanding Nitrous Oxide Emissions in Bioanodic Ammonium Oxidation and Strategies for Mitigation

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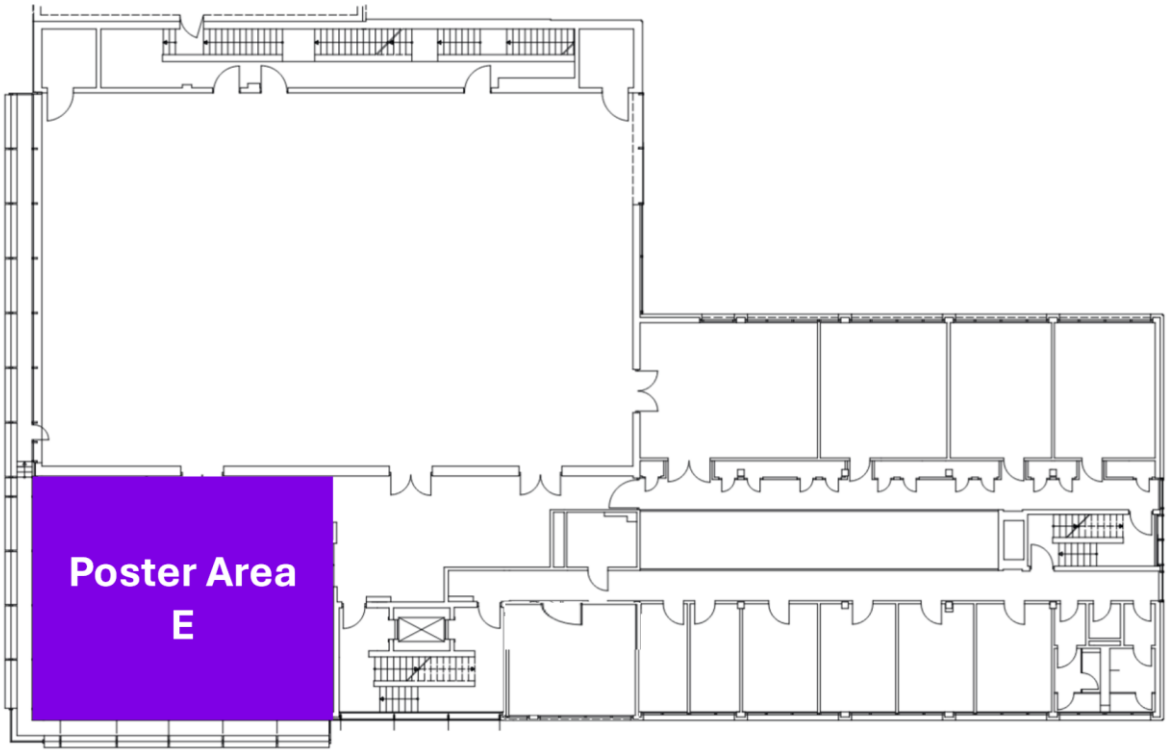
Keywords: Ammonium oxidation, bioanodes, nitrous oxides emission, nitrifying bacteria.

Anoxic ammonium (NH_4^+) oxidation at bioanodes offers an alternative to biological nitrogen removal (BNR) process by eliminating aeration and enabling direct nitrogen gas (N_2) production without additional treatments. However, the production of nitrous oxide (N_2O), an unwanted byproduct from the BNR process that contributes to global warming, is not yet fully understood. This study first assesses N_2O emissions during anoxic NH_4^+ oxidation at a polarized bioanode (+0.55 V vs Ag/AgCl) and investigates the underlying pathways through batch experiments using key intermediates, hydroxylamine (NH_2OH) and nitrite (NO_2^-). The involvement of different microbial in N_2O production was also analyzed through communities analysis and nitrifier inhibitor tests. Results indicate that up to 40% of oxidized NH_4^+ is converted into N_2O, and nitrifying bacteria are the core functional group for N_2O production. NH_2OH oxidation was identified as the dominant pathway, likely involving nitric oxide (NO) as an intermediate, with its reduction leading to N_2O production. NO_2^- reduction also contributed to N_2O generation but likely to a lesser extent. N_2O reduction is observed in situ and can be enhanced by increasing electron donor (H_2) availability, effectively eliminating N_2O to an undetectable level (<0.01 mg-N/L), which suggest potential strategies for mitigating N_2O emissions, such as reactor configuration adjustments to incorporate cathodic H_2 evolution. Overall, this study provides key insights into N_2O formation pathways, offering guidance for advancing bioelectrochemical systems toward more sustainable ammonium removal technologies.

POSTER PRESENTATIONS

Microbial Electrosynthesis
- Area E -



2nd Floor

Poster-E14: Sequential C1-Fermentation of *Acetobacterium woodii* and *Corynebacterium glutamicum* for CO_2-based biomass and amino acid production in an H-type microbial electrosynthesis cell

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Climbing global temperatures caused by increasing atmospheric CO_2 -levels demand a multitude of diverse technologies capable of fixating CO_2 from already existing point sources, e.g. biogas plants, fossil fueled power plants, or fossil fueled transportation. Microbial electrosynthesis (MES) is a promising technology to tackle this problem, as it relies on the capabilities of auto- and mixotrophic microorganisms to fixate CO_2 into organic compounds driven by regenerative electricity serving as energy source for their metabolism. However, two important aspects have not been addressed in detail yet: narrow product spectra and unexploited oxygen in the anodic chamber. Hence, this work focuses on establishing a CO_2 -based sequential C1-fermentation that utilizes both MES chambers to produce biomass and amino acids.
At this point in time, the H-type MES has been constructed and a glucose-based cultivation of *Corynebacterium glutamicum* in the anodic chamber could be implemented without additional oxygen supply. Parallel, first trials for co-substrate utilization of wild-type *C. glutamicum* have shown the capability to consume different C1/C2 acids and alcohols, e.g. acidic acid, formic acid, ethanol, and methanol, within certain boundaries. Furthermore, cultivations of *C. glutamicum* with C1/C2 acids and alcohols as sole carbon sources will be implemented to validate the feasibility of the sequential C1-fermentation. In subsequent experiments the cultivation of *Acetobacterium woodii* in the cathodic chamber will be conducted. As the cultivation progresses, the cathodic broth will enrich with organic acids, mainly acidic acid, through CO_2-fixation. This broth will then be extracted and delivered into the anodic chamber. Here, it can be metabolized by *C. glutamicum* into biomass and amino acids.
A CO_2-based sequential C1-fermentation within a MES cell provides an important step towards a more holistic utilization of the MES-technology, as it requires both chambers and potentially reduces oxygen diffusion into the cathodic chamber. Additionally, the utilization of the anodic chamber offers the implementation of traditional biotechnological productions hosts as oxygen is prevalent such fermentations.

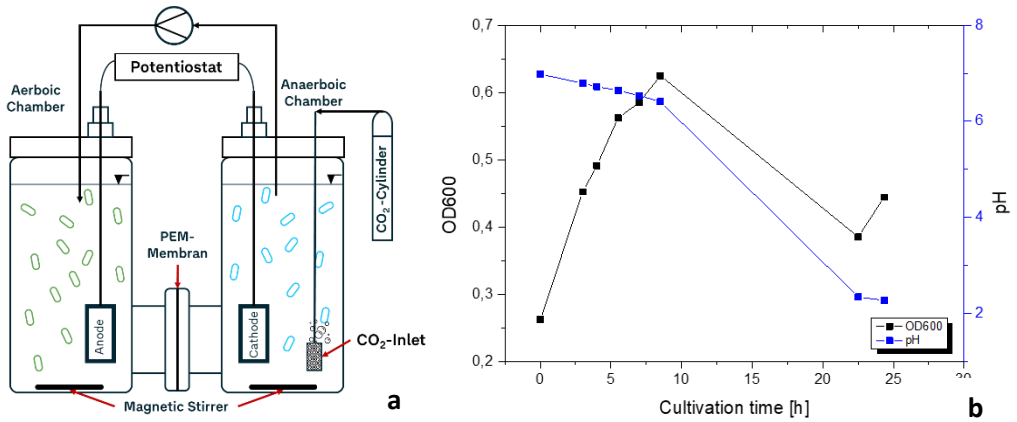

Figure 1: Depiction of current reactor setup and cultivation trial. a) Schematic H-type reactor setup with anodic or aerobic chamber and cathodic or anaerobic chamber. Both, anode and cathode are depicted in the corresponding chamber. CO_2 is delivered via gas cylinder and aeration stone into the cathode chamber. In future cultivations, culture broth can be pumped from the cathodic to the anodic chamber by peristaltic pump. b) Growth profile of wild-type Corynebacterium glutamicum grown in the aerobic chamber of the reactor configuration. Showing the optical density (OD) at 600 nm and the pH values of the cultivation broth over cultivation time.

Poster-E15: Improved reactor design enables productivity of microbial electrosynthesis on par with classical biotechnology

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Microbial electrosynthesis (MES) converts (renewable) electrical energy into CO_2-derived chemicals including fuels. To achieve commercial viability of this process, improvements in production rate, energy efficiency, and product titer are imperative. Employing a compact plate reactor with zero gap anode configuration and NiMo-plated reticulated vitreous carbon cathodes substantially improved electrosynthesis rates of methane and acetic acid. Electromethanogenesis rates exceeded 10 L $L^{-1}_{catholyte}$ d^{-1} using an undefined mixed culture. Continuous thermophilic MES by *Thermoanaerobacter kivui* produced acetic acid at a rate of up to 3.5 g $L^{-1}_{catholyte}$ h^{-1} at a titer of 14 g/L, surpassing continuous gas fermentation without biomass retention and on par with glucose fermentation by *T. kivui* in chemostats. Coulombic efficiencies reached 80 %–90 % and energy efficiencies up to 30 % for acetate and methane production. The performance of this plate reactor demonstrates that MES can deliver production rates that are competitive with those of established biotechnologies exceeding this limit may be not considered.

Poster-E16: Enhancing Dark Fermentation of Cheese Whey via Bioelectrochemical Systems

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Dark fermentation is a promising anaerobic process for converting organic wastes—such as industrial byproducts—into biohydrogen and volatile fatty acids (VFAs). However, its efficiency is often limited by metabolic bottlenecks, including byproduct accumulation and incomplete substrate conversion. The integration of a bioelectrochemical system (BES) to modulate redox conditions has emerged as a strategy to overcome these challenges by steering microbial metabolism toward higher yields and selectivity.

This study implemented a 0.5 L glass bottle BES reactor with a working electrode (anode) poised at +1 V vs. the counter-electrode (cathode), for the dark fermentation of cheese whey using digested sewage sludge as inoculum. The system's headspace was connected to a gas trap for continuous volume quantification and composition analysis, enabling real-time monitoring of hydrogen evolution. Comparative experiments evaluated conventional dark fermentation (DF) against bioelectrochemically-assisted dark fermentation (BEDF) under identical organic loading conditions.

Remarkably, the BEDF system achieved a 202% increase in cumulative hydrogen production relative to the non-electrified control, demonstrating the profound influence of electrochemical stimulation on microbial activity. Additionally, shifts in the VFA profile suggest that the applied potential redirects metabolic fluxes, offering further opportunities for process optimization (Fig. 1). These findings highlight the potential of electrofermentation to transform organic waste streams into higher-value products while improving process efficiency.

Future work will focus on elucidating the microbial mechanisms behind the observed metabolic shifts and optimizing operational parameters (e.g., applied potential, hydraulic retention time) to maximize both hydrogen yield and product selectivity. This research provides critical insights into sustainable waste-to-energy technologies, bridging the gap between bioelectrochemistry and industrial bioprocessing.

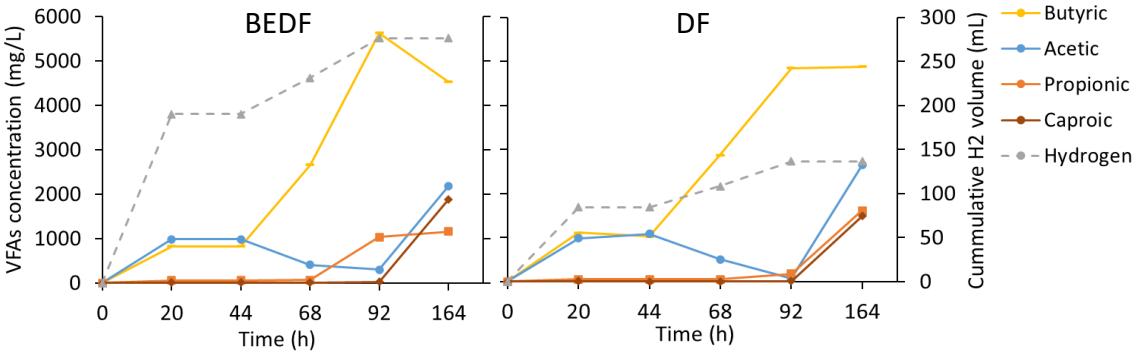

Fig. 1. VFAs concentration and cumulative hydrogen production in bioelectrochemical dark fermentation (BEDF) vs. conventional dark fermentation (DF).

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Poster-E17: Utilizing Humin as Insoluble Extracellular Electron Mediator to Enhance Hydrogen-Mediated Microbial Electrosynthesis of Organic Compounds from CO_2

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The potential of the hydrogen-mediated microbial electrosynthesis system (HMES) has been shown as a reliable and eco-friendly approach for reducing anthropogenic CO_2 emissions and production of CO_2- driven green organic compounds. However, challenges such as low productivity and energy efficiency have hindered the scalability of HMES for practical applications (Aryal et al., 2017). Humin, insoluble extracellular electron mediator, has been used to enhance various microbial activities such as microbial reductive dechlorination of pentachlorophenol, denitrification, nitrogen and CO_2 fixation (Zhang, D. et al., 2014; Xiao et al., 2016; Dey et al., 2022; Laskar et al., 2020). This study aimed to increase performance of HMES with addition of humin. The study utilized HMESs, which were H-shaped reactors, that were inoculated with *Acetobacterium woodii* DSM 1030 to investigate the impact of humin on organic compounds production efficiency from CO_2. The HMESs were operated in a semi-batch mode over a period of 98 days, divided into four stages under different conditions named semi-batch 1, semi-batch 2, semi-batch 3, and semi-batch 4. The initial cathodic potential in all semi-batches was set at -1010 mV vs. Ag/AgCl, then different cathodic potentials were applied: -810 mV vs. Ag/AgCl in semi-batches 1 and 2, -900 mV in semi-batch 3, and constant at -1010 mV vs. Ag/AgCl in semi-batch 4. The primary products in HMESs were acetate and formate. In all tested batches, acetate and/or formate production were found to be higher in HMESs with humin compared to HMESs without humin. The performance of acetate and/or formate production in semi-batch 1 and 2 was significantly lower than in semi-batch 3 and 4. The highest titer of acetate production, reaching 5.19 g/L, and acetate production rate of 188 mg/L/day were observed in semi-batch 3. Similarly, the highest titer of formate production, with 8.44 g/L, and formate production rate of 323 mg/L/day were observed in semi-batch 4. These findings suggest that the addition of humin to HMESs is an effective strategy to enhance HMES performance.

Key words: humin, CO_2, hydrogen-mediated microbial electrosynthesis system (HMES).

