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ISAM 2025

13th International Symposium on Anaerobic Microbiology

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ISAM 2025

MIBI TECH

e-Book of Abstracts

Photos by Marcell Nikolausz

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13th International Symposium on Anaerobic Microbiology
ISAM2025

Editors: Marcell Nikolausz & Sabine Kleinsteuber
Helmholtz Centre for Environmental Research – UFZ
Department of Microbial Biotechnology

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WELCOME ADDRESS

On behalf of the Local Organising Committee, we warmly welcome you to the 13th International Symposium on Anaerobic Microbiology (ISAM2025), organised by the Helmholtz Centre for Environmental Research – UFZ. ISAM2025 is one of the most important international events on the microbiology of anaerobes, where the latest findings, developments and innovations in the realm of anaerobic microorganisms are discussed. This year's topics include: Gut Microbiology, Syntrophic Bacteria, Biogas Microbiology, Anaerobic Fungi, Enzymes of Anaerobes, Microbes in Anaerobic Environments, Biotechnological Application of Anaerobes, Gas Fermentation, and Power-to-Gas approaches.

The ISAM conference series has a long tradition of bringing together researchers from all areas of anaerobic microbiology and offering young scientists in particular the opportunity to present their work. Initially, this conference series focused mainly on developments in Central European countries, particularly research into rumen and gastrointestinal microorganisms. Gradually, the main organisers decided to broaden the topics and involve more countries in the organisation, making ISAM one of the most important international conferences in the field of anaerobes. The following symposia were held to date:

- 1st ISAM, Ljubljana (Slovenia), 1998
- 2nd ISAM, Prague (Czech Republic), 2000
- 3rd ISAM, Košice (Slovakia), 2003
- 4th ISAM, Warsaw (Poland), 2005
- 5th ISAM, Domžale (Slovenia), 2007
- 6th ISAM, Liblice (Czech Republic), 2009
- 7th ISAM, Smolenice Castle (Slovakia), 2011
- 8th ISAM, Innsbruck (Austria), 2013
- 9th ISAM, Portorož (SIovenia), 2015
- 10th ISAM, Liblice (Czech Republic), 2017
- 11th ISAM, online (hosted by the University of Innsbruck), 2021
- 12th ISAM, Innsbruck (Austria), 2023

The following pages provide an overview of the conference programme, abstracts and list of authors. The programme includes four invited keynote lectures, 48 short oral presentations in 11 sessions and 55 poster presentations. In addition to the scientific programme, the welcome reception and the conference dinner at Restaurant Felix, with its fantastic view over the city centre, offer opportunities for further discussions in a relaxed atmosphere. The conference ends with optional post-conference activities, including a visit to the research biogas plant of the Deutsches Biomasseforschungszentrum (DBFZ) or the ProVIS Centre for Chemical Microscopy at the UFZ. Alternatively, conference attendees may participate in a bioinformatics workshop – Unlocking the Power of Omics in Anaerobic Microbiology.

In addition to the conference programme, Leipzig offers many touristic attractions. The city has a unique atmosphere, which can be attributed to its important role in the commercial and intellectual history of Europe and its current renaissance as one of the most dynamic cities in Europe.

We would like to thank our sponsors for their important contribution: the journal Frontiers in Microbiology and Umwelt- und Ingenieurtechnik GmbH Dresden. We wish them many new contacts and successful business ventures.

Finally, we would like to thank the editorial board of Frontiers in Microbiology for agreeing to publish selected contributions from ISAM2025 under the special Research Topic 'Advances in Anaerobic Microbiology: Insights from 13th International Symposium on Anaerobic Microbiology (ISAM2025)'.

We hope that the year-long hard work and dedication of the organising committee will result in a memorable and interesting event. We look forward to fruitful discussions and the making of not only new collaborations, but also new friendships. Finally, we would like to thank our colleagues from the Scientific Committee, the Local Organising Committee and the student volunteers for their continuous support throughout this event.

We wish all the participants a pleasant and memorable scientific stay.

Sabine Kleinsteuber & Marcell Nikolausz

ISAM2025 COMMITTEE

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PROGRAMME

MONDAY, 22 September		
12:30 – 18:00	Registration (KUBUS Foyer)	
14:30 – 14:45	Arrival (KUBUS Foyer)	
14:45 – 15:15	**WELCOME AND OPENING SESSION** (KUBUS Room 1AB) Chairs: Marcell Nikolausz / Sabine Kleinsteuber	
15:00 – 15:15	**Heribert Insam** Biotreat GmbH, Innsbruck	MikroMondo, a science center featuring microorganisms
15:15 – 16:00	**KEYNOTE 1: Andreas Brune** MPI for Terrestrial Microbiology, Marburg	Lateral gene transfer as evolutionary driver of reductive acetogenesis in termite guts
16:00 – 16:30	Coffee Break (KUBUS Foyer)	
16:30 – 18:00	**SESSION 1: Gut Microbiology (Lectures L1 – L6)** (KUBUS Room 1AB) Chairs: Andreas Brune / Nico Jehmlich	
16:30 – 16:45	**L1: Lilian Prinsen** KU Leuven	Anaerobes in the gut microbiome of wood-eating beetle larvae for unlocking the potential valorisation of residual lignocellulosic biomass
16:45 – 17:00	**L2: Stéphanie Perret** Aix-Marseille University – CNRS	Physiological and proteomic studies show that *Ruminiclostridium cellulolyticum* is specialized in the utilization of xylan
17:00 – 17:15	**L3: Samantha Joan Noel** Aarhus University	Isolation of hydrogenotrophic bacteria and their impact on hydrogen levels in rumen fluid under inhibited methanogenesis conditions
17:15 – 17:30	**L4: Maximilienne Allaart** University of Tübingen	Lactate stereoisomers are metabolized differently in the gut microbiome
17:30 – 17:45	**L5: Tomaž Accetto** University of Ljubljana	*Prevotella* sweet tooth may just not be the only answer
17:45 – 18:00	**L6: Blaz Stres** National Institute of Chemistry, Ljubljana	Integrating gut microbiome information layers: from constructed variables to single mutations and high-throughput microbiome activity kinetics
18:00	Welcome Reception (KUBUS Foyer)	

TUESDAY, 23 September

8:30 – 10:30	Registration (KUBUS Foyer)	
9:00 – 9:45	**KEYNOTE 2: Maria Westerholm** Swedish University of Agricultural Sciences, Uppsala	Syntrophic bacteria and syntrophic interactions in anaerobic digestion systems
9:45 – 10:30	**SESSION 2: Syntrophic Bacteria (Lectures L7 – L8)** Chairs: Maria Westerholm / Sabine Kleinsteuber	
9:45 – 10:00	**L7: Yanlin Cai** University of Konstanz	Amino acid metabolism of the thermophilic acetogen *Thermacetogenium phaeum*
10:00 – 10:15	**L8: Minjae Kim** Pukyong National University, Busan	Effect of magnetite nanoparticles on recovery of continuous anaerobic digestion from propionic and acetic acids accumulations at varying pH conditions
10:15 – 10:30	**POSTER PITCH 1**	
10:30 – 11:15	Coffee Break & Poster Session (KUBUS Foyer)	
11:15 – 12:45	**SESSION 3: Biogas Microbiology I (Lectures L9 – L13)** Chairs: Andreas Wagner / Marcell Nikolausz	
11:15 – 11:30	**L9: Seonmin Kang** Pukyong National University, Busan	Improved process stability and methanogen enrichment in magnetite-supplemented anaerobic sequencing batch reactor treating catechol wastewater
11:30 – 11:45	**L10: Christian Margreiter** University of Innsbruck	Evaluating the impact of bio-based gasification chars and activated carbon on anaerobic digestion in wastewater treatment
11:45 – 12:00	**L11: Elliot Guerra-Blackmer** UFZ Leipzig	Impact of increasing ratio of sugar beet silage feed in laboratory scale biogas reactors' microbial community structure
12:00 – 12:15	**L12: Frederik Bade** UFZ Leipzig	Hydrolytic enzyme activities during increasing organic loading rate in laboratory scale biogas reactors
12:15 – 12:30	**L13: Maria Chiara Valerin** University of Padua	Microbiological and physicochemical surveillance of full-scale biogas plants
12:30 – 12:45	**POSTER PITCH 2**	
12:45 – 14:45	Lunch Break & Poster Session (KUBUS Foyer)	
14:45 – 16:15	**SESSION 4: Anaerobic Fungi (Lectures L14 – L19)** Chairs: Sabine Podmirseg / Nico Jehmlich	
14:45 – 15:00	**L14: Sabine Podmirseg** University of Innsbruck	Anaerobic gut fungi: the story of a unique research relationship
15:00 – 15:15	**L15: Etelka Kovács** University of Szeged	The happy marriage of methanogenic archaea and anaerobic fungi
15:15 – 15:30	**L16: Anke Neumann** Karlsruhe Institute of Technology	Physiological and metabolic analysis of six anaerobic gut fungi

15:30 – 15:45	**L17: Sophia Strobl**, University of Innsbruck	Gut feeling: Isolation of anaerobic gut fungi from unconventional hosts
15:45 – 16:00	**L18: Jolanda van Munster** Scotland's Rural College, Edinburgh	Anaerobic fungi *Neocallimastix, Caecomyces* and *Piromyces* each have a distinct role in the degradation of plant biomass
16:00 – 16:15	**L19: Louisa Edge** Scotland's Rural College, Edinburgh	*Neocallimastix frontalis* effectively degrades starch via multiple amylases; an initial look into the diversity of starch metabolic capability between genera of anaerobic gut fungi
16:15 – 16:45	Coffee Break (KUBUS Foyer)	
16:45 – 18:00	**SESSION 5: Biogas Microbiology II (Lectures L20 – L24)** Chairs: Florin Musat / Carsten Vogt	
16:45 – 17:00	**L20: Beatriz Diniz** TU Delft	Unravelling the influence of pH and alkalinity in haloalkaliphilic methanogenic community dynamics
17:00 – 17:15	**L21: Olivier Chapleur** INRAE Antony	Longitudinal multiomics analysis of anaerobic microbial community to a pulse salinity disturbance
17:15 – 17:30	**L22: Roland Wirth** HUN-REN Biological Research Centre, Szeged	Comparative meta-omics analysis of antibiotic resistance in anaerobic systems treating agricultural biomass or municipal wastewater
17:30 – 17:45	**L23: Herald Wilson Ambrose** Aarhus University	Impact of chemical and biological additives on microbial community and methane emissions in pig slurry storage
17:45 – 18:00	**L24: Marcell Nikolausz** UFZ Leipzig	Microbial community dynamics triggered by in situ biomethanation