References

Aryal, N., Ammam, F., Patil, S.A. and Pant, D., 2017. An overview of cathode materials for microbial electrosynthesis of chemicals from carbon dioxide. Green chemistry, 19(24), pp.5748-5760.
Zhang, D., Zhang, C., Li, Z., Suzuki, D., Komatsu, D.D., Tsunogai, U. and Katayama, A., 2014. Electrochemical stimulation of microbial reductive dechlorination of pentachlorophenol using solid-state redox mediator (humin) immobilization. *Bioresource technology*, *164*, pp.232-240.
Xiao, Z., Awata, T., Zhang, D., Zhang, C., Li, Z. and Katayama, A., 2016. Enhanced denitrification of Pseudomonas stutzeri by a bioelectrochemical system assisted with solid-phase humin. Journal of bioscience and bioengineering, 122(1), pp.85-91.
Dey, S., Kasai, T. and Katayama, A., 2022. Promotion of nitrogen fixation of diverse heterotrophs by solid-phase humin. Frontiers in Microbiology, 13, p.853411.
Laskar, M., Kasai, T., Awata, T. and Katayama, A., 2020. Humin assists reductive acetogenesis in absence of other external electron donor. International Journal of Environmental Research and Public Health, 17(12), p.4211.

Poster-E18: Electro-biocatalytic Process for CO_2 Conversion to Glycolic Acid

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Rising atmospheric CO_2 levels and their negative impacts on the global climate require innovative mitigation strategies. Electrochemical CO_2 reduction (eCO_2R) offers a promising route for converting CO_2 into valuable chemicals under mild conditions. However, eCO_2R is often limited to producing simple C_1 and C_2 compounds like carbon monoxide, formic acid, and ethylene[1]. Combining eCO_2R with whole-cell biocatalysis presents an attractive approach for synthesizing more complex and valuable chemical compounds. Harnessing the synthetic capabilities of aerobic microbial fermentation can upgrade eCO_2R products into high-value-added compounds[2-3]. Formic acid, produced from CO_2 with high yields, is chemically stable and easier to handle than gaseous feedstocks, serving as an ideal platform for microbial C_1 utilization[3]. In this context, this study proposes an electro-biocatalytic cascade process for converting CO_2 to glycolic acid. We investigated electrolyte conditions for CO_2 conversion to formate and direct transfer to a bioreactor, where an engineered *Methylorubrum extorquens* TK0001 strain enabled glycolic acid production[4]. The development of a compatible electrolyte involved the optimization of phosphate buffer concentrations (KPi)[5], operating temperature, and using a defined minimal media suitable for fermentation. Electrolysis in defined minimal media decreased formate production by 31 % without significant loss of faradaic efficiency. Formate utilization with *M. extorquens* for glycolic acid synthesis was demonstrated in batch and fed-batch bioreactors. The integrated electro-biochemical reaction cascade highlights the potential of using CO_2-based formate as a sustainable carbon source for microbial synthesis, enabling the production of valuable products from CO_2.

References

[1] P. De Luna, C. Hahn, D. Higgins, S. A. Jaffer, T. F. Jaramillo, E. H. Sargent, Science 2019, 364, eaav3506.
[2] J. T. Fabarius, V. Wegat, A. Roth, V. Sieber, Trends in Biotechnology 2021, 39, 348-358.
[3] R. Takors, M. Kopf, J. Mampel, W. Bluemke, B. Blombach, B. Eikmanns, F. R. Bengelsdorf, D. Weuster-Botz, P. Dürre, Microbial Biotechnology 2018, 11, 606-625.
[4] K. Dietz, C. Sagstetter, M. Speck, A. Roth, S. Klamt, J. T. Fabarius, Microbial Cell Factories 2024, 23, 344.
[5] L. Vieira, J.T. Fabarius, G. R. Piton, B. Bohlen, D. Pangotra, M. Speck, C. Sagstetter, V. Sieber, A. Roth, 2025, Electrocatalytic CO2 reduction coupled to formate fermentation: an electro-bio-cascade approach in biocompatible electrolytes [Submitted].

Poster-E19: Fostering Ethanol Production From CO_2 by Digital In-line Control in Bench Scale MES

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Microbial electrosynthesis (MES) is an emerging technology for CO_2 utilization, striving to improve its performance and progress towards higher development levels. Although the primary target products are acetic acid and methane, ethanol holds significant promise. However, CO_2 conversion to ethanol in MES suffers from low efficiency and selectivity obtained. This study addresses these two challenges by implementing a bench scale CO_2 recycling plant featuring an electrically efficient, low-gap MES stack. The system allows to control key operation parameters previously indicated to favor ethanol production, including pH (< 5.0), and low CO_2 partial pressures (< 0.2 atm)(Romans-Casas et al., 2023). Acetic acid was the single product until the control parameters were switched. The strict in-line parameter control included pH (between 4.5 and 4.7), dissolved CO_2 concentration (ranging between 200 and 800 ppm) and total pressure (1.8 atm). This alteration activated solventogenesis and an ethanol production rate of 5.69 g m^{-2} d^{-1} was achieved with an average conversion of 59 %, improving previous results reported by Srikanth et al. (2018). Overall, this work advances the MES technology by implementing an automated control system to precisely regulate operational parameters for optimal ethanol production from CO_2 in MES cells.

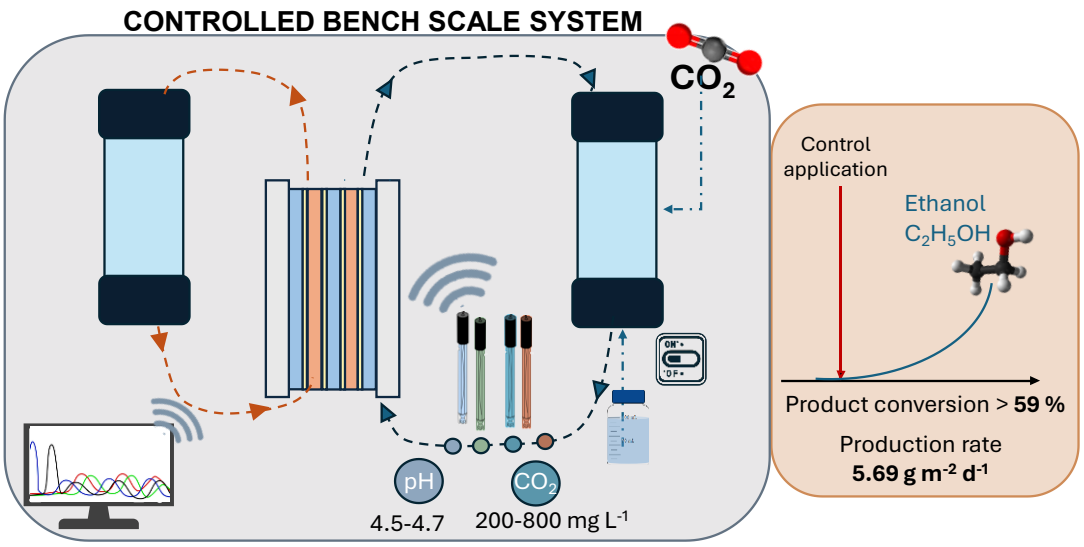

Figure. 1. Schematic representation of the bench scale-controlled system and the key conditions selected.

References

Romans-Casas, M., Perona-Vico, E., Dessì, P., Bañeras, L., Balaguer, M. D., & Puig, S. (2023). Boosting ethanol production rates from carbon dioxide in MES cells under optimal solventogenic conditions. *Science of the Total Environment*, *856*(September 2022), 1–8.

Srikanth, S., Singh, D., Vanbroekhoven, K., Pant, D., Kumar, M., Puri, S. K., & Ramakumar, S. S. V. (2018). Electro-biocatalytic conversion of carbon dioxide to alcohols using gas diffusion electrode. *Bioresource Technology*, *265*(February), 45–51.

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Poster-E20: At the edge of electrofermentation and bioelectrochemical synthesis: towards optimization of microbial mediated synthesis of surfactants

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Bioelectrochemical systems (BES) offer a promising platform for integrating sustainable electricity generation with organic molecule synthesis, a process known as bioelectrosynthesis. Electrofermentation, on the other hand, involves modulating redox potential through an external electrode to influence existing metabolic pathways. This study explores the microbially mediated electrosynthesis of biosurfactants in the anodic chamber of microbial fuel cells (MFCs). While these secondary metabolites are not directly synthesized by accepting electrons from the electrode, our research demonstrates a direct correlation between biosurfactant production (including various rhamnolipids) and current generation. Experiments using petrochemicals and waste cooking oil showed a strong link between MFC power performance and surface tension activity, which is directly influenced by biosurfactant concentration. Our results suggest that the major contributors to surface tension changes belonged to the group of various types rhamnolipids. The synthesis of these compounds can be optimized and we have improved the process by varying the macroelelemental composition of the electrolyte. The underlying mechanism, requiring detailed investigation of metabolic interactions, exhibits features of both bioelectrosynthesis and electrofermentation.

Poster-E21: Continuous Microbial Electrosynthesis to Characterize Reaction Kinetics and Product Formation

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Recent years have shown the great potential of microbial electrosynthesis (MES) as a future technology for the production of low-molecular platform chemicals. In contrast to conventional chemical electrosynthesis, MES allows working at considerably lower overpotentials and thus higher energy efficiency as well as achieving complex syntheses. One of the current limitations of cathodic MES is the comparatively low substrate turnover rate, which is 2 to 3 orders of magnitude lower than that of high-performance anodic processes in microbial fuel cells. One reason for the low performance is that the great majority of MES processes do not utilize the highly efficient extracellular electron transfer (EET) machinery found in anodic electroactive bacteria, resulting in very thin biofilms. This and the comparatively long cultivation periods make it more challenging to establish continuous synthesis processes. Continuous MES processes offer significant advantages over conventional batch operations as they enable enhanced productivities through pH control and product extraction, thus mitigating potential feedback product inhibition. Additionally, continuous processes provide an ideal experimental framework to study the metabolic performances and physiology of microbial systems as well as to determine reaction kinetic parameters from steady state substrate/biomass/product concentrations, aspects that have seldom been explored in the context of MES so far (Kubannek *et al.*, 2022).
The acetogen, *Clostridium ljungdahlii*, is a well-known model organism for MES. However, there is a considerable gap in the existing literature regarding the knowledge of reaction kinetic models that effectively characterize its product formation processes within the context of MES. The required reaction kinetic parameters can be determined using chemostat and retentostat cultivations. This requires a gaseous feed flow of CO_2 for the formation of the main product acetate and other by-products from CO_2 and H_2, which is formed directly at the cathode. H_2 production can be monitored and controlled electrochemically through cyclic voltammetry and chronopotentiometry as well as by off-gas analysis. The efficiency of electrochemical H_2 production was improved by employing H_2-catalyzing electrode materials such as platinum instead of graphite. To optimize acetate production, different CO_2 feeding strategies were developed and compared. The first strategy, CO_2 is supplied *indirectly* via a permeable membrane, which reduces the formation of gas bubbles and the hydrodynamic induced shear stress on shear-sensitive organisms. The indirect CO_2 supply resulted in low acetate production of 1 mM, probably due to insufficient CO_2 supply. The second strategy involves the continuous *direct* injection of CO_2 into the catholyte via a gas sparger at different CO_2 volume flows, mimicking a continuous feeding process. When comparing acetate production under a continuous gas flow of 30 mL/min with 20 % CO_2 in the inflow to a five-fold reduced gas flow (6 mL/min and 100 % CO_2) resulting in the same total CO_2 content, the acetate concentrations after 7 days of cultivation were 25.9 ± 1.7 and 31.5 ± 5.7 mM, respectively, suggesting a negative impact of high hydrodynamical induced shear stress. However, a statistically significant negative influence of high shear stress due to higher gas flux has not yet been observed.
Varying the substrate supply of CO_2 and H_2, significantly impacts the reaction kinetics and the product yields. Relevant reaction kinetic parameters can be derived from quasi-steady state continuous conditions, which will be used to develop a product formation model for the cathodic MES based on the combination of biochemical and electrochemical model terms.

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References

Kubannek F, Block J, Munirathinam B, Krull R, Eng Life Sci 22 (2022), 152–164, https://doi/10.1002/elsc.202100088.

Poster-E22: Production of Microbial Protein via Bio-electrochemical Nitrogen and Carbon Fixation using 3D Electrodes

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Microbial protein produced from renewable resources is an alternative to conventional non-sustainable food and feed protein sources such as soy and fish meal (1). Here, the hydrogen-oxidizing bacterium *Xanthobacter autotrophicus* (*X. autotrophicus*) was used for microbial protein production. *X. autotrophicus* can simultaneously fix atmospheric CO_2 and N_2 while using hydrogen as sole energy and electron source and O_2 as final electron acceptor (2). To avoid dependency on the slow expanding hydrogen infrastructure (3), *in situ* hydrogen production via water splitting reaction was used in a bio-electrochemical system (BES). Since nitrogen reduction is more energy demanding than CO_2 reduction (4, 5), a simple setup of the BES using conventional graphite rod electrodes as cathode material proved insufficient for growth under nitrogen-fixing conditions. Therefore, 3D electrodes made of reticulated vitreous carbon (RVC) foam were tested in a small-scale one chamber BES. In comparison with rod electrodes, RVC foam exhibit an increased active surface-to-volume-ratio, thereby facilitating enhanced current flow.

Prior to conducting biotic BES experiments, the influence of the pore size of the RVC foam cathode was evaluated using linear sweep voltammetry with a platinated titanium mesh anode. This analysis revealed that a small pore size impedes current flow due to the entrapment of gas bubbles within the 3D structure, thereby decreasing the active surface area of the cathode. For the biotic BES experiments, platinated titanium mesh was used as anode. The water splitting reaction was achieved by applying an external voltage of 5 V. The BES was continuously flushed with N_2, CO_2, and air (80/10/10). The growth of *X. autotrophicus* in nitrogen-free minimal medium was measured over a 6-day period.

In these BES experiments it was demonstrated that *X. autotrophicus* can successfully be grown in a BES with RVC foam cathodes under nitrogen-fixing conditions. A cumulative charge of 13,000 ±2,000 C was required to achieve an optical density of 1.6 ± 0.2 AU. This results in an energy demand of 8,000 ± 3,000 C/AU while consuming up to 99 % of the produced hydrogen. To reduce the energy demand, the over potential for the water splitting reaction should be decreased by screening different electrode materials or coating the RVC foam with a suitable catalyst.

References:

1. Ritala A, Häkkinen ST, Toivari M, Wiebe MG. Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. Frontiers in Microbiology. 2017;8.
2. Wiegel J, Schlegel HG. Enrichment and isolation of nitrogen fixing hydrogen bacteria. Archives of Microbiology. 1976;107(2):139-42.
3. DBI Gas- und Umwelttechnik GmbH GfA-uR, Deutscher Verein des Gas- und Wasserfaches e.V. PORTALGREEN II - Entwicklung von Wasserstoff-Netzinfrastrukturen in Deutschland bis 2030. 2023.
4. Dixon R, Kahn D. Genetic regulation of biological nitrogen fixation. Nature Reviews Microbiology. 2004;2(8):621-31.
5. Hudson EP. The Calvin Benson cycle in bacteria: New insights from systems biology. Seminars in Cell & Developmental Biology. 2024;155:71-83.

Poster-E23: Dark Electrodriven PHA production from CO_2 by *Rhodopseudomonas palustris.* Tuning biopolymer characteristics through electrochemical conditions

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Among the innovative technologies for carbon sequestration, the use of *Rhodopseudomonas palustris*—a photosynthetic, Gram-negative, purple non-sulfur bacterium (PNSB) from the family Nitrobacteraceae, stands out as particularly promising. This bacterium was selected due to its remarkable adaptability to diverse environmental conditions and its metabolic versatility. It can utilize light, as well as inorganic and organic compounds, as sources of carbon and energy under both anaerobic and aerobic conditions. Most importantly, R. palustris is capable of fixing CO_2 via the Calvin-Benson-Bassham (CBB) cycle, contributing to its cellular growth and metabolism. One significant advantage of using a pure culture of R. palustris is the ability to precisely control and optimize growth and CO_2 fixation conditions, ensuring consistent performance and product quality. Moreover, *R. palustris* holds promise as a source of single-cell protein (SCP), a novel alternative protein source based on microorganisms rich in proteins, vitamins, and lipids, used as supplements in human and animal nutrition. Recent research has focused on microorganisms capable of producing polyhydroxyalkanoates (PHAs) as part of SCP. PHA is a water-insoluble, biodegradable polymer stored in bacterial cells, and when present in SCP, it has been shown to promote growth and enhance immune function, increasing resistance to pathogens.