WEDNESDAY, 24 September

9:00 – 9:45	**KEYNOTE 3: Alexander Loy** University of Vienna	Hunting for hidden sulfur-cycling microbes and metabolisms
9:45 – 10:30	**SESSION 6: Anaerobic Sulfur Cycle (Lectures L25 – L26)** Chairs: Alexander Loy / Carsten Vogt	
9:45 – 10:00	**L25: Faith I. Oni** UFZ Leipzig	Functional generalists and key degraders: Genome-resolved niche analysis in a long-term sulfate-reducing benzene-degrading consortium
10:00 – 10:15	**L26: Vít Procházka** Masaryk University, Brno	Evaluating the biotechnological potential of anoxygenic phototrophic bacteria for desulfurization
10:15 – 10:30	**POSTER PITCH 3**	
10:30 – 11:15	Coffee Break & Poster Session (KUBUS Foyer)	
11:15 – 12:45	**SESSION 7: Enzymes of Anaerobes (Lectures L27 – L31)** Chairs: Darja Deobald / Ivonne Nijenhuis	
11:15 – 11:30	**L27: Johann Heider** Marburg University	Tungsten-dependent aldehyde oxidoreductases: reaction mechanism and applications
11:30 – 11:45	**L28: Marie Eberwein** UFZ Leipzig	Cobalt complexes enable anodic cultivation of *Dehalococcoides mccartyi* strain CBDB1 in a bioelectrochemical cell
11:45 – 12:00	**L29: Jesica M. Soder-Walz** UFZ Leipzig	Anaerobic fermentation of dichloromethane in *Dehalobacterium formicoaceticum* strain EZ94 is driven by methyltransferases
12:00 – 12:15	**L30: Fidel Ramirez** Marburg University	The ATP-dependent methyl-coenzyme M reductase activation complex from *Methanococcus maripaludis*
12:15 – 12:30	**L31: Andja Mulllaymeri** University of Innsbruck	Cofactor F420-polyglutamate-chain-length profiles in methanogens
12:30 – 14:30	Lunch Break & Poster Session (KUBUS Foyer)	
14:30 – 16:00	**SESSION 8: Microbes in Anoxic Environments (Lectures L32 – L37)** Chairs: Heribert Insam / Ivonne Nijenhuis	
14:30 – 14:45	**L32: Giulia Fiorito** ETH Zurich	Microbial communities influencing methane cycling in temperate Swedish peatlands
14:45 – 15:00	**L33: Marion Holmière** Aix-Marseille University	How to handle the lignin in absence of oxygen: a bacterial lesson
15:00 – 15:15	**L34: Kristine Røsdal** NMBU, Ås	Deciphering microbial strategies for lignocellulose degradation under denitrifying conditions
15:15 – 15:30	**L35: Shuting Li** UFZ Leipzig	Anaerobic metabolization of ^{13}C-labeled *Pseudomonas alloputida* cells by thermophilic microorganisms from aquifer sediments
15:30 – 15:45	**L36: Antonio Grimalt-Alemany** DTU Lyngby	Quorum-sensing-mediated biofilm formation in anaerobic microbial communities

15:45 – 16:00	**L37: Chang Ding** UFZ Leipzig	Reactor-based enrichment of an autotrophic *Paracoccus*-dominated culture using hydrogen and nitric oxide
16:00 – 16:30	Coffee Break (KUBUS Foyer)	
16:30 – 17:30	**SESSION 9: Biotechnological Applications of Anaerobes (Lectures L38 – L41)** Chairs: Stefano Campanaro / Heike Sträuber	
16:30 – 16:45	**L38: Mathilde Bourgeois** INRAE Narbonne	Eco-engineering strategies to target propionic acid production from food waste fermentation
16:45 – 17:00	**L39: Selina Haller** University of Innsbruck	BIOcubed: Biosynthesis of green hydrogen using a newly discovered microorganism
17:00 – 17:15	**L40: Maria Gaspari** Hellenic Agricultural Organisation Dimitra	Investigating resilience of biological methanation to prolonged hydrogen starvation
17:15 – 17:30	**L41: Omer Uzun** Boğaziçi University, Instanbul	Unlocking the potential of lignocellulosic waste: Alkali pre-treatment to sustainable chemicals in the circular bio-economy context
19:00	Conference Dinner at FELIX	

THURSDAY, 25 September

9:30 – 10:15	**KEYNOTE 4: Diana Sousa** Wageningen University & Research	Reimagining syngas fermentation: Innovative production routes through microbial discovery and synthetic design
10:15 – 11:00	**SESSION 10: Gas Fermentation (Lectures L42 – L44)** Chairs: Diana Sousa / Heike Sträuber	
10:15 – 10:30	**L42: Giacomo Antonicelli** Istituto Italiano di Tecnologia, Turin	Unlocking sustainable bio-hexanol production with *Clostridium carboxidivorans* through adaptive evolution and process optimization
10:30 – 10:45	**L43: Kira Sofie Baur** Ulm University	Quantification of an anaerobic synthetic co-culture containing *Acetobacterium woodii* and *Clostridium drakei*
10:45 – 11:00	**L44: Estelle M. Goonesekera** DTU Lyngby	A new point of view: studying syngas biomethanation biofilms in flow-chambers
11:00 – 11:30	Coffee Break (KUBUS Foyer)	
11:30 – 12:15	**SESSION 11: Power-to-Gas (Lectures L45 – L47)** Chairs: Marcell Nikolausz / Sabine Kleinsteuber	
11:30 – 11:45	**L45: Wenyo Gu** EPFL, Lausanne	From methanogen physiology to a robust Power-to-Gas process: addressing intermittent substrate supply and oxygen
11:45 – 12:00	**L46: Xavier Goux** LIST Hautcharage	Microbiology of the mesophilic ex-situ biological methanation: influence of operating parameters such as inoculum and scaling up
12:00 – 12:15	**L47: Stefano Campanaro** University of Padua	Phage-driven microbial adjustments under different gas retention times in pilot-scale CO_2 biomethanation
12:15 – 12:30	**CLOSING CEREMONY**	
12:30 – 13:00	Take Away Lunch (KUBUS Foyer)	
13:15	Post Conference Activities	

ORAL PRESENTATIONS

Opening session

MikroMondo, a science center featuring microorganisms

H. Insam[*a], C. Ebner[b], T. Pümpel[a], S. Strobl[c], J. Ascher-Jenull[d], S. Hirschl-Neuhauser[e], C. Griesbeck[e], M. Stüttler[f], M. Nagler[c], S. Podmirseg[c]

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MikroMondo, an upcoming science center in Austria, will immerse visitors in the fascinating world of microorganisms. The center aims to enhance public microorganism literacy through innovative exhibits, including Europe's tallest Winogradsky column, world-record attempts with fungi, displays of plant-microbe interactions, bioreactor simulations, a laboratory-scale wastewater treatment plant and food microbiology.

Designed to inspire curiosity and deepen understanding of microbiology, MikroMondo will also offer interactive activities for students, educators, and the public. Participants can explore microbiological processes such as nutrient cycling, plant-microbe symbiosis, and microbial diversity through guided microscopy, microbial cultivation, Winogradsky column creation, fermented food and gas production.

By making the hidden world accessible and captivating, MikroMondo seeks to foster appreciation also for the anaerobic world and inspire the next generation of science stewards and microbial enthusiasts, based on a storyline of everyday encounters of people with microorganisms.

Keynote 1

Lateral gene transfer as evolutionary driver of reductive acetogenesis in termite guts

Andreas Brune

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Termite guts are abundantly colonized by a diverse assemblage of spirochetes. It is generally assumed that 'termite gut treponemes' are responsible for reductive acetogenesis from $H_2 + CO_2$, an important step in the symbiotic digestion of lignocellulose. However, the origin of reductive acetogenesis in termite gut treponemes and its distribution across different lineages are unclear because most representatives are uncultured. We assessed the capacity for reductive acetogenesis in the spirochete family *Breznakiellaceae* [formerly 'termite (*Treponema*) cluster I'] by comparatively analyzing 292 metagenome-assembled genomes (MAGs) obtained from 43 termite species. The results showed that a complete Wood–Ljungdahl pathway is encoded only by three distinct subgroups of this family. The key enzymes of the pathway, namely hydrogen-dependent CO_2 reductase (HDCR) and CO dehydrogenase/acetyl-CoA synthase, are absent from all basal lineages. Phylogenetic analysis of these enzymes suggests that *Breznakiellaceae* acquired the capacity for reductive acetogenesis by lateral gene transfer from acetogenic *Clostridiales* several times during the evolutionary radiation of their host. The absence of a complete Wood–Ljungdahl pathway in all spirochetes from higher termites (family Termitidae) indicates that the high rates of reductive acetogenesis observed in this family must be attributed to members of other bacterial phyla. We found compelling evidence that additional transfers of HCDR genes conferred the capacity for reductive acetogenesis also to a termite-specific clade of so-far uncultured *Desulfobacterota*. We isolated *Acetispira formosa*, the first representative of the new order *Acetispirales*, from the gut of a cockroach and confirmed its homoacetogenic metabolism. Our findings underscore that lateral gene transfer among the intestinal microbiota is a major evolutionary driver of the termite gut symbiosis.

Keynote 2

Syntrophic bacteria and syntrophic interactions in anaerobic digestion systems

Maria Westerholm

Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Syntrophic bacteria perform essential biochemical steps in anaerobic digestion, enabling the complete breakdown of complex organic matter and playing a pivotal role in biogas production. Despite their significance, many fundamental questions about their metabolism and interactions remain unanswered, even as advanced technologies increasingly uncover their presence across a wide range of anaerobic systems.