Studies also investigate PHA production, a metabolic pathway known to be active in *R. palustris* under light conditions but not yet studied in the dark. Traditionally, research on *R. palustris* has focused on its phototrophic growth, often overlooking its capacity for diverse metabolic lifestyles. However, phototrophic cultivation presents major challenges, including the need for artificial illumination, the difficulty of distributing light evenly to all biomass, and the optimization of light/dark cycles. These factors add complexity, increase operational costs, and reduce the scalability of the process.

Some studies have suggested that *R. palustris* is capable of chemoautotrophic growth in the dark by fixing CO_2 through H_2 oxidation coupled with O_2 reduction. However, these studies relied on externally supplied O_2 + H_2, which poses several limitations due to its low solubility, safety risks, and high costs.

To address these issues, this research explores an innovative approach using a bioelectrochemical system (BES) to enable in situ O_2 and H_2 generation, providing a sustainable electron donor and acceptor for *R. palustris* overcoming solubility issues. Furthermore, R. palustris has shown the ability to directly uptake electrons from electrodes, offering an additional energy source.

In this study, 0.5 L single-chamber reactors were operated under both potentiostatic and galvanostatic conditions to evaluate SCP and PHA production. PHA content was later analyzed via gas chromatography to quantify total polymer yield, as well as the composition in hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers. Throughout the experiment, the reactors were continuously sparged with a CO_2/N_2 gas mixture to provide a carbon source while preventing the formation of an explosive atmosphere inside the reactors. Its ability to growth in the dark, without any supply of organic carbon highlights the versatility of the metabolism underscoring its potential for SCP and PHA production using only waste gases and renewable energy resources.

Poster-E24: Microbial Fuel Cells and Microbial Electrosynthesis: Transforming Glycerol-rich Wastewater and CO_2 into Valuable Products with Future Integration Potential

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Bioelectrochemical systems (microbial fuel cells (MFCs) and microbial electrosynthesis (MES)) have emerged as a promising and innovative technology to tackle two critical global issues: the wastewater treatment, and the production of valuable bioproducts from CO_2[1]. This study explored the use of MFC and MES to treat glycerol-rich wastewater and convert CO_2 and glycerol waste into valuable chemicals, respectively. The initial chemical oxygen demand (COD) of the wastewater used in this study was 20,900 ppm, primarily composed of glycerol (~3000 ppm) along with other alcohols and carboxylic acids. MFCs utilise microorganisms to degrade organic pollutants while generating electricity. In this study, MFCs with carbon brush anodes and FePc/C carbon paper cathodes, inoculated with activated sludge, reduced the COD from 2028 ppm to 287 ppm (86% reduction) in 10 days. This significant reduction demonstrated the MFCs' efficacy in treating highly polluted wastewater. The performance of the MFCs was also validated through polarization and power density curves, achieving a maximum power density of 7.6 $\mu W/cm^2$, confirming the generation of electrical energy during the treatment process. For MES systems, glycerol-rich wastewater was diluted to 140 ppm glycerol which served as an extra electron donor to enhance microbial CO_2 reduction and produced various organic compounds[2]. MES operated in a dual-chamber setup with a carbon brush cathode, Pt@Ti mesh anode, and an Ag/AgCl reference electrode at -1V vs. Ag/AgCl, primarily produced acetate, reaching a maximum concentration of 3840 ppm and production rate of 284.5 ppm/day. Propionate (700 ppm) and formate (550 ppm) were also produced, along with butyrate (81 ppm), methanol (290 ppm), and ethanol (121 ppm) in lower concentrations. Future microbial community analysis will focus on understanding the key microbial species and their metabolic interactions in these systems, particularly how microbial consortia contribute to wastewater treatment efficiency and CO_2 reduction pathways. The future integration of MFC and MES can demonstrate the potential of using glycerol-rich wastewater. The effluent from MFCs, with significantly reduced COD levels, can be directly utilised as the substrate for MES. This integration can utilise the treated wastewater's residual glycerol as an electron donor in MES, enhancing the microbial reduction of CO_2 to high-value chemicals. This synergistic approach can provide a combined solution for wastewater treatment and CO_2 conversion.

References

1. S. Jung, J. Lee, Y. Park, and E. E. Kwona, Bioresour. Technol., 2020, 300, 122748.
2. Z. Gong, H. Yu, J. Zhang, F. Li and H. Song, Synth Syst Biotechnol., 2020, 5, 304-313.

Poster-E25: Rethinking Methanogenesis Control: Acid-Induced Inhibition in Microbial Electrosynthesis

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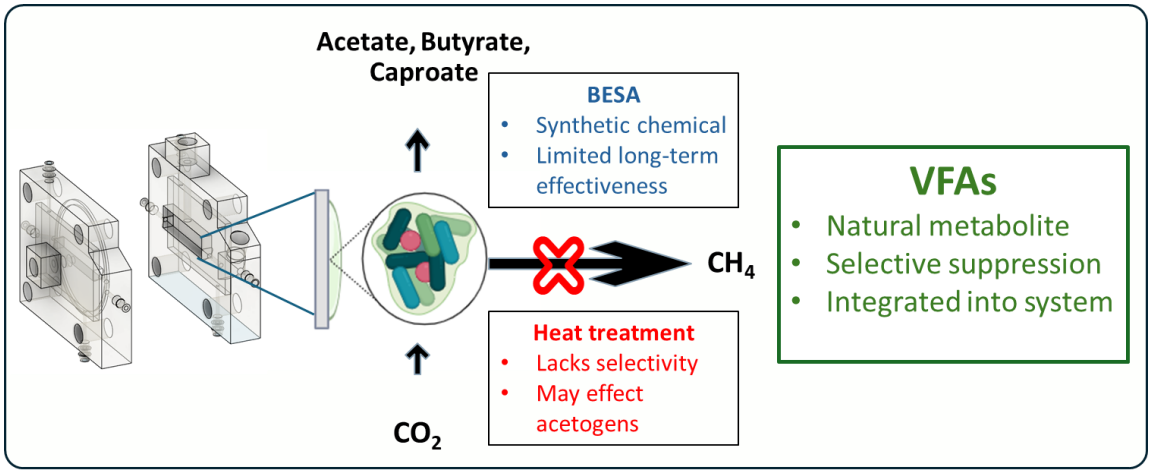
Microbial electrosynthesis (MES) is an emerging carbon capture and utilization (CCU) technology that harnesses renewable electricity to drive microbial catalysis, enabling the conversion of CO_2 into value added chemicals such as acetate, butyrate, and caproate. A major challenge in MES systems is methanogenesis, which competes for electrons and carbon, diverting these resources toward methane, a low-value end product [1]. Conventional suppression strategies including heat shock and chemical inhibition with 2-bromoethanesulfonic acid (BESA), have shown limited long-term effectiveness and each have limitations [2,3]. Heat shock can effectively reduce methanogen abundance but lacks specificity and may impair beneficial microbial populations. In contrast, BESA which is widely used in MES studies offers greater target specificity, yet prolonged application can result in a rebound of methanogen activity.

Therefore, this study investigates volatile fatty acids (VFAs) as a selective alternative for inhibiting methanogenesis in a biofilm-driven MES system. Incremental increases in acetate and propionate concentrations achieved complete suppression of methane production at a total free-acid concentration of 139 mM. Methanogenesis inhibition was accompanied by a recovery in acetate synthesis when solely fed with CO_2, reaching a maximum rate of 3.15 g L^{-1} d^{-1}. Notably, comparable acid concentrations have previously been reported during steady-state MES operation, which suggests that methanogenesis could be effectively in-situ suppressed by the produced VFAs [3]. These findings advance the development of MES technology by improving carbon conversion to high-value products while minimizing reliance on non-specific or synthetic inhibitors and leveraging the system's inherent characteristics.



References

1. Park, S.G., Rhee, C., Shin, S.G., Shin, J., Mohamed, H.O., Choi, Y.J. and Chae, K.J., 2019. Methanogenesis stimulation and inhibition for the production of different target electrobiofuels in microbial electrolysis cells through an on-demand control strategy using the coenzyme M and 2-bromoethanesulfonate. *Environment international*, *131*, p.105006.
2. Mills, S., Dessì, P., Pant, D., Farràs, P., Sloan, W.T., Collins, G. and Ijaz, U.Z., 2022. A meta-analysis of acetogenic and methanogenic microbiomes in microbial electrosynthesis. *npj Biofilms and Microbiomes*, *8*(1), p.73.
3. Cabau-Peinado, O., Winkelhorst, M., Stroek, R., de Kat Angelino, R., Straathof, A.J., Masania, K., Daran, J.M. and Jourdin, L., 2024. Microbial electrosynthesis from CO2 reaches productivity of syngas and chain elongation fermentations. *Trends in Biotechnology*, *42*(11), pp.1503-1522.

Poster-E26: Coupling microbial electrosynthesis with mixotrophic microalgae cultivation as a strategy for future sustainable aviation fuels production

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The current fossil fuel and environmental crisis is driving the research for alternative energy sources, especially in aviation, with a strong focus on sustainability and CO_2 capture and utilization (CCU). Microbial electrosynthesis (MES) is an innovative CCU technology for biofuel production. However, its reported productivities remain low, making it uncompetitive. In contrast, acetate production via MES is well-documented and achieves high yields, but acetate itself has limited economic value. Microalgae are a known source of neutral lipids, which could be transformed into sustainable aviation fuel (SAF). To expand the growth of microalgae beyond daylight, a viable approach is coupling their metabolism with MES. The MES effluent, rich in VFA (volatile fatty acid), could be used as a heterotrophic carbon source for microalgae growth during the dark metabolic phase, enhancing CO_2 capture and biomass productivity while reducing the use of agricultural-competitive carbon sources (Bolognesi et al. 2021).

To optimize this technology, two zero-gap MES reactors were operated at a hydraulic residence time (HRT) of 15 days) and under galvanostatic control (12 A/m^2), using a mixed microbial culture enriched in acetogenic microorganisms. This setup achieved an average productivity of up to 71±24 g/(m²·d) (selectivity: 97±0,3%) and a high coulombic efficiency of 89±33%. Preliminary tests were conducted by feeding *Chlorella sorokiniana* with different MES effluent/synthetic acetate at concentrations ranging from 1-5 g/L) under mixotrophic conditions (12h light/12h Dark, 25ºC, constant aeration) prior to the coupling with a 5 L bioreactor. An autotrophic control achieved a biomass productivity of 0,04 g/(L·d), while in samples supplemented with 1 $g_{acetate}$/L of MES effluent, productivity increased to 0,17 g/(L·d), demonstrating the potential of integrating MES and mixotrophic microalgae for enhanced biomass production. Further studies will investigate the quality and quantity of the SAF produced.

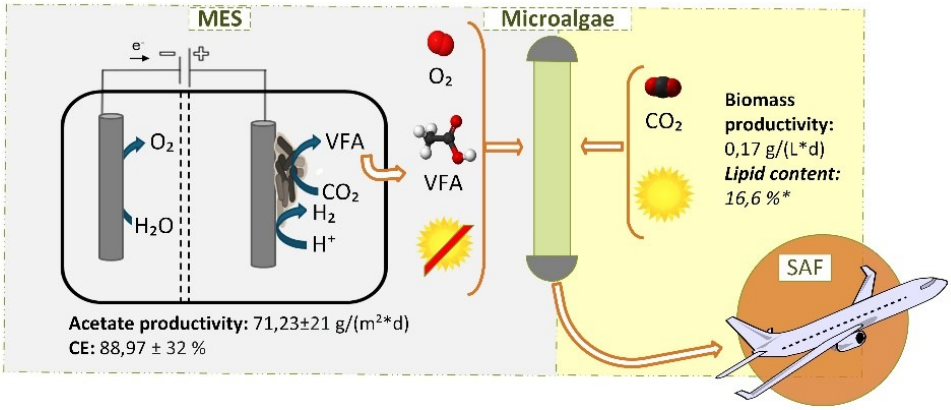

Figure 1. Scheme of the proposed process for CO_2 bio-electro conversion into SAF.

Acknowledgments: The ALGAESOL project (https://algaesol.eu/) has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101147112. S.P. is a Serra Hunter Fellow (UdG-AG-575) and acknowledges the funding from the ICREA Academia award. LEQUIA has been recognized as "consolidated research group" (Ref 2021 SGR01352) by the Catalan Agency of Research and Universities.

References:

1. Bolognesi, S., Bañeras, L., Perona-Vico, E., Capodaglio, A. G., Balaguer, M. D., Puig, S. 2022 Carbon dioxide to bio-oil in a bioelectrochemical system-assisted microalgae biorefinery process. Sustainable Energy & Fuels, 6(1), 150–161.

Poster-E27: e-BNF: Powering Nitrogen Fixing Microbes with Electrons

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Bioelectrochemical Nitrogen Fixation (e-BNF) process uses bioelectrochemical systems (BES) to enhance the natural nitrogen fixation capabilities of microbes, providing a more energy-efficient and environmentally friendly alternative to traditional ammonia production methods. In this study, a pure culture of *Xanthobacter autotrophicus*, a CO_2 fixing, diazotroph grown on a cathode to fix dinitrogen gas to ammonia. The effect of polarization (-0.5 V, 1 V, -1.25 V) as well as electrode materials (carbon felt, and graphite) on the fixation efficiency are currently being investigated. We observed a maximum current density ranging from -12.5 to 25 $\mu A/cm^2$ indicating that electrons are being taken up by Xanthobacter demonstrating efficient fixation with in a span of 15 d, when the cathode (carbon felt) is polarized to -0.5 V. With this study, we aim to identify the optimal trade-offs between polarization and yield, paving the way for the effective development and scaling of this technology.

Poster-E28: Analysing the Microbial Energy Metabolome to Improve the Efficiency of Carbon Conversion in Bioelectrochemical Systems

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Introduction

Increasing interest is being shown in using microbial processes to produce chemicals and fuels to replace those produced from fossil hydrocarbons. However, to reach the volumes required at an economically viable cost point, significant improvements in yield and carbon efficiency need to be achieved. One way of achieving this is to develop bioelectrochemical systems (BES) either directly to convert carbon dioxide or integrated with conventional fermentations to improve carbon dioxide utilisation. Although there are a number of strategies that can be applied to achieve this goal based on a robust understanding of the energy metabolome of industrially exploited microbes to direct these efforts. A typical aim of integrating electrochemistry is to alter the NAD/NADH ratio of microbial cultures. However to validate the mechanism of these approaches knowledge of microbial energy metabolome is required using a quick, robust, and high throughput analytical methods of determining the widest possible aspect of the energy metabolome. Although NAD/NADH, NADP/NADPH, and ADP/ATP are structurally similar, these redox pairs have critical but specific metabolic functions which result in great chemical diversity and an even greater range in concentrations present in cells. Therefore, the development of specific yet sensitive methodologies for the detection and quantification of these metabolites that can be transferred to a variety of microbial systems is needed. These could include pure cultures that can be electrochemically stimulated such as Yeast, E.coli or mixed cultures found in BES.