In this talk, I will present recent advances in our understanding of the factors that shape and regulate syntrophic interactions. I will discuss what drives species dominance, how syntrophs interact or compete with other microbes, and how environmental stressors, such as elevated temperature, stirring, and ammonia, affect their stability and function.

Beyond their crucial role in biogas production, syntrophic interactions are of considerable interest from a fundamental microbiological perspective. Key knowledge gaps remain regarding how these partnerships are established and maintained, whether there are shared or distinct traits among syntrophic species, and what cooperative strategies they use to enhance resilience and metabolic efficiency. For instance, evidence suggests that cooperation may go beyond interspecies electron transfer, potentially involving cross-feeding of amino acids or other compounds between syntrophs and surrounding microbes. Understanding how syntrophs adjust their metabolic behavior in response to microbial community context is a promising avenue for further exploration.

There is also increasing reliance on bioinformatic tools to predict syntrophic function. While such tools offer valuable insights, their predictions ultimately require experimental validation. However, cultivation and isolation remain technically challenging due to the strong interdependence between syntrophic bacteria and their microbial partners. In this context, deeper insights from bioinformatics could help unravel key physiological traits and interaction patterns, potentially guiding more targeted and successful cultivation strategies.

As our insights into syntrophic communities deepen, the key challenge becomes translating this knowledge into practice. Although it is possible to steer syntrophic interactions under simplified systems, achieving similar outcomes in complex microbial communities remains difficult.
However, considering the typically low abundance and activity of syntrophs relative to the overall microbial community in anaerobic degradation systems, a bottom-up approach appears most promising. It may offer a path toward developing management strategies that harness or suppress syntrophic cooperation, depending on the objectives of the system or environment.
To fully realize this potential, fundamental and applied research must be aligned and mutually informative. We should ask ourselves how we can better bridge this gap and ensure that knowledge from both domains supports the development of robust and sustainable microbial processes.

Keynote 3

Hunting for hidden sulfur-cycling microbes and metabolisms

Alexander Loy

Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria

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Microorganisms have been key drivers of Earth's sulfur cycle since the origin of life. However, our understanding of their capacity to metabolize sulfur compounds – particularly organic sulfur compounds – remains incomplete. Moreover, the integration of sulfur metabolism with the cycling of other elements such as nitrogen, carbon, and iron at the level of individual organisms has not been comprehensively explored.

In this talk, I will present a large-scale genomic analysis of sulfur metabolism across the prokaryotic tree of life. Using improved tools for detecting over one hundred genes and proteins involved in the dissimilatory transformation of both inorganic and organic sulfur compounds, we recorded sulfur-metabolizing potential across nearly all bacterial and archaeal phyla.

I will highlight two examples supported by experimental validation: (i) the role of sulfoquinovose – an environmentally ubiquitous sulfonated sugar derived from the thylakoid membrane lipid sulfoquinovosyl diacylglycerol – as a selective nutrient shaping the composition of the animal and human gut microbiota; and (ii) the discovery of a novel microbial metabolism that couples sulfur and iron cycling in globally significant anoxic environments.

Together, these findings broaden our understanding of sulfur-based metabolisms of microorganisms and their roles in global biogeochemical cycles as well as in host-associated microbial communities.

Keynote 4

Reimagining syngas fermentation
Innovative production routes through microbial discovery and synthetic design

Diana Z. Sousa

Laboratory of Microbiology, Wageningen University & Research, The Netherlands

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Syngas fermentation is a promising approach for converting carbon-rich waste streams and CO-rich industrial gases into valuable chemicals and fuels. To expand the product spectrum beyond conventional outputs like ethanol, multiple complementary strategies are emerging. One option is the genetic engineering of model acetogens to broaden their metabolic capabilities. Alternatively, discovering novel carboxydotrophs or designing synthetic microbial co-cultures offers routes to access unexplored pathways and new functional synergies.

At our group we design synthetic microbial co-cultures that leverage the synergistic metabolism of acetogens and partner organisms. For example, *Clostridium autoethanogenum* co-cultivated with *Clostridium kluyveri* or *Anaerotignum neopropionicum* enables the production of even- and odd-chain medium-chain carboxylic acids and alcohols. We have also developed hybrid systems combining acetogens with non-sulfur purple phototrophic bacteria to convert CO into polyhydroxyalkanoates (PHAs). These synthetic consortia demonstrate the potential to broaden the product range of syngas fermentation.

In parallel, we explore natural environments to uncover the hidden diversity of CO-utilizing microbes. Metagenomic analyses reveal numerous organisms with the genetic potential for CO metabolism, far beyond what cultivation studies have captured. Guided by these insights, we isolate novel mesophilic and thermophilic strains – representing new genera and species – with diverse CO-utilization pathways. These newly isolated microbes may offer exciting opportunities to develop next-generation synthetic engineering approaches, including their integration into novel co-culture systems to further diversify and optimize syngas fermentation.

SESSION 1: Gut Microbiology

L1

Anaerobes in the gut microbiome of wood-eating beetle larvae for unlocking the potential valorisation of residual lignocellulosic biomass

Prinsen L.[a*], González Álvarez Á.[a], Horemans B.[a,b]

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In the transition towards a biobased circular economy, there has been a growing interest in the use of biological conversion routes to produce materials and chemicals from residual lignocellulosic biomass. Driven by specific host-microbiome interactions in specialized micro-aerobic and anaerobic gut compartments (mid- and hindgut, respectively), wood-eating beetle larvae can feed on nutrient-poor lignocellulosic biomass, converting it to compounds for energy and nutrition.

By utilizing this 'natural biorefinement' as a blueprint, we aim to harness the diverse functionalities of both anaerobes and aerobes found in this gut system to produce value-added biochemicals from residual lignocellulosic biomass. As a first step in the translation from an *in vivo* to an *in vitro* system, we taxonomically and functionally characterized the microbial gut community of our model species *Pachnoda sinuata flaviventris* (PSF), thereby exploring its lignin degradation potential and the combined role of aerobes and anaerobes in the formation of fermentation products from lignocellulosic biomass. We also relied on traditional culturing techniques to isolate, identify and characterize bacteria with lignin conversion potential.

Using amplicon and shotgun metagenome sequencing, we observed distinct differences in the bacterial community present in mid- and hindgut. The anaerobic hindgut harbored a highly diverse community, especially within the class Clostridia. The orders Oscillospirales and Lachnospirales, which have been previously reported as typical of carboxylate chain elongation, were particularly present within this class. Also, the genus *Proteiniphilum* (phylum Bacterioidota) was highly abundant in the hindgut and is hypothesized to play an important role in proteolytic fermentation. Moreover, inference of genes involved in lignin degradation indicates that lignin degradation might not be limited to the micro-aerobic midgut. Preliminary anaerobic plate cultures led to the isolation of several strains belonging to the *Citrobacter* and *Enterococcus* genera. The aerobic isolates were mainly representative of the *Bacillus*, *Pseudomonas*, *Sphingobacterium*, *Microbacterium* and *Stenotrophomonas* genera, with *Delftia acidovorans* as one of the most abundant species. In particular, some strains from the facultative anaerobic genera *Pseudomonas* and *Bacillus* showed promising results for the utilization of different lignin derivatives in subsequent *in vitro* screening assays.

These insights and initial isolation efforts contribute to our current work, which focuses on the culturing of these PSF mid- and hindgut communities by using a Biolector XT microbioreactor platform. This allows for high-throughput testing and optimisation while maintaining consistent micro-aerobic and anaerobic conditions. This should improve our understanding of PSF gut microbiome functioning and facilitate the *in vitro* engineering of those microbiomes.

L2

Physiological and proteomic studies show that *Ruminiclostridium cellulolyticum* is specialized in the utilization of xylan

Liu N[a]., Fierobe H.P.[a], Perret S.[a*]

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The anaerobic Gram-positive bacterium *Ruminiclostridium cellulolyticum* was isolated from decayed grass biotope. It grows on straw and diverse plant cell wall polysaccharides like cellulose or xyloglucan. The growth relies on oligosaccharides ABC-transporters which possess a typical Family- 1 solute-binding protein responsible for the specificity of the ABC oligosaccharide transporter. In *R. cellulolyticum*, oligosaccharides comprising up to nine monosaccharides can be imported by these systems before to be degraded by a set of cytosolic enzymes. The genes involved in regulatory, import and degradation functions of these systems are grouped together in so-called Gram-positive polysaccharide utilization loci (GpPULs).

In the present study, we investigated the diversity of GpPUL through an integrative approach combining genomic, physiological, and proteomic analyses. We were able to link the function of the newly identified loci to their corresponding polysaccharide substrates. Affinity and specificity of the solute-binding protein toward specific oligosaccharides confirmed those results. From this study, *R. cellulolyticum* is revealed as a specialist in the degradation of a diversity of xylan polysaccharides comprising glucuronoxylan, arabinoxylan, xylan. Highly decorated arabinoxylodextrins can also be imported and processed in the cell by a set of glycoside hydrolases and carbohydrate esterases.

This study provides a basis for the exploration of novel GpPUL functions in Gram-positive anaerobic bacteria. A better understanding of GpPULs function is of significant interest from a predictive and physiological standpoint. Indeed, these “selfish” systems provide a competitive advantage by scavenging oligosaccharides before their complete extracellular degradation and avoiding competition for monosaccharide uptake with other species in the biotope. Furthermore, importing large oligosaccharide rather than monosaccharide is energy saving, a property that is particularly important for anaerobic bacteria.

L3

Isolation of Hydrogenotrophic Bacteria and their impact on Hydrogen Levels in Rumen Fluid Under Inhibited Methanogenesis Conditions