Materials and Methods

The instrumentation used consists of the Waters Acquity UPLC H-Class PLUS system fitted with a binary solvent manager, sample manager, 6-capacity column manager, and PDA detector. Paired with the Xevo TQ-S Cronos triple quadrupole mass spectrometer. The column specification is the Atlantis Premier BEH Z-HILIC column (95 Å 1.7 µm, 2.1 mm x 150 mm) coated with a zwitterionic functional group of sulfobetaine, fitted with an Atlantis Silica HILIC VanGuard Cartridge (100 Å, 3 µm, 2.1 mm x 5 mm) column protection. The optimised quenching, extraction, detection, and analysis protocols were applied to further Saccharomyces cerevisiae samples to determine the intracellular concentration of metabolites of the wider energy metabolome at varying growth points. Organisms analysed included *Saccharomyces cerevisiae* and *E.coli.*

Conclusions

A relatively simple, fast, and accurate methodology for the determination of nucleotide concentrations from the wider energy metabolome of *Saccharomyces cerevisiae* using a zwitterionic column and UHPLC-MS instrumentation was developed and robustly validated. This is, to the best of our knowledge, a methodology that provides the lowest LOD and LOQ for metabolites in a singular sample and the only methodology that does not require samples to be pre-spiked with the metabolites intended for detection and quantification. This methodology has demonstrated real-time comparison of energy metabolites in relation to platform chemical production.

Furthermore, the analysis has shown that the concentration of ethanol production fluctuates in response to environmental changes and metabolic manipulation, as reflected in shifts in intracellular nucleotide ratios. These variations provide insight into how microbial metabolism adapts under different growth conditions and electrochemical stimulations, offering potential avenues for optimisation in biofuel and biochemical production. Organisms analysed included *Saccharomyces cerevisiae* and *E.coli* but could include mixed cultures such as found in BES.

Poster-E29: Acetate production from CO_2 using microbial electrosynthesis

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Carbon dioxide (CO_2) is on one hand a powerful greenhouse gas contributing to climate change and on the other hand an abundant source of carbon, that can be utilized as feedstock to produce valuable carbon-based products such as acetate (Yang et al., 2021). Acetate is an important end-product as well as a platform chemical for further synthesis into plastics or medicine. Using microbial electrosynthesis (MES), electroactive microbes can convert CO_2 into acetate in the cathode chamber of a bioelectrochemical system (Figure 1). In this work, the conversion from CO_2 to acetate by MES was investigated using pure- and mixed cultures in two-chamber cells. The MES performance was monitored regularly and corresponding parameters such as acetate production rate, coulombic efficiency and current density were evaluated and compared. Furthermore, the CO_2 consumption and hydrogen production were measured using a gas chromatograph to find optimal experimental settings, bearing in mind the overall goal to maximize acetate production and CO_2 conversion. Preliminary results showed that ≥90% of gaseous CO_2 was removed from the gas-phase within a three-day batch cycle using a mixed culture MES.

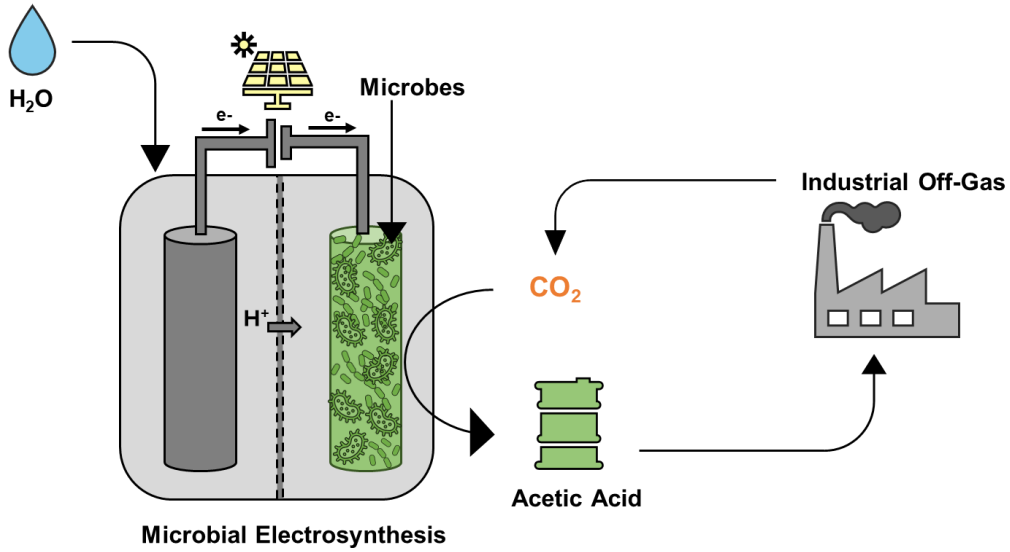

Figure 1: Scheme of CO_2 conversion from industrial off-gas into acetic acid through microbial electrosynthesis.

References:
Yang, H. Y., Hou, N. N., Wang, Y. X., Liu, J., He, C. S., Wang, Y. R., Li, W. H., & Mu, Y. (2021). Mixed-culture biocathodes for acetate production from CO2 reduction in the microbial electrosynthesis: Impact of temperature. Science of the Total Environment, 790. https://doi.org/10.1016/j.scitotenv.2021.148128

Acknowledgement:
The authors gratefully acknowledge the funding support of K1-MET GmbH. K1-MET is a COMET Centre within the COMET – Competence Centers for Excellent Technologies Programme and funded by the Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Federal Ministry of Labour and Economy, the provinces, Upper Austria, Styria, and Tyrol as well as the the Steirische Wirtschaftsförderungsgesellschaft m.b.H. (SFG) and the Standortagentur Tyrol. Furthermore, we thank Upper Austrian Research GmbH for the continuous support. The COMET Programme is managed by FFG.

Poster-E30: GDE-directed flow microbial electrosynthesis cell increases acetate production from CO_2

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Introduction: Carbon Capture and Utilization (CCU) technologies like microbial electrosynthesis (MES) can convert CO_2 into value-added chemicals. MES utilizes microbial biocatalysts under mild conditions, providing high selectivity and sustainability. However, its scalability is limited by mass transfer constraints and reactor design. To address this, we designed a three-chamber flow-pattern MES reactor and applied computational fluid dynamics (CFD) modelling to optimize electrolyte and CO_2 circulation. The flow-pattern cell was then compared to the original [1], square-shaped cell in terms of efficiency for CO_2 conversion to acetic acid.

Materials and methods: The CFD was based on the Fluid Flow Fluent analysis (ANSYS) with a pressure-based coupled solver using the k-ε standard turbulence model. Boundary conditions were set to operation parameters (inlet electrolyte and CO_2 flow, pressure resistance, temperature, and operating pressure). A microbial community previously enriched in H-type cells was used to inoculate both three-chamber reactors operated galvanostatically (1.0 mA cm^{-2}) and fed with pure CO_2 (2.0 mL min^{-1}).

Results: The CFD model demonstrated a decrease in dead space in the flow pattern cell, facilitating the exploitation of the entire electrode area. This leads to improved CO_2 delivery to the GDE and enhanced contact between CO_2, catholyte, and microorganisms on the GDE surface. As a result, an average production rate of 17.12 g m^{-2} d^{-1} was achieved, on a 16 days linear production period, with a CE of 25.7%, compared to 7.46 g m^{-2} d^{-1} and a CE of 16.31% obtained in the square-shaped cell. These findings highlight the potential of CFD for optimizing MES reactor design. Ongoing studies are evaluating the performance of the flow pattern cell in continuous mode and using gas mixtures that mimic flue gas from concrete plants.

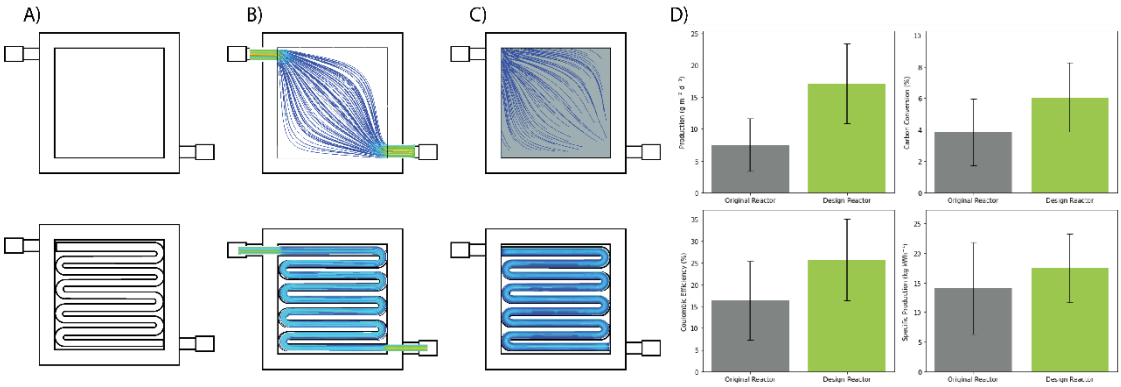

Figure 1 A) Scheme of the square-shaped and flow-pattern cells; B) Liquid flow dynamics; C) Contact CO_2-GDE; D) Average performance parameters obtained with the two cell designs.

Acknowledgments: The financial support by the Irish Research Council under grant number GOIPG/2022/2272 is greatly appreciated.

[1] P. Dessì *et al.*, "Microbial electrosynthesis of acetate from CO2 in three-chamber cells with gas diffusion biocathode under moderate saline conditions," *Environmental Science and Ecotechnology*, p. 100261, Mar. 2023, doi: 10.1016/J.ESE.2023.100261.

Poster-E31: Rapid biocathode activation using synergistic methanogenic and electroactive inocula to increase bioelectrochemical efficiency

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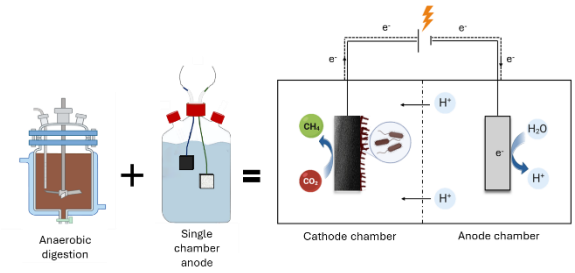
Biocathodes play a key role in bioelectrochemical systems, and their rapid activation is a key challenge to improve the efficiency of these systems in environmental and energy applications. In this study, an approach based on the use of a mixed inoculum designed to promote synergy between electroactive bacteria and methanogenic communities was evaluated with the aim of reducing the start-up time and improving the electrochemical activity of the biocathode.



Inoculation was conducted using a pre-designed mixture of two effluents, with the objective of promoting the development of a synergistic electroactive microbial consortium[1]. The first effluent was derived from a single-chamber bioelectrochemical cell that had been operating stably for a period exceeding three months at a potential of 1 V against an Ag/AgCl reference electrode. This single chamber gave rise to a high concentration of electroactive microorganisms, with a majority presence of *Geobacter*, a genus characterised by its ability to transfer electrons extracellularly via conductive pili and membrane cytochromes. The second effluent was originated from an anaerobic digestion process developed under controlled conditions to maximise the concentration of methanogenic bacteria, mainly *Methanolinea*, a key genus in the production of methane from hydrogen and organic compounds. The homogenisation of these effluents facilitated the generation of an optimal inoculum, thereby ensuring the establishment of a robust electroactive microbial community and the subsequent initiation of the bioelectrochemical system in an efficient manner. The inoculum was introduced into an H-type two-chamber system, with a volume of 500 mL, separated by a cationic membrane. The cell was configured with a three-electrode system. The working electrode was a 3×5 cm^2 pre-treated carbon felt, while the counter electrode was a platinum mesh of similar size. The reference electrode was an Ag/AgCl (3 M KCl) electrode, to which a potential of -1 V was applied to promote bioelectrochemical activity [2].
This study highlights the crucial role of a tailored inoculum composition in the start-up of a bioelectrochemical cell. This strategic microbial mix promoted rapid colonization of the electrode surface, significantly reducing start-up times while enhancing the productivity and efficiency of the biocathode. These results represent a significant improvement over previous studies, where conventional inoculum required longer adaptation periods and exhibited inferior electrochemical performance.

[1] Rotaru AE, Calabrese F, Stryhanyuk H, et al. Conductive particles enable syntrophic acetate oxidation between Geobacter and methanosarcina from coastal sediments. mBio. 2018;9(3).
[2] Cheng S, Xing D, Call DF, et al. Direct biological conversion of electrical current into methane by electromethanogenesis. Environ Sci Technol. 2009;43(10):3953–3958.

Acknowledgements: PID2023-147848OB-I00, financing by MCIU/AEI/10.13039/501100011033 and by FEDER, EU. In addition, the authors acknowledge the research contract of T. Joglar (INVESTIGO PROGRAM, Next Generation UE).

Poster-E32: Boosting Organic Acid Production from CO_2 in a Continuous Electro-Bioreactor: The Role of Dilution Rate

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The pursuit of environmentally friendly technologies has driven society toward closing the carbon cycle by converting CO_2 into products through bioelectrochemical approaches. Here, we investigated an electro-driven bioreactor, operated under a constant potential (300 mV) in continuous mode, to promote the conversion of CO_2 into products. By varying the dilution rate (D), we aimed to enrich a specialized mixotrophic community and enhanced high-added products concentration. The electro-bioreactor was a single vessel (1.1 L) inoculated with an anaerobic sludge from a sugarcane vinasse treatment plant and operated over 1200 hours at increasing D values: 0.03, 0.053, and 0.071 h^{-1}. Graphite electrodes (1.5 × 13.0 × 0.5 cm) were placed into the bioreactor, as anode and cathode. The system was fed with M9 mineral medium supplemented with 3.6 $g{\cdot}L^{-1}$ fructose and 4.0 $g{\cdot}L^{-1}$ $NaHCO_3$ (pH 6.3). CO_2 was sparged into the medium to ensure anaerobiosis and a CO_2-rich atmosphere. Samples were taken every 24 h to quantify carboxylic acids (Acetic/HAc, Propionic/HPr, Butyric/HBut, Formic/HFor acids) by high-performance liquid chromatography (HPLC) (Shimadzu LC-20 AT – photo diode array detector). Also, inorganic carbon (TIC), and volatile solids (TVS) were quantified. The microbial diversity from the inoculum and cell biomass developed under different D were assessed using 16S rRNA gene sequencing. First, the electro-bioreactor was operated at D = 0.03 h^{-1} with an initial biomass (TVS) at 2.65 ± 0.13 $g{\cdot}L^{-1}$. Over the first 144 hours, biomass washout occurred, reducing it to 1.99 ± 0.05 $g{\cdot}L^{-1}$; however, after 192 h, biomass recovered and reached 2.66 ± 0.11 $g{\cdot}L^{-1}$. When D was increased to 0.053 h^{-1}, biomass has diminished from 2.91 ± 0.12 $g{\cdot}L^{-1}$ to 2.19 ± 0.33 $g{\cdot}L^{-1}$, but increasing to 2.49 ± 0.21 $g{\cdot}L^{-1}$, after 408 h of operation. The highest biomass washout arose at D = 0.071 h^{-1}, where TVS decayed from 4.19 ± 0.06 $g{\cdot}L^{-1}$ to 2.36 ± 0.17 $g{\cdot}L^{-1}$. Variations in D values significantly influenced the microbial community structure and the products profile. The inoculum was dominated by *Trichococcus* (54.22%) and *Advenella* (10.41%). After 192 hours at D = 0.03 h^{-1}, *Trichococcus* decreased to 44.78%, while *Klebsiella* emerged at 27.51%. At the end of the operation at D = 0.03 h^{-1}, Hac, HPr, and HBut emerged as products at 0.85 ± 0.0005 $g{\cdot}L^{-1}$, 0.782 ± 0.002 $g{\cdot}L^{-1}$, and 0.100 ± 0.0005 $g{\cdot}L^{-1}$, respectively. At D=0.053 h^{-1} the microbial community underwent a marked shift, with *Enterococcus*, *Arcobacter*, and *Robinsoniella* emerging at 25.21%, 22.25%, and 18.9%, respectively. After 408 h at D = 0.053 h^{-1} the concentrations of HAc, HPr, and HBut reached 1.17 ± 0.0005 $g{\cdot}L^{-1}$, 0.79 ± 0.0013 $g{\cdot}L^{-1}$, and 0.12 ± 0.0005 $g{\cdot}L^{-1}$, respectively. Also, HFor was detected in low concentration (0.0054 $g{\cdot}L^{-1}$). Upon increasing the D to 0.071 h^{-1} applied to the electro-bioreactor, *Enterococcus* remains as most abundant at 26.05% and *Clostridium* emerges in the microbial community at 15.75% of relative abundance. At this dilution rate, HAc increased 73% compared to the initial flow rate (D = 0.03 h^{-1}), reaching 1.47 ± 0.0005 $g{\cdot}L^{-1}$. In contrast, HPr decreased 42% to 0.45 ± 0.0005 $g{\cdot}L^{-1}$, while HBut increased 150% (0.25 ± 0.003 $g{\cdot}L^{-1}$), compared to the lowest D (0.03 h^{-1}). Also, TIC concentrations during the operation at D = 0.071 h^{-1} dropped from 453 ppm (in the culture medium) to 47.3 ± 13.8 ppm, reflecting inorganic carbon assimilation by the stablished microbial community. In conclusion, higher D values induced shifts in the microbial structure, with *Enterococcus* and *Clostridium*, as leading representants, and acetate and butyrate, as main products. In essence, our study demonstrates the successful cultivation of a tailored microbial consortium within a continuous electro-bioreactor, effectively unlocking the potential for sustainable CO_2 valorization into valuable carboxylic acids.