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Normal fermentation by the rumen microbiota produces hydrogen (H_2) as a byproduct. Hydrogenotrophic archaea (methanogens) metabolize H_2 and carbon dioxide (CO_2) to form the greenhouse gas, methane (CH_4). The rumen also contains hydrogenotrophic bacteria that can metabolize H_2; including acetogens via the acetogenesis pathway, sulfate reducing bacteria (SRB) via sulfate reduction and some bacteria reduce H_2 via fumarate or nitrate reduction. Although the pathways harbored by these bacteria are not the dominant means of H_2 removal under normal conditions, they could come into play when methanogens are inhibited. Further, redirecting H_2 away from methanogenesis into e.g., acetogenesis, could have twofold benefit of reducing CH_4 production as well as more efficiently utilizing the ingested feed for ruminant energy. Acetogens are of diverse phylogenetic affiliation and only a few have been characterized from animal gut environments. We aimed to 1) isolate and characterize novel hydrogenotrophic bacteria and 2) to assess if adding live hydrogenotrophic bacteria to *in vitro* rumen fermentations where methanogenesis is inhibited can counteract H_2 accumulation and shift fermentation metabolite profiles. We used selective media with a combination of enrichments and plating to isolate a culture collection of 173 potential hydrogenotrophic bacteria. They were isolated predominantly from bovine rumen fluid, but also from pig gut content and from rumen enrichment cultures of methanogens. Volatile fatty acids (VFA) were measured when isolates were grown using H_2/CO_2 as the sole energy source and 46% produced acetate. Selected isolates were screened for their capacity to utilize H_2 by measuring the remaining dissolved H_2 after 5 days growth. Thirty-two bacteria (29 new isolates) were able to significantly reduce the dissolved H_2 ranging from 7.7 to 84.6%. The isolates with the largest H_2 reducing capacity were assessed in an *in vitro* rumen simulation system (ANKOM^{RF}) with a potent methane inhibitor (iodoform) included and corn silage as substrate. Iodoform inclusion reduced CH_4 production by 62% and increased H_2 accumulation 200-fold, however challenges remain, as even the most promising candidates were not able to significantly reduce CH_4 or H_2 or change VFA production in the *in vitro* rumen fermentation with methane inhibition.

L4

Lactate stereoisomers are metabolized differently in the gut microbiome

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The gut is home to trillions of anaerobic microbes involved in digestion. An important intermediate in this microbial breakdown process is lactate, which is produced in two enantiomeric forms. L-lactate is native to the human body, whereas D-lactate is neurotoxic. In healthy individuals, lactate does not accumulate, but the fate of the individual stereoisomers in the gut remains underexplored. This work aims to ecologically and physiologically profile the conversion of D- and L-lactate in the gut microbiome.
Two identical anaerobic chemostat bioreactors (V= 1L) were used to enrich lactate-consuming microbial communities at pH 7.0, T = 37°C, and a retention time of 4 days. The bioreactors were inoculated with a human-derived gut microbiome and fed with DL-lactate. After steady-state was reached, we inoculated duplicate chemostats fed with exclusively D- or L-lactate with the DL-community. After maintaining steady-state for >3 SRTs, D-lactate-fed reactors were switched to L-lactate and vice-versa. The biochemical conversion profiles and microbial community composition of D-, L-, and DL-lactate were monitored using off-gas mass spectrometry, HPLC measurements, biomass quantification, and 16S rRNA gene amplicon sequencing.
D-, L- and DL-lactate were predominantly converted into acetate, propionate and CO2. Butyrate, valerate, and hexanoate were measured in small amounts. The microbial community of the DL-enrichments predominantly contained the *Anaerostignum* and *Acidipropionibacterium* genera. In the D-enrichments, *Anaerostignum* became the dominant genus, whereas *Acidipropionibacterium* initially dominated the L-enrichments. However, *Propionibacterium* outcompeted *Acidipropionibacterium* in time. Remarkably, the biomass yield on D-lactate was less than half of the biomass yield on L-lactate, even though the product spectrum was the same. Switching from L-lactate to D-lactate feeding caused no change in the observed metabolic activity but coincided with a steady drop in biomass yield and a community shift to *Anaerostignum*. Switching from D-lactate to L-lactate, however, caused an immediate drop in lactate consumption, leading to the accumulation of L-lactate in the bioreactor. After three days, L-lactate consumption re-started, concurrent with an increased biomass yield and a community shift to predominantly *Acidipropionibacterium*.
Our results show that different microbes consume the stereoisomers of lactate. The significant difference in biomass yield between D- and L-lactate, combined with the highly similar product spectrum suggests that the isomers are metabolized through different biochemical routes with the same metabolic output, but a significantly different biochemistry. Overall, our results shed light on the ecological strategies of the underexplored functional group of gut lactate consumers and increase our fundamental understanding of the gut microbiome.

L5

Prevotella sweet tooth may just not be the only answer

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Bacteria, formerly known as *Prevotella* (recently classified in *Xylanibacter*, *Segatella*, *Hoylesella*, *Hallella*, *Leyella* and *Prevotella* proper), and *Bacteroides* are among the most abundant bacterial groups in mammalian gut. Although colonizing most parts of the gut, they are known best as major contributors to plant polysaccharide breakdown in the hind gut of primates and the rumen (genera *Xylanibacter* and *Segatella* of the former *Prevotella*). There, they may occupy similar niches since the seemingly inverse relationships in abundance of these genera both in humans and in rumen has been reported. In the former case, a major driver in favour of *Bacteroides* may be higher bile acid release in persons consuming "westernized" diet and consequent inhibition of *Prevotella*. In the rumen, *Bacteroides* colonise early on in life to high relative shares, but are replaced with *Prevotella* upon introduction of fibre-based food. The reason for this is currently unknown.