Acknowledgement: FAPESP PROCESS: 2024/00725-0.

Poster-E33: Bioelectrochemical selective purification of hydrolysate from food waste to achieve recovery of propionate and gaseous biofuels

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The dark fermentation process is a biological hydrogen production technology and carried out as partial anaerobic digestion without methanogenesis as last step. H_2 is generated as the targeted product with considerable amounts of short-chain fatty acids (SCFAs) remaining in the hydrolysate (Venetsaneas et al., 2009). However, purification of the produced hydrolysate to harvest a single acid for further industrial refinement still remains challenging.

In this study, a microbial electrolysis cell (MEC) (as shown in Fig. 1 (a)) in the form of a 10-L single-chamber rotating disc bioelectrochemical reactor (RDBER) was applied in order to recover gaseous biofuels and propionate from food waste hydrolysate, which contained a mixture of mainly butyrate, lactate, propionate and acetate. The RDBER was inoculated with *Geobacter sulfurreducens* and a newly published butyrate-oxidizing microbial consortium (Elreedy et al., 2024). Different hydraulic retention times (HRTs) were tested to investigate the impact on propionate purity and biogas composition.

In Fig. 1 (b), when the pH was stabilized at around 7 and an HRT of 8 days was applied, a propionate purity of up to 95.5 % (as propionate content per total SCFAs concentration) can be achieved. At the same time, the propionate concentration in the outflow was at least doubled with the maximum concentration reaching 30.6 mmol L^{-1}. No obvious impact of HRT on biogas composition was observed, which indicated the robustness of methanogenesis in the process, however, hydrogen concentration (%, v/v) constantly decreased with cultivation time while methane was dominant.

Thus, it could be proven that the combination of dark fermentation and microbial electrolysis is an effective option to treat food waste to sustainably recover single precursors for subsequent production of bio-based chemicals.

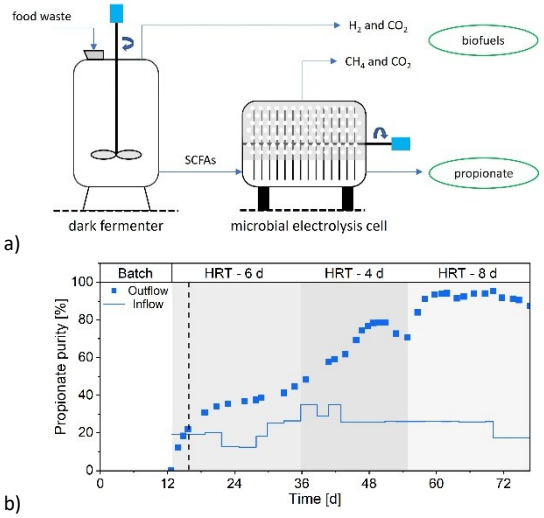

Figure 1. a) Dark fermenter and microbial electrolysis cell for recovery of propionate and biofuels from food waste; (b) Propionate purity development in the RDBER (dashed line refers to the start of pH regulation to prevent the pH of dropping below 7).

Elreedy, A., Härrer, D., Ali, R., Hille-Reichel, A., Gescher, J. 2024. Efficacious enrichment of butyrate-oxidizing exoelectrogens upgrades energy recovery in relevant bioelectrochemical systems. *Environmental Technology & Innovation*, 36, 103871.

Venetsaneas, N., Antonopoulou, G., Stamatelatou, K., Kornaros, M., Lyberatos, G. 2009. Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. *Bioresource technology*, 100(15), 3713-3717.

Poster-E34: A stepwise enhancement of acetate production via microbial electrosynthesis

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The microbial conversion of CO_2 via microbial electrosynthesis (MES) holds potential as a sustainable technology for producing fossil-free chemicals. A major product of MES is acetate, a platform chemical used to produce, for example, various polymers. In addition, production of long alkyl esters, i.e. wax esters, through combination of MES and aerobic bioprocess with *Acinetobacter baylyi* (Lehtinen et al., 2017). Through the years, the efficiency of MES systems has improved, but the utilization of MES in a larger scale is hindered by low production rates and yields and high capital cost (Prévoteau et al., 2020). When it comes to increasing the efficiency of MES and decreasing the costs, reactor and electrode design as well as operational conditions play an integral role (Jourdin et al., 2020).

The aim of this research is the enhancement of acetate production rates and yields in MES by optimizing operational conditions and electrode materials. To optimize operational parameters, the effects of varying temperatures (23 and 35°C) and pressures (1-2 atm, with H_2/CO_2 ratio of 80/20) were determined in serum bottles and varying pH values (5.5-7.0) and semi-continuous or continuous CO_2 feeding were studied in MES fed with CO_2 and methanol. A microbial culture dominated by *Eubacterium* was used as inoculum (Yao et al., 2024).

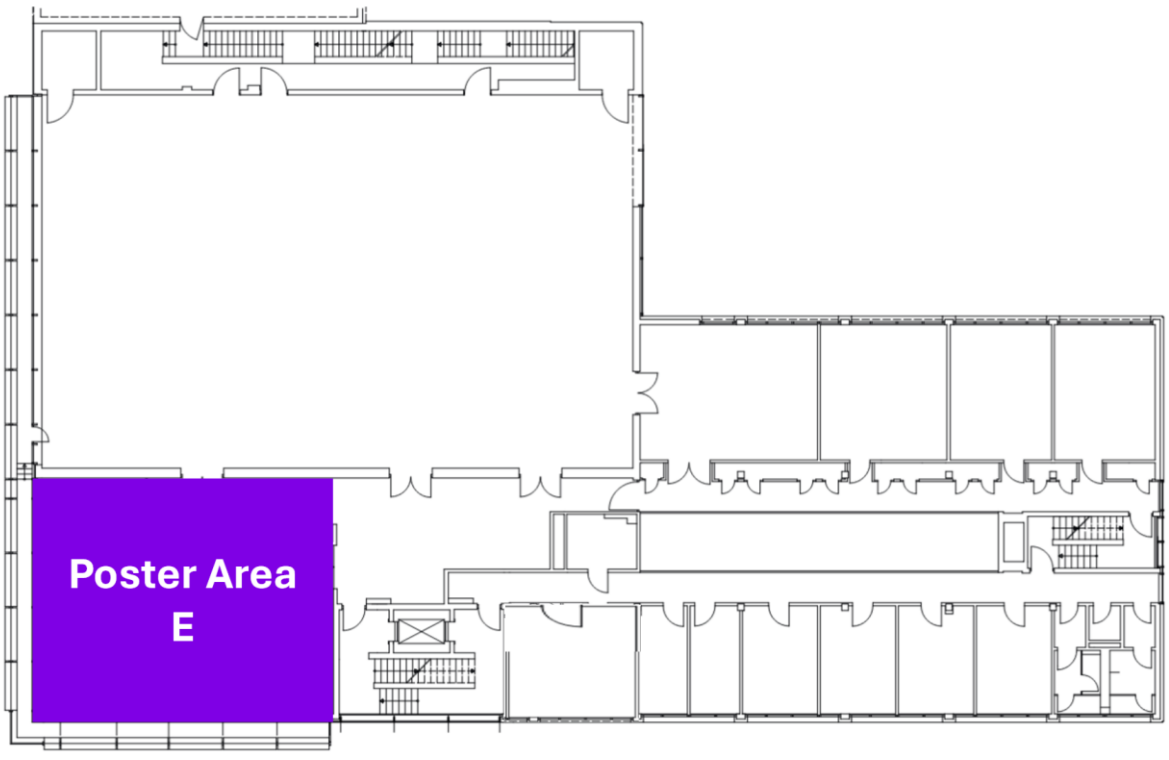
Initial tests in serum bottles indicated that similar acetate production was observed at 23 and 35°C, 7.7±0.4 g/L and 8.2±0.4 g/L, respectively, while higher selectivity toward acetate was obtained at 25°C (93% vs. 51-83%). Acetate yield decreased with the elevated pressures from 1 atm (0.24 mol/mol) to 1.5 atm (0.15 mol/mol) and 2 atm (0.17 mol/mol). In this study fed with CO_2 and methanol, increasing the pH resulted in higher acetate titers and production rates. Under semi-continuous CO_2 feeding, pH increase improved the acetate yield from 1.3 to 5.8±1.8 g/L and acetate production rate from 0.03±0.01 to 0.2±0.1 g/L/d. Changing the CO_2 feeding from semi-continuous to continuous further improved the acetate yield and production rate at pH 7 to 14.4 g/L and 0.48 g/L/d, respectively. Furthermore, the selectivity of acetate production was increased from ca. 30% to 89%. In the next step, novel carbon foam electrodes are characterized electrochemically and structurally, and their utilization in MES reactors is investigated.

References

Jourdin, L., Sousa, J., Stralen, N. van, & Strik, D. P. B. T. B. (2020). Techno-economic assessment of microbial electrosynthesis from CO2 and/or organics: An interdisciplinary roadmap towards future research and application. *Applied Energy*, *279*, 115775.
Lehtinen, T., Efimova, E., Tremblay, P.-L., Santala, S., Zhang, T., & Santala, V. (2017). Production of long chain alkyl esters from carbon dioxide and electricity by a two-stage bacterial process. *Bioresource Technology*, *243*, 30–36.
Prévoteau, A., Carvajal-Arroyo, J. M., Ganigué, R., & Rabaey, K. (2020). Microbial electrosynthesis from CO2: Forever a promise? *Current Opinion in Biotechnology*, *62*, 48–57.
Yao, H., Rinta-Kanto, J. M., Vassilev, I., & Kokko, M. (2024). Methanol as a co-substrate with CO2 enhances butyrate production in microbial electrosynthesis. *Applied Microbiology and Biotechnology*, *108*(1), 372.

POSTER PRESENTATIONS

Reactor Engineering and Materials
- Area E -



2nd Floor

Poster-E35: Hydrogen production in Microbial Electrolysis Cells using stainless steel and nickel foam as cathode materials

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Hydrogen is the energy vector that can support the energy transition to replace fossil fuels and mitigate the effects of climate change. Agroindustrial wastewaters containing carbohydrates can be used as feedstock to obtain biohydrogen through dark fermentation. However, at the end of fermentation, between 60% and 70% of the initial organic matter is transformed into volatile fatty acids (VFAs). VFAs can be used in a subsequent step using Microbial Electrolysis Cells (MECs) to produce hydrogen, increasing the overall hydrogen yield that can be obtained from the initial substrate. MECs consist of an anode and a cathode in two chambers separated by an ion-exchange membrane. At the anodic chamber an electroactive biofilm with exoelectrogenic bacteria metabolize the VFAs and transfers electrons to the anode. At the cathode water is reduced to produce high-purity molecular hydrogen. Unlike a conventional electrolysis cell (2-3 V), MECs require less power (1-1.5 V). However, the bottleneck for MECs implementation lies in incorporating low-cost materials that provide long-term operation. In this work we evaluate hydrogen production in MECs using an acetate(1.5g/L) in a first phase and acidogenic effluent from dark fermentation of brewery wastewater in a second phase. The brewery wastewater had a COD of 3.24g/L, 0,17 ± 0,02 g/L of carbohydrates, 662 mg/L of ethanol, 978 mg/L of acetic acid, 762mg/L of propionic acid and 66 mg/L of butyric acid. Anaerobic granular sludge was used as inoculum. Two cathode materials were tested, nickel foam and stainless steel and the catholyte was NaCl 125mM. MECs were operated at 30°C at a missing rate of 120rpm. Gas volume produced in both chambers was collected and measured in an inverted graduated cylinder. In the first phase (with acetate) H_2 yield was not significantly different between both cathode materials (1011 ± 85 mL/L for stainless steel and 990 ± 94 mL/L for nickel foam. However, the applied potential was higher for stainless steel than for nickel foam (1.29 ± 0.17 vs 1.12 ± 0.03 respectively) which could indicate that a more active electrogenic biofilms were formed in the MEC with nickel foam cathode. Coulombic efficiency (81-84%) was not significantly different between MECs however electric efficiency was higher using nickel foam as cathode (92 ± 5% vs 84 ± 3%). In the first 10 operation cycles, COD removal was less of 60% for both cathode materials. This was due to a decrease of pH in the anodic chamber which affected the electroactive biofilm. In the following cycles, pH was adjusted every day and the COD removal increased to 90% and hydrogen production up to 1600 mL/L for both cathodes evaluate. After 25 cycles operated with acetate the substrate was changed to real brewery wastewater. Hydrogen production was higher using stainless steel (621 ± 178 mL/L) than nickel foam (546 ± 112 mL/L). Electrical efficiency was around 84 % in both cases. However, for both configurations, the organic matter removal was lower than 50 %, and coulombic efficiency was lower than 75 %. At the same time, both cathode materials have a limit for recovering electrons and transforming them into hydrogen (cathodic efficiency) less than 80%. Microbial communities of the anodes are being analyzed by 16S amplicon sequencing.

Poster-E36: Not quite Völki-sized: Scale up of Electrochemical CO_2 Reduction Reaction (eCO_2RR) to 300 cm^2 scale

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Carbon Conversion and Utilization (CCU) Technologies are an important step in re-using greenhouse gas emissions and assisting in the move towards a net zero society and industry. Thereby, CCU technologies follow many of the Principles of Green Chemistry.[1] To ensure these technologies are practical for use, they must first be scaled up, with their performance at scale carefully evaluated and optimized. Continuing previous works on this field, the study presented here is departing from an established 10 cm^2 tin-based gas-diffusion electrode (GDE) setup for the electrochemical CO_2 Reduction Reaction (eCO_2RR) to formate.[2] This setup was already scaled up to 100 cm^2 in previous works. Continuing from there, a stack assembly of three 100 cm^2 GDEs totaling a surface area of 300 cm^2 was established. To keep comparisons consistent, operational parameters such as current density (100 mA cm^{-2}) and ratio of catholyte volume to electrode surface area (50 ml cm^{-2}) remained constant.
Results showed that even in the, as of yet, unoptimized 300 cm^2 scale electrode stack systems, coulombic efficiencies for formate production ($CE_{Formate}$) of $CE_{Formate} \leq 65$ % could be achieved. This shows promise, that upon further optimization, similar values as in the optimized 10 cm^2 and 100 cm^2 scale ($CE_{Formate} \geq 85$ %) can reasonably be reached.[2] Additionally, formate production rates ($r_{Formate}$) of $r_{Formate} \leq 370$ mmol h^{-1} could be achieved at the 300 cm^2 scale. This is an almost 6-fold increase from the 10 cm^2 scale setup ($r_{Formate} \approx 64$ mmol h^{-1}), which this design was based on.[2] Thereby, the immense potential of scaling up reaction setups beyond ordinary lab scale is shown, arguably making up for the observed reductions in efficiencies, as is expected when carrying out scale-up work.[3] In the work presented here, the challenges, outlook and opportunities involved with scaling up laboratory scale experiments into semi-industrial scales and stack assemblies will be discussed.

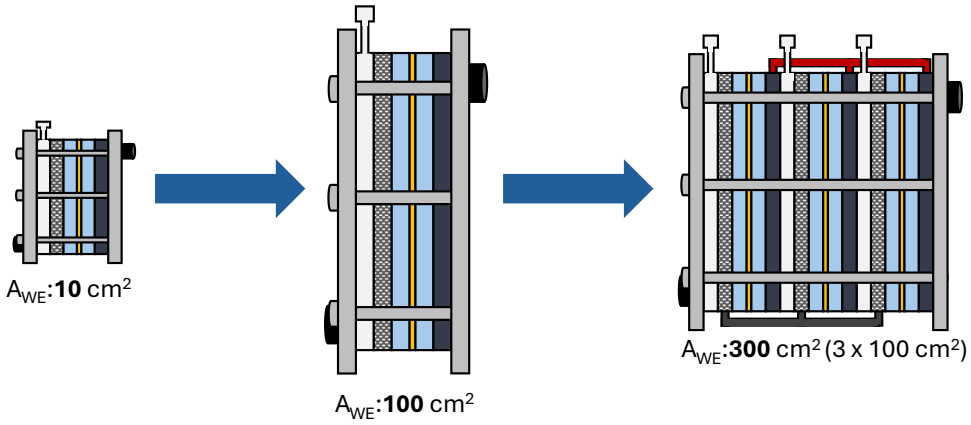

Figure 1: Schematic progression of reaction setup scale-up from a GDE working electrode surface area (A_{WE}) of 10 cm^2, to 100 cm^2 and finally 300 cm^2. Figure adapted from [4].

References

[1] P. T. Anastas, J. C. Warner, *Green Chemistry : Theory and Practice*, Oxford University Press, Oxford, 1998.
[2] P. Izadi, J. Song, C. Singh, D. Pant, F. Harnisch, *Advanced Energy and Sustainability Research* 2024, *5*, 1–7.
[3] M. N. Gey, C. Schneider, U. Schröder, *React Chem Eng* 2025, *10*, 417–427.
[4] P. Izadi, S. Varhade, C. Schneider, P. Haus, C. Singh, A. Guruji, D. Pant, F. Harnisch, 2025, *in preparation.*

Poster-E37: Optimization of 3D Electrodes Design for Enhanced Bioanode Performance: Bridging Numerical Modeling and Experimental Validation.

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The geometric design of electrodes in bioelectrochemical systems is critical to maximise the performance of bioelectrooxidation and the free transfer of soluble chemical species (substrates, ions and products including H^+). It is sometimes imagined quite simply that the larger the colonisable surface area or available surface area of the electrode, the higher the anodic bioelectrooxidation kinetics [2]. The problem is more complex because the electric current production kinetics of microbial bioanodes are dependent on (i) the exchange surface between the electrode and the electroactive biofilm, (ii) the supply of organic substrates to and in the biofilm, (iii) the removal of toxic reaction products, (iv) the easy circulation of ions in the electrolyte and the biofilm [1]. My thesis work consists of using numerical simulation (Comsol Multiphysics software) to **design innovative 3D electrodes** using a systemic approach for bioanodes and the phenomena that modulate their electroactivity. Experimental work in 3-electrode electrochemical cells and under model conditions is also used for validating the applicability of these designs. Directions for improvement include design upgrades based on 3D geometry [2], surface topography [1] and the porous properties of the electrodes.

More concretely, the first objective is to develop and perfect a numerical model that is as realistic as possible (tertiary current distribution) in order to test, by simulation, different 3D geometries with increasing degrees of complexity and optimization. Complex designs are created using Autodesk Inventor 3D modeling software and then imported into Comsol Multiphysics for analysis. The most promising electrode geometries are subsequently manufactured using carbon-based additive 3D printing before being tested experimentally. The numerical model developed incorporates the electrochemical real kinetics of bioanodes, determined from cyclic voltammetries carried out on flat electrodes in optimized and reproducible bioelectrochemical reactors (stirred medium, controlled substrate concentration).

The preliminary findings from the simulation work, which guided the optimization of electrode design, validated previously established knowledge:

- **Impact of electrode spacing:** Based on a primary current distribution model, the electrolyte's conductivity—assimilated to wastewater—plays a key role in the ohmic drop. Increasing the distance between electrodes raises resistance, thereby reducing overall current density.
- **Pore depth in porous electrodes:** The depth of anode pores influences local ohmic drop due to the greater distance between deep-pore regions and the counter-cathode. This weakens the expected correlation between the developed electrode surface area and the predicted current density. In short, deeper pores lead to lower average current density in the bioanode.

These first steps in simulation indicate that, to maximize the anode's active surface area while minimizing current density loss, shallow pores should be distributed across a large 2D surface positioned as close as possible to the counter-cathode, reducing ohmic drop effects. With this objective in mind, one of the targeted geometries is a symmetrical 2D anodic "zigzag" structure with a shallow structured porous surface. This "zigzag" design is based on "folding patterns" formed after the additive manufacturing of electrode materials. This approach optimizes the ratio between the anode surface area and the electrolyte volume (m^2/m^3).

References.

[1] P. Champigneux, M.-L. Delia, et A. Bergel, « Impact of electrode micro- and nano-scale topography on the formation and performance of microbial electrodes », *Biosensors and Bioelectronics*, vol. 118, p. 231-246, oct. 2018, doi: 10.1016/j.bios.2018.06.059.

[2] X. Xie, C. Criddle, et Y. Cui, « Design and fabrication of bioelectrodes for microbial bioelectrochemical systems », *Energy Environ. Sci.*, vol. 8, n° 12, p. 3418-3441, 2015, doi: 10.1039/C5EE01862E.

Poster-E38: Heavy Metal Meets Heavy Science: The influence of Leipzig's Hardcore - Death Metal band Chaver on MES Reactor Design

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In the spirit of Leipzig's hardcore-death metal band Chaver, known for their relentless energy and innovative sound, our work in the ESCAPE 2.0 project (Establishing a scalable bioprocess reactor platform for cathodic obligate anaerobic electrobiosynthesis) embodies a similar drive for innovation and precision in the realm of microbial electrosynthesis (MES). Our efforts focus on the design and optimization of reactors and process parameters for pure-culture MES with obligate anaerobic microorganisms, as well as the characterization of stress factors for the microbial hosts in our system.
At the heart of our project is the development of a scalable and efficient electrobioreactor platform[1], designed to overcome existing limitations in MES technology. Our primary goal is to enhance the performance of acetogens, like *Clostridium ljungdahlii*[2], by addressing critical challenges such as oxygen intrusion and hence stress and limited electron donor availability in the bioreactors. Through an iterative design approach, integrating both experimental testing and computational modeling, we aim to optimize reactor configurations and operational parameters.
A significant aspect of our research involves exploring alternative anodic reactions (besides the classical oxygen evolution reaction) to mitigate oxygen toxicity, thereby improving microbial viability and productivity. We systematically evaluate and optimize reactor-specific parameters, such as electrode surface area and gas transfer rates, mass transfer kinetics, and mixing time, using a Design of Experiments methodology. The optimized platform will be benchmarked across different operational modes and microbial hosts to assess its versatility and efficiency.
Our work is driven by the same unyielding pursuit of excellence that characterizes Chaver's music, as we strive to push the boundaries of MES technology. By combining the intensity of heavy metal with the rigor of scientific inquiry, we aspire to establish a robust and scalable MES process, contributing to sustainable bioproduction from CO_2. Just as Chaver's music resonates deeply with its audience, we aim for our research to resonate within the scientific community, driving forward the field of microbial electrosynthesis.
Funding: This research is funded by the Deutsche Forschungsgemeinschaft (DFG) - Project number 422694804

References:
[1]Rosa, L. F. M., et al. (2019). "Integrating Electrochemistry into Bioreactors: Effect of the Upgrade Kit on Mass Transfer, Mixing Time and Sterilizability." Frontiers in Energy Research **Volume 7 - 2019**.
[2]Boto, S. T., et al. (2023). "Microbial electrosynthesis with Clostridium ljungdahlii benefits from hydrogen electron mediation and permits a greater variety of products." Green Chemistry **25**(11): 4375-4386.

Poster-E39: Development of carbon-efficient self-powered disinfection system based on on-site microbial electrochemical system

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Human development has accelerated climate change, causing extreme weather events and water scarcity. Simultaneously, human-generated waste contributes to water pollution, with pathogenic microorganisms in wastewater posing serious concerns. Conventional wastewater treatment facilities commonly use chlorine gas (Cl_2) for disinfection due to its high efficacy and cost-effectiveness. However, Cl_2 generates disinfection by-products (DBPs) that can be harmful to human health, and issues with the transportation and storage of chlorine remain problematic. Recently, hypochlorous acid (HOCl), produced via electrolysis, has gained attention as a potential alternative to chlorine. HOCl generated through electrolysis is a disinfectant that operates at low concentrations, producing fewer disinfection by-products compared to conventional disinfectants. Since HOCl is produced on-site, transportation and storage costs can be reduced. However, its generation through the electrolysis of solutions containing dilute sodium chloride requires an external power source. Microbial fuel cells (MFCs) are a technology capable of supplying electricity for HOCl production. These systems utilize electrochemically active microorganisms to convert organic matter in wastewater into electricity. In this study, we propose an MFC-based disinfection system capable of on-site generation of disinfectants. The proposed system is evaluated for its effectiveness in inactivating representative pathogenic microorganisms, and its potential for field application is discussed.

Poster-E40: Development of a Biohybrid Solar Power Plant (BISON) by The New Leipzig School of Reactor Design

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The **Bi**ohybrid **S**olar **P**ower Pla**n**t project (BISON) [1] aims at developing a combined biophotovoltaic-electrochemical prototype for the synthesis of hydrogen and formate from sunlight, CO_2 and water. Photosynthesising cyanobacteria are employed to harvest sunlight energy by water splitting which is then transferred to reduce redox shuttles. Those redox shuttles have been chemically tailored to increase bio-compatibility and reduction/oxidation rates and are subsequently channeled to an electrochemical module where they are anodically oxidized. At the cathode, hydrogen or formate are produced depending on the used electrode material. The complete prototype is supposed to host $1m^3$ of cyanobacterial culture medium and represents an autarkic, decentralized system for producing simple energy carriers.
Our role (i.e. work packages 7 & 8) in the project is to design, build and benchmark a scalable electrochemical module with a dual functionality combining two separated streams: On the one hand, it serves to oxidise the redox shuttles at the anode so that oxidized redox shuttles are continuously provided to the cyanobacteria to keep their metabolism continuously running. In addition, the bacterial electrons are used to electrochemically make hydrogen and/or reduce CO_2 to formate which can be easily harvested.
Design-criteria for the electrochemical module are portability, autonomy, and simplicity as well as energy and cost efficiency.
In order to meet these criteria, the electrochemical module will be equipped with a tailored instrumentation and control unit and a CO_2 capture unit. We have employed 3D-resin-printing for rapid-prototyping of a continuous flow electrochemical plate reactor which allows for swift optimisation of the design. In a nutshell, BISON combines established biological and electrochemical processes into a novel concept. The developed reactor shall be used as a blueprint beyond the use case of BISON – hence we call it the New Leipzig School of Reactor Design.

Acknowledgment: This project (No. 100702610) is co-financed by means of taxation based on the budget adopted by the representatives of the Landtag of Saxony.

[1] BISON website: https://www.ufz.de/index.php?en=52030

Poster-E41: Bypassing the limits of hydrogen solubility in biomethanation with conductive materials and redox mediators

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Ex-situ biomethanation implies a conversion of carbon dioxide (CO_2) and hydrogen (H_2) into methane (CH_4) by hydrogenotrophic microorganisms in a separate reactor. Being able to upgrade raw biogas to near natural gas quality and utilize renewable energy in the form of H_2, biomethanation holds great promise as a method of CO_2 recycling and Power-to-X technology. It can be carried out in reactors of various designs. Arguably, one of the most suitable options is a trickle bed reactor: inoculate carrier materials inside it with methanogenic biofilms, feed it with biogas, and it produces CH_4 at a remarkable overall rate up to 12.6 L L^{-1} d^{-1}, achieving complete conversion of H_2 [1]. However, although this system performs gas fermentation, it remains largely limited by the very low aqueous solubility of H_2, which must diffuse through the liquid sheath surrounding the biofilms. This leads to an uneven distribution of the methane production rate within the reactor, restricting the system's performance and likely complicating scaling up.

Various approaches could alleviate the H_2 solubility issue by providing methanogens with electron donors other than H_2. For example, conductive granular carbon as a support material can not only increase active areas and enrich surface chemistry, but also offer a platform for interspecies electron transfer, so that methanogens could receive electrons from syntrophic microorganisms [2]. Redox mediators work in much the same way. They could be reduced either by electrons from the electrodes (i.e. electrochemically) or other microorganisms, and then oxidised by methanogens, thus - again - bridging them to new electron sources [3].

Therefore, this study explores the possibility of boosting biomethanation in trickle-bed reactors using conductive support materials with immobilized redox mediators. As the former, it screens different granular carbon materials compared to other conductive and non-conductive supports, while the library of redox mediators consists of various anthraquinones and phenazines. The prospects of different combinations are assessed in a series of batch measurements, where the kinetics of ongoing biomethanation are continuously tracked by pressure measurements supported by gas chromatography data. Meanwhile, the redox behavior of the support materials is scrutinized through various electrochemical measurements (cyclic voltammetry, square wave voltammetry, chrono methods, etc.) performed before and after the CO_2-to-CH_4 conversion. Overall, the data presented highlights the potential for methanogenic organisms to utilize electron donors other than H_2, thereby reducing the dependence of biomethanation productivity on constrained mass transfer.

The study is funded under the HORIZON MSCA Postdoctoral fellowship program (HORIZON-MSCA-2023-PF-01-01, Project BES.WIRE)

[1] Sieborg, M. U., Ottosen, L. D. M., & Kofoed, M. V. W. (**2023**). *Bioresource Technology*, *384*
[2] Caizán-Juanarena, L., Sleutels, T., Kokko, M., Berenguer, R., & ter Heijne, A. (2024). In *Emerging Trends and Advances in Microbial Electrochemical Technologies* (pp. 425–478). Elsevier.
[3] Gemünde, A., Lai, B., Pause, L., Krömer, J., & Holtmann, D. (2022). *ChemElectroChem*, *9*(13).

Poster-E42: Evaluation of Graphite-Based Cathode Materials for Autotrophic Denitrification in Bioelectrochemical Systems

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Elevated nitrate levels, especially in groundwater, challenge global drinking water supplies. Autotrophic denitrification via bioelectrochemical systems (BES) offers a promising alternative, enabling nitrate removal with low energy demand and minimal sludge production. However, large-scale implementation is still hindered for example by high ohmic resistances[1]. While BES research has explored various operational parameters, the impact of electrode material selection on autotrophic denitrification remains unclear. This study provides the first lab-based comparative analysis of graphite-based electrode materials for nitrate reduction in BES.