In the absence of any reported antagonism between these bacteria, we: (i), examined fundamental microbiological nutritional requirements of 23 former *Prevotella* and 16 *Bacteroides* species; (ii), performed a 16S NGS monitored coculture experiments involving eleven species and three media variants suggested by (i); and (iii), did a mRNAseq experiment of cocultures involving two former *Prevotella* ruminal species, for which polysaccharide utilization loci (PULs) were previously characterized. We used medium with the complex mixture of polysaccharides and its variant suggested by results of (i) and (ii).

The nutritional requirements survey showed that *Bacteroides* species formed a coherent separate group, while in *Prevotella*, strains were quite variable depending on their origin. In cocultures, the *Prevotella* prevailed despite similar growth rates in pure cultures of *Prevotella* and *Bacteroides*. mRNAseq experiments showed coexistence of strains and simultaneous yet differential induction of several PULs in both strains.

The competition for resources among *Prevotella* was not defined solely by available carbohydrates. Other features, not just PUL complements determine the strain success. The *Prevotella* may utilise nutrients more efficiently than *Bacteroides*.

L6

Integrating gut microbiome information layers: from constructed variables to single mutations and high-throughput microbiome activity kinetics

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Complex systems operate over large scale differences in time and space and are made of units that interact in nonlinear way to produce emergent properties not possessed by any of the units before. Microbiology has shifted towards analyses of large data sets containing thousands of samples utilizing high performance computing (HPC) coming close to resemble astronomy.

The term "Microbiome" contains various layers of information for which there is inherently vague understanding of their information content. Conversely, being inherently linked to the vast amount of chemical and physical signatures that change over time and space in response to variations in thermodynamic conditions of the environment, microbiome information is coupled to health and disease. In order to tackle this, machine learning is being heavily coupled to deconvolution of large microbiome data matrices (taxonomy, diversity, functional genes, enzymatic reactions, metabolic pathways, metabolomics, environmental chemistry (organic, inorganic), thermodynamic conditions, patient metadata, and other), amenable to extensive biomarker search and mechanistic insight that have all revolutionized our established views of health and disease.

To address bottlenecks and improve reproducibility, high-performance computing (HPC)-compatible pipelines were developed for microbial dataset analysis: (i) GUMPP (General Unified Microbiome Profiling Pipeline) processes amplicon sequencing data using taxonomic analysis, various alpha- diversity indices and predicts microbiome functional genes, enzymatic reactions, and metabolic pathways. (ii) MetaBakery employs BioBakery tools for large-scale metagenomic sequencing data analysis, generating species-level taxonomic profiles, diversity estimates, microbiome functional genes, enzymatic reactions, metabolic pathways, and human gut-associated metabolites. (iii) MAGO (Metagenome Assembled Genomes Orchestra) expands metagenomic analysis through assembly, binning, and evolutionary interpretation of metagenome-assembled genomes. (iv) GMRC (Gut Microbiome Response Chip) assesses the influence of initial conditions and their interactions on microbiome physiological responses, capturing the response chemical space (including metabolomic signatures and chemical bonds) in real time.

The resulting data matrices are further deployed to batch-correction pipeline BERNARD before entering the machine learning for training or for classification of unknown samples into 18 noncommunicable disease classes. International Classification of Diseases codes enable linking the microbiome information to Unified Medical Language System (disease-disease similarity network) and DrugBank Knowledgebase (multi-state drug-drug contra/indications) to build knowledge graph giving rise to the condensed clinical report. In parallel, the identified biomarkers are used to ask relevant mechanistic questions. In conclusion, these results show that human gut microbiome layers contain wealth of valuable information that can be distilled into diagnostic and therapeutic action plans for a number of noncommunicable diseases.

SESSION 2: Syntrophic Bacteria

L7

Amino acid metabolism of the thermophilic acetogen *Thermacetogenium phaeum*

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The thermophilic, acetogenic bacterium *Thermacetogenium phaeum* (*T. phaeum*) is known for its capacity to degrade C1 and C2 compounds via the Wood-Ljungdahl pathway (WLP). Methylene- tetrahydrofolate (methylene-THF) plays a central role as an intermediate in the WLP during acetogenesis. As previously observed in the mesophilic *Eubacterium acidaminophilum*, methylene-THF is also a product of the glycine cleavage system (GCS) and therefore links amino acid degradation to acetogenesis.

The genome of *T. phaeum* contains all the genes encoding the GCS and serine converting enzymes, which were previously postulated to be involved in syntrophic acetate oxidation in the mesophilic *Syntrophaceticus schinkii*. However, when grown syntrophically with acetate, production of the corresponding enzymes could not be demonstrated in the thermophilic *T. phaeum*. Therefore, the purpose of the genes for amino acid converting enzymes in *T. phaeum* was investigated in this study.

T. phaeum was cultivated either in axenic culture or in syntrophic culture with *Methanothermobacter thermautotrophicus* supplied with amino acids or non-amino acid compounds as substrates. Growth yields and substrate degradation in syntrophic and axenic cultures were analyzed. Additionally, proteome analysis was performed to compare the abundances of relevant enzymes under different incubation conditions. Moreover, *in vitro* enzyme assays were conducted to investigate the activity