Batch experiments (245 mL working volume, 31.3 ± 0.1 cm² working electrode surface) were conducted in duplicates for graphite rod, graphite granules, and graphite felt over four days at a poised potential of -0.853 V vs. Ag/AgCl. Nitrate and nitrite concentrations in synthetic groundwater were measured twice daily, while total cell count (TCC) was determined once daily. Electrodes were characterized, among other methods, using electrochemical impedance spectroscopy (EIS) and flow cytometry.

Denitrification rates varied significantly depending on the electrode material. The figure below illustrates the denitrification rates and nitrite accumulation over time. It is evident that graphite felt exhibited the best denitrification performance, achieving both high denitrification rates and low nitrite accumulation. The high denitrification rate in graphite felt reactors can be attributed, among other factors, to the highest TCC in both the water and on the working electrode compared to the other materials. Material properties such as high specific surface area promote biomass growth. Additionally, graphite felt electrodes exhibited relatively low charge transfer and diffusion resistances, leading to improved electron transfer to nitrate and thereby enhancing denitrification kinetics. By identifying materials that enhance microbial growth and minimize resistances, this study provides key insights toward improving BES performance. While further studies on system scalability are needed, the results suggest that material selection is a critical factor in enabling the upscaling of autotrophic denitrification processes.

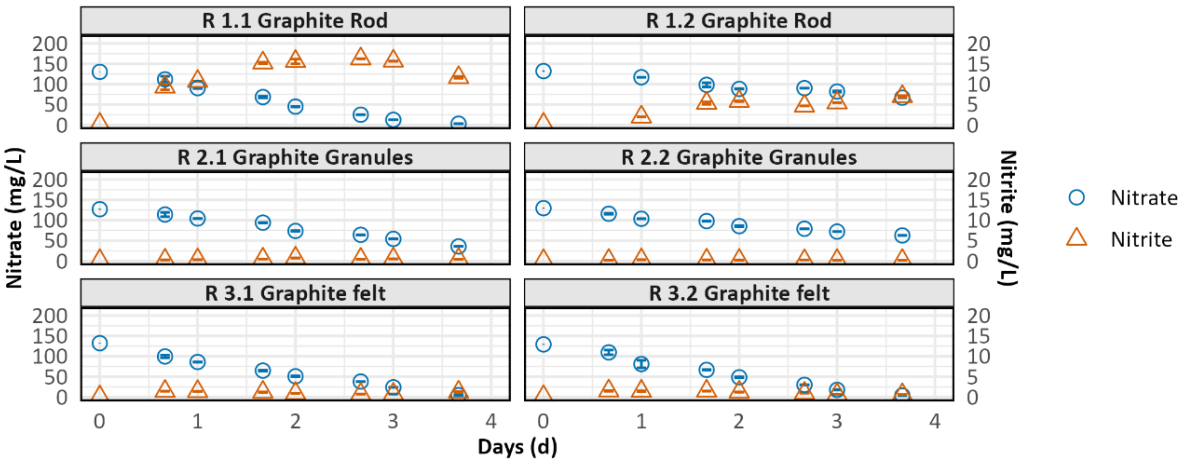

Figure 1: Nitrate reduction and nitrite accumulation over time for different graphite-based electrodes.

1. Ortega-Martínez, E., Toledo-Alarcón, J., Fernández, E., Campos, J.L., Oyarzún, R., Etchebehere, C.,6+ Cardeña, R., Cabezas, A., Koók, L., Bakonyi, P., Magdalena, J.A., Trably, E., Bernet, N., Jeison, D., 2024. A review of autotrophic denitrification for groundwater remediation: A special focus on bioelectrochemical reactors. Journal of Environmental Chemical Engineering 12, 111552. https://doi.org/10.1016/j.jece.2023.111552

Poster-E43: Bioelectrochemical *In-Situ* Regeneration of Granular Activated Carbon for Greywater Treatment

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Greywater reuse is a reliable strategy for addressing water scarcity in hydric-stressed zones. For this purpose, biological treatment systems based on granular activated carbon (GAC) represent a promising alternative due to high GAC adsorptive capacity, adaptability to high organic matter loads, no chemical requirement, and no byproduct generation. An emerging challenge associated with the operation of these systems occurs when GAC reaches its maximum adsorptive capacity (saturation). This subsequently results in a detrimental impact on the treatment performance. Therefore, it is essential to restore the properties of GAC to ensure the continued effectiveness of greywater treatment systems. To extend the operational lifespan of the GAC, several regeneration techniques have been employed, including electrochemical, thermal, and biological. While traditional techniques such as thermal and electrochemical regeneration are highly effective, they are also energy-intensive. They can lead to the depletion of microbial communities due to elevated temperatures, drastic pH fluctuations, and electricity application. Conversely, biological techniques comprise an eco-friendly approach for GAC regeneration but suffer from slow regeneration rates and irregular efficiencies.

An emerging technology with significant potential for the treatment of greywater is the bioelectrochemical systems (BESs). BESs are based on the principle of electrochemically active microorganisms (EAMs) capable of degrading pollutants to produce electric current. While the efficacy of BES in treating greywater has been established, a notable potential exists for the technology to be employed in the field of *in-situ* regeneration of GAC. The present study focuses on assessing the efficiency of BESs as a technology applied for *in-situ* regeneration of GAC from the biological greywater treatment and its effect on bacterial community diversity. In this regard, the study determines the optimal operational conditions of BESs for GAC regeneration and the impact of bacterial community diversity on the effectiveness of GAC regeneration. This is achieved by conducting effluent water quality analyses and GAC adsorption isotherms for organic loads before and after regeneration. Bioinformatic bacterial community analyses (i.e., alpha diversity, non-metric multidimensional scaling, taxonomic assignment) are performed to evaluate the variance between electrochemical and nonelectrochemical GAC regeneration methods. Finally, this research offers crucial insights into utilizing BES as an effective approach for *in-situ* GAC regeneration and the synergistic interaction between GAC and EAMs for the prospective applications of GAC.

Poster-E44: Deploying Power Management Systems Using Maximum Power Point Tracking for the Implementation and Scale Up of Microbial Fuel Cell Systems

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Microbial Fuel Cells (MFCs) can be used as remote sensors, for the treatment of pollution and for energy harvesting. However, to realise this potential, the electrical monitoring and control of these systems needs to be implemented via an effective and low cost power management systems. The Power Management System (PMS) for MFCs should perform three essential tasks: optimising the power generation of MFCs by operating them at their Maximum Power Point (MPP), efficiently capture and store the energy generated by the MFCs, and maintain the output voltage within the desired range to deliver the primary objective of the MFCs either as biosensor, pollution removal technology or for energy harvesting. Consequently, there is a growing demand for PMSs that can efficiently extract and harvest energy from multiple MFCs, each operating at its respective MPP, while effectively converting the harvested energy into a usable form for powering external devices. However, in applications where a single MFC unit is required, such as in low-power biosensors or standalone monitoring devices, an efficient PMS is equally crucial. In these cases, the PMS must be designed to maximise the energy extracted from a single MFC, ensuring stable operation and high efficiency. Whether managing a single MFC or multiple MFC modules, an effective PMS is essential for optimising power extraction, improving system efficiency, and enabling practical deployment.

A PMS was modeled using MATLAB Simulink with the optimum circuit design implemented in a with an ultra-low power microcontroller (STM32L452RE6T, ARM) to measure directly the voltage of the MFC with a current sense amplifier (Isense, INA190A4IDDFR, Texas Instruments) with a shunt resistor (Rs) which is connected in series with the inductor to measure the current. The resulting PCB integrates the PMS, capable of performing MPPT for up to five MFCs, along with energy harvesting, voltage regulation, and power management functions. The designed PMS was employed in the operation of five tubular MFCs connected hydraulically in series. Five tubular MFC were assembled, each with 5 MFC modules. These MFCs used a cation exchange membrane (CMI7000S, Membrane International Inc., NJ, USA), with a 6 mm rolled carbon cloth rod anodes and a 0.5 mg cm^{-2} Pt carbon cloth cathode. The five tubes were hydraulically connected in series. Each tubular MFC had an approximate volume of 1.6 L (8 L in total). The MFC assembly was batch fed on a continuous loop from a 20 litre reservoir with a 1:1 blend of untreated domestic wastewater and synthetic sewage prepared according to the OECD guidelines, which had a Chemical Oxygen Demand (COD) strength of approximately 1000 mg/L. More recently, a second PMS was developed, incorporating a novel Parallel-Boosted Double Dual Converter (PBDDC) alongside an Adaptive Differential Evolution with Localized Exploration (ADELE) MPPT algorithm.

In conclusion a comprehensive PMS was designed and tested to effectively track the MPP and recover energy from up to five tubular MFCs reactors. The energy obtained from these MFCs was used to provide power to the PCB, with any excess energy being directed towards illuminating an LED. This PMS provided distinct advantages compared to previously reported systems such as individual and rapid MPPT for up to 5 MFCs with energy harvesting capabilities with a voltage up to 3.3 V at 5.8 mW, a high efficiency of 87%, self-sustaining operation when MFCs have sufficient feed COD, a backup power source to ensure uninterrupted operation in all scenarios, and a compact PCB design housing all necessary components for ease functionality. A second innovative PBDDC PMS was developed to track the MPPT of a single MFC, addressing the low efficiencies typically encountered when achieving voltage gains greater than 10 from a single MFC. By implementing the optimized ADELE algorithm, efficiencies of up to 92% were achieved at an output voltage of up to 3.3 V. The PMS PCB is now being investigated further at the USW and with collaborators in South Korea.

Poster-E45: Tailoring Glubugraphite (GG) via ZnO Template Engineering for Enhancing Electrochemical Performance

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The development of efficient and stable carbon-based electrodes is crucial for enhancing bioelectrochemical systems, where key properties such as electrical conductivity, porosity, surface area, and mechanical strength determine performance. This study presents the synthesis, refining porosity and electrochemical evaluation of Glubugraphite (GG), a novel porous carbon foam fabricated through ZnO templating [1,2]. ZnO templates were prepared under different sintering patterns, varying temperature and holding time. This template engineering strategy allowed control over the pore size and total porosity of the resulting GG structures. A photograph of a representative tablet-shaped GG electrode is shown in Figure 1a. The final GG materials were characterized using SEM, XRD, BET and MIP revealing well-connected porous carbon frameworks. Specific surface area varied between 536.5 to 819.1 m^2/g which was found to be mainly mesoporous. However, the total skeletal porosity remains almost same at 98% and total accessible porosity of 79.0% was achieved.
Electrochemical performance was evaluated using cyclic voltammetry, demonstrating the effect of tailoring on the final GG electrochemical performance and charge transport behavior. Preliminary electroenzymatic assessments also showed differences in reactivity, further highlighting the role of structural tuning.

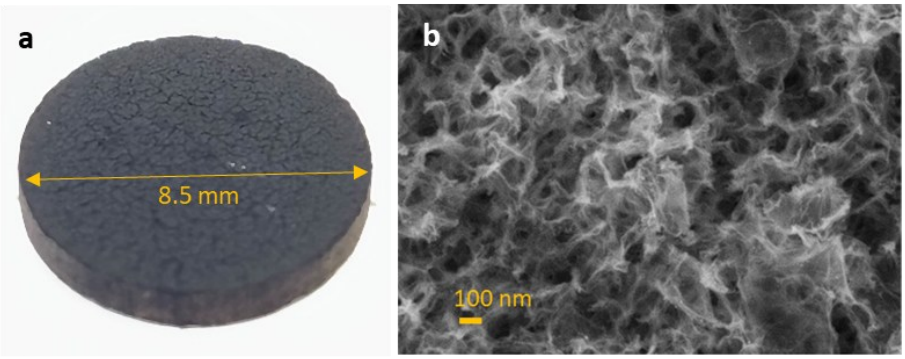

Figure 1: Photograph of a tablet-shaped Glubugraphite (GG) electrode. (b) SEM image showing the highly porous and interconnected nanostructure of GG

References:

[1] Beisch, H., & Fiedler, B. (2020). Nanocarbon Aerogels and Aerographite. In *Synthesis and Applications of Nanocarbons* (pp. 247–274). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119429418.ch8
[2] Marx, J., Beisch, H., Garlof, S., & Fiedler, B. (2017). Structural improvement of a bio-inspired 3D globular carbon foam by a continuously thermal treatment: A comprehensive study. *Advanced Material Science*, *2*(4). https://doi.org/10.15761/AMS.1000132

Poster-E46: Establishing jet loop reactors as scalable bioelectrochemical reactor systems for anodic and cathodic production processes

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Microbial electrolysis cells (MEC) and oxic microbial electrosynthesis (OMES) offer promising approaches for the sustainable production of hydrogen and platform chemicals. However, establishing these technologies faces challenges related to mass transfer, scalability, and process optimization. The microbes need a well-defined low-shear environment while facing mass transfer limitations regarding substrate and dissolved gas supply [1]. To address these issues, we present a novel reactor concept that ensures efficient mixing and nutrient supply while maintaining a laminar environment to support biofilm growth. The targeted systems are MEC with exoelectrogenic Shewanella oneidensis and OMES with electroautotrophic Knallgas bacterium Kyrpidia spormannii.

The double jet-loop reactor (Fig. 1) is a two-phase jet loop reactor with integrated bioelectrochemical modules, where microorganisms colonize 3D-printed lattice electrodes as biofilms. It features an inner loop for gas dispersion, mass transfer, and mixing [2], and an outer loop with low shear forces for biofilm cultivation. The triply periodic minimal surface electrodes optimize fluid flow, substrate accessibility, and surface area [3]. Functionalization with cell cross-linkers enhances biofilm stability.

We employ a two-step approach, starting with an automated two-dimensional microfluidic platform (Fig. 2) equipped with a potentiostat, an optical coherence tomograph and a sampler to enabler a detailed investigation of bioelectrochemical processes including productivity as well as biofilm stability at different flow rates. In the second step, fluid dynamics within the 3D structure are analyzed using magnetic resonance imaging (MRI), enabling targeted scaling to larger systems. A vertical MRI setup further allows comprehensive characterization of the entire reactor concept.

The insights gained from this approach support the optimization of mass transfer and flow rates to establish ideal conditions for microbial growth and activity. By integrating microfluidic testing, MRI-based flow analysis, and scalable jet loop reactors, we propose an innovative strategy to enhance the efficiency of microbial electrochemical systems, ultimately maximizing hydrogen production or the synthesis of valuable platform chemicals.

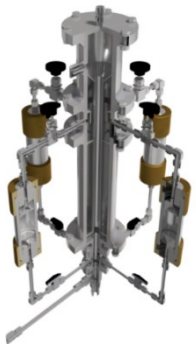

Figure 1: Double jet-loop reactor

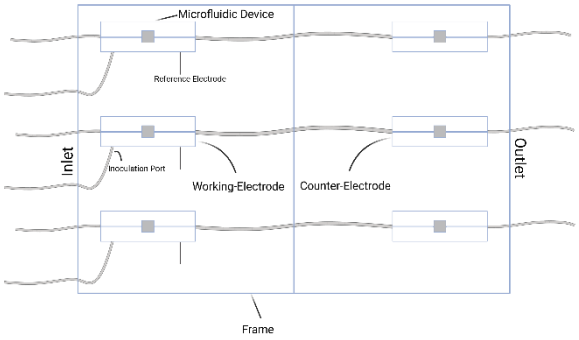

Figure 2: Microfluidic platform, created with Biorender

References
[1] Hackbarth, M., Wagner, M. (2020). Chemical engineering journal, 390, 124604.
[2] Maly, M., Schlüter, M. (2022). Processes, 10(8), 1531.
[3] Eckendörfer, L., (2024). Annual Review of Chemical and Biomolecular Engineering, 15(1), 163–186.

Poster-E47: Tailored functional electrode structures for SMART bioreactors

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This project aims to characterise electrode materials in cathodic Microbial Electrosynthesis Systems (MES) as substrata for hydrogenotrophic biofilms, leading to adaptive continuous biotechnological processes. The desirability of continuous biofilm-based processes stems from their ability to behave as a natural retentostate and self-optimize over time. Biofilms are defined by a robust extracellular polymeric substance (EPS) matrix and inherent heterogeneity, making them highly desirable and advantageous for designing sustainable and scalable bioprocesses [1]. The electrode material of choice was graphite, in order to ensure good electrical conductivity and durability. Different graphite electrode forms were evaluated on the basis of porosity and electrochemical activity. These electrode materials were extensively tested in the MES with the model knallgas bacterium *Cupriavidus necator* H16 to observe the biofilm growth characteristics via optical coherence tomography (OCT), a non-invasive imaging technique [2]. Globugraphite is an aerogel made up of spherical, hollow carbon shells that are interconnected resulting in a highly porous three-dimensional material [3]. It exhibited an electroactive surface area 1.36 times greater than that of solid graphite, attributing to its porous structure featuring macropores of diameter 500 µm. Initial experiments were conducted using plain graphite as the benchmark electrode material. *C. necator* was first cultivated under heterotrophic conditions for six days, with fructose serving as the primary carbon source. Following the onset of biofilm formation, the system was transitioned to electroautotrophic conditions by applying a constant current of –15 µA and introducing carbon dioxide as the sole carbon source to support microbial activity. During the 10-day electroautotrophic phase, the biofilm reached a maximum thickness of 250–300 µm, indicating that the shift from heterotrophic to autotrophic conditions fostered biomass growth. Furthermore, the introduction of a recirculation loop enabled the detection of acetoin, a key platform chemical produced during the process. This finding, alongside future investigations, will advance our understanding of biofilm behavior and metabolic dynamics in bioelectrochemical systems.

References:

[1] C. Windhorst and J. Gescher, “Efficient biochemical production of acetoin from carbon dioxide using Cupriavidus necator H16,” *Biotechnol Biofuels*, vol. 12, no. 1, Jun. 2019, doi: 10.1186/s13068-019-1512-x.
[2] E. Klein, R. Wurst, D. Rehnlund, and J. Gescher, “Elucidating the development of cooperative anode-biofilm-structures,” *Biofilm*, vol. 7, Jun. 2024, doi: 10.1016/j.bioflm.2024.100193.
[3] J. Marx, H. Beisch, S. Garlof, and B. Fiedler, “Structural improvement of a bio-inspired 3D globular carbon foam by a continuously thermal treatment: A comprehensive study,” *Advanced Material Science*, vol. 2, no. 4, 2017, doi: 10.15761/ams.1000132.

Poster-E48: Optimization of Redox Mediator Reduction within Capillary Biofilm Reactor for Application in Biophotovoltaics: Numerical Simulation

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Biophotovoltaics (BPV) is a mediator-based extracellular electron transfer (MEET) process. It enables photoautotrophic cyanobacteria to use light energy for water splitting and electrochemical reactions. This results in the sustainable production of value-added chemicals like hydrogen gas (H_2)[1]. MEET helps extract electrons from water splitting using redox mediators[2]. Ferricyanide $[Fe(CN)_6]^{3-}$ (FeCN(III)) is used here due to its stability, solubility, and robust redox behavior in BPV systems[3]. This study aims to optimize FeCN(III) reduction in a capillary biofilm reactor (CBR) with cyanobacterial monocultures. The reduced form, ferrocyanide $[Fe(CN)_6]^{4-}$ (FeCN(II)), transfers electrons to the anode in a bioelectrochemical system (BES) for potential H_2 production at the cathode. Experimental studies of BPV systems are time- and resource-intensive. Complex reactor dynamics often make data interpretation difficult. Mathematical modeling offers a faster and more cost-effective alternative. It helps evaluate FeCN(III) reduction by cyanobacteria and guides experimental design and system optimization. In this study, the governing equations were solved using MATLAB©. A linear kinetic model was developed and validated with batch reactor data. FeCN(III) reduction was tested across different cyanobacterial strains. Parameter fitting used the Nelder-Mead simplex algorithm. Reduction constants varied by an order of magnitude, with *Anabaena sp*. showing the highest activity. Next, a one-dimensional model of the CBR was created using *Anabaena* biomass. It includes advection, diffusion, and reaction kinetics. The model was solved using a tri-diagonal matrix algorithm, with Robin and Neumann boundary conditions at the inlet and outlet. Model results were verified against analytical solution[4] for stability and accuracy. Simulation results show FeCN(III) reduction improves with reactor length. At 0.2 m length, reduction is ~30%, rising to ~80% at 0.8 m. A similar ~80% reduction can be achieved at 0.2 m by lowering the flow rate (F_V) from 52 to 13 µL min^{-1}. However, low F_V may increase diffusion dominance, lowering FeCN(III) availability and increasing residence time, which may reduce efficiency. Thus, an optimal balance between reactor length and F_V is needed. Future improvements include modeling simultaneous FeCN(III) and cyanobacteria injection. This will offer deeper insight into spatial dynamics and performance limits. Overall, the model enables efficient optimization of the BPV system by guiding parameter selection and reducing experimental effort and cost.

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References

1. Lai, B. et al. 2021. Technical-scale biophotovoltaics for long-term photo-current generation from *Synechocystis sp.* PCC6803. *Biotechnol Bioeng.* **118**: 2637 – 2648.
2. Gemünde, A. et al. 2022. Redox mediators in microbial electrochemical systems. *ChemElectroChem* **9**: e202200216.
3. Reilly-Schott, V. et al. 2024. Electron leaks in biophotovoltaics: A multi-disciplinary perspective. *ChemCatChem* **16**: e202400639.
4. van Genuchten, M. Th., and Alves, W. J. 1982. Analytical solutions of the one-dimensional convective-dispersive solute transport equation. U.S. Department of Agriculture, Technical Bulletin No. 1661, 151 p.

Poster-E49: Additively manufactured metallic 3D-lattice bioanodes for Microbial Electrochemical Technologies

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Microbial electrochemical technologies (METs) offer a promising approach for wastewater treatment while simultaneously generating electrical current or energy carriers. However, their practical implementation remains limited due to low current densities and challenges in scaling up. In this context, 3D-additively manufactured metallic electrodes are an exciting opportunity, as they enable a large degree of freedom in tailoring the electrode macro- and meso-structure to improve current densities. This study evaluates the potential application of additively manufactured metallic 3D-lattice bioanodes for METs, assessing their bioelectrochemical performance and the influence of alloying elements.
Electrodes were additively manufactured via powder bed fusion with a laser beam (Aconity Mini, Aconity3D GmbH, Herzogenrath, Germany) and subsequently cleaned by sonication (20 min in 70 % isopropanol, followed by two 10 min sonication in deionized water). Two single-chamber battery glass reactors were assembled [1] to test six electrodes simultaneously under identical conditions. One reactor contained duplicates of three stainless steels (316l, EOS254 and F53) while the other included duplicates of two stainless steels and a titanium alloy (EOS254, F53 and Ti6Al4V). The reactors were filled with 1 l anaerobic medium [2], supplemented with 15 ml beer and 2 ml of 10 % m/V yeast extract (only for startup), and inoculated with *G. sulfurreducens* to an OD_{600} of 0.06, along with 10 ml brewery wastewater. After 18 h at open circuit potential, bioanodes were polarized at -300 mV vs. SCE and operated under strong convective flow (500 rpm, magnetic stirring). Electrodes were characterized by scanning electron microscopy, capacitance measurements (double-layer analysis), corrosion behavior (linear sweep voltammetry), and electrochemical performance at different potentials (cyclic voltammetry and stepwise polarization).
Surface characterization revealed that F53 exhibited the highest surface and electrocatalytic areas, while EOS254 had the lowest. Corrosion potential measurements showed that 316l had the lowest (-479 mV vs. SCE) and EOS254 the highest (-317 mV vs. SCE), suggesting better corrosion resistance of EOS254. 316L also exhibited the highest corrosion current at -300 mV vs. SCE, though values were three orders of magnitude lower than those observed with biofilms. In four-week potentiostatic experiment, F53 bioanode achieved the highest current density (3.2 mA cm^{-3}), followed by EOS254 and 316L. Ti alloy showed a continuously increasing current over the four weeks but reached a maximum of only 0.3 mA cm^{-3}. These results surpass previously reported performances of additively manufactured 316L 3D-lattice bioanodes which produced up to 1.18 mA cm^{-3} [3], and align well with the performance of layered corrugated carbon anodes (one of the highest reported) with low number of layers [4]. Regarding alloying elements, while Ni affects surface finish during manufacturing, it had no significant influence on bioelectrochemical performance. In contrast, Cr and Mo impacted corrosion resistance, with Cr also potentially affecting the bioelectrochemical performance.

References

[1] Erben, J., Pinder, Z.A., Lüdtke, M.S., Kerzenmacher, S. (2021). Front. Microbiol. 12, 660474. doi:10.3389/fmicb.2021.660474
[2] Coppi, M.V., Leang, C., Sandler, S.J., Lovley, D.R. (2001). Appl. Environ. Microbiol. 67, 3180–3187. doi:10.1128/AEM.67.7.3180-3187.2001
[3] Yamashita, T., Yamashita, Y., Takano, M., Sato, N., Nakano, S., Yokoyama, H. (2023). Environ. Technol., 44(21), 3229-3235. doi: 10.1080/09593330.2022.2056083
[4] Chen, S., He, G., Liu, Q., Harnisch, F., Zhou, Y., Chen, Y., Hanif, M., Wang, S., Peng, X., Hou, H., Schröder, U. (2012). Energy Environ. Sci. 5(12), 9769-9772. doi: 10.1039/C2EE23344D

Poster-E50: Polymer-Coated Bioelectrode for Enhanced Bacterial Proliferation in Bioelectrochemical Systems

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Carbon dioxide (CO_2) reduction is a fundamental process for emissions mitigation. Microbial electromethanogenesis (EM) allows the conversion of CO_2 to methane (CH_4) by specific microorganisms, achieving high efficiencies under controlled conditions. Nevertheless, the implementation of these systems is complex [1] due to various factors, including inoculum selection, electrode design and acclimatisation procedures. Consequently, optimisation and large-scale application are difficult. Carbon felt is one of the most widely used materials in biocathodes due to its high conductivity, chemical stability, large surface area and compatibility with methanogens. However, their hydrophobicity hinders the adhesion of microbial biofilms, affecting colonisation and delaying system set-up. To address this limitation, this study proposed the use of polymer-based hydrogel coatings, whose hydrophilic structure favours water retention and cell adhesion [2]. In addition, the incorporation of a dissolved carbon source into the coatings was proposed with the aim of enhancing the proliferation of electroactive biofilms and, thus, improving the efficiency of the system in the conversion of CO_2 to CH_4 by microbial electromethanogenesis.

This approach was evaluated in two-chamber systems, separated by a cationic membrane, with a three-electrode configuration. The working electrode, which acted as a biocathode, had a surface area of 4 cm^2. Three types of electrodes were tested: Carbon felt (control), hydrogel-coated carbon felt and hydrogel-coated carbon felt with a carbon source incorporated. As a counter electrode, a platinum grid of similar dimensions was used. A potential of -1 V was applied in front of an Ag/AgCl (3M KCl) reference electrode. The experiments were carried out with a continuous CO_2 feed, constant stirring at 200 rpm and controlled temperature of 30 °C.

The results showed that by coating the electrodes with a polymer-based hydrogel, it was possible to achieve 30% higher current densities from the start of operation compared to the system without hydrogel. This improvement was attributed to the increased affinity of the hydrogel for microorganisms, promoting their adhesion to the electrode surface. In particular, the hydrogel favoured the establishment of a more specific microbial community, with over 90% dominance of Methanobacterium, a key genus in electromethanogenesis. In addition, the production of value-added compounds such as caproic acid (which persists as an intermediate in metabolic pathways) was observed on the coated electrode. Furthermore, the incorporation of a carbon source into the hydrogel matrix enabled sustained methane production, demonstrating its positive effect on the stability and efficiency of the system. Therefore, this study suggests that the integration of polymer-based hydrogel coatings with dissolved carbon sources is a promising strategy to optimise bacterial proliferation and performance in electromethanogenesis systems.

[1] Mateos R, Sotres A, Alonso RM, et al. Impact of the start-up process on the microbial communities in biocathodes for electrosynthesis. Bioelectrochemistry. 2018;121:27–37.
[2] Jiménez-Rosado P;, Romero M; Novel Trends in Hydrogel Development for Biomedical Applications: A Review. Polymers (Basel) [Internet]. 2022;2022:3023.

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Poster-E51: Surfaces need standards like measuring at the Leipzig Trade Fair: Defining the Relevant Surface Area of Graphite Granules for Fixed-Bed Electrode Reactors

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Graphite granules (GGs), a three-dimensional carbon-based material, are widely employed in fixed bed electrode reactors for microbial electrochemical technologies (MET)[1] as it is a cheap and biocompatible electrode material. Furthermore, their granular structure provides huge reaction zones for (bio)electrochemical processes leading to a high electrode surface-to-reactor volume ratio. While GGs have been increasingly studied in MET over the past decade for applications such as wastewater treatment and electrobioremediation, defining the *relevant* surface area for electroactive biofilms is not trivial[2]. The standard method for quantifying the total surface area of graphite-based electrodes is gas adsorption-desorption coupled with Brunauer-Emmett-Teller (BET) analysis, which can lead to overestimation of the areas as it also includes pores inaccessible to electroactive microorganisms. In contrast, calculating the surface area assuming ideal spheres likely underestimates the accessible area. This discrepancy leads to significant inaccuracies in normalizing electrochemical performance metrics such as current density, hindering reliable comparisons with literature data[3] and realistic performance assessments of fixed bed electrode reactors. It was the same for traders from different regions at the Leipzig Fair – a common standard for length and weight was needed to allow proper comparison and business.
Therefore, we compared different surface area determination methods for individual GGs. Four techniques were compared: (1) BET analysis (gas adsorption-desorption), (2) underpotential copper deposition, (3) cyclic voltammetry with ferro/ferri cyanide, and (4) profilometry combined with water displacement. The accuracies of these methods were tested by cultivating *Geobacter sulfurreducens* biofilms at single GG and graphite plates and calculating current density based on the four different methods for area determination. The determined mass-specific surface area considerably varied between 0.8 ± 0.2 m^2 g^{-1} (BET analysis) and 0.0015 ± 0.0004 m^2 g^{-1} (profilometry with water displacement).
Profilometry coupled with water displacement resulted in the most realistic estimation of the accessible surface area of GG based on calculations of current density and a comparison with graphite plates. Helium Ion Microscopy of *G. sulfurreducens* biofilms of different maturity states showed that the microorganisms colonized only the outer GG surface, avoiding pores of up to a size of ca. 10 µm confirming that profilometry yielded a realistic assessment of the microbially accessible area.
These findings underscore the necessity of defining GG surface area metrics based on microbial accessibility rather than total porosity. BET analysis, though standard for abiotic systems, is unsuitable for biofilm electrodes due to its inclusion of inaccessible regions.

References

(1) Rodrigo Quejigo, J.; Tejedor-Sanz, S.; Esteve-Núñez, A.; Harnisch, F. Bed Electrodes in Microbial Electrochemistry: Setup, Operation and Characterization. *ChemTexts* 2019, *5* (1), 4.
(2) Caizán-Juanarena, L.; Sleutels, T.; Borsje, C.; Ter Heijne, A. Considerations for Application of Granular Activated Carbon as Capacitive Bioanode in Bioelectrochemical Systems. *Renew. Energy* 2020, *157*, 782–792.
(3) Rotaru, A.-E.; Woodard, T. L.; Nevin, K. P.; Lovley, D. R. Link between Capacity for Current Production and Syntrophic Growth in Geobacter Species. *Front. Microbiol.* 2015, *6*.

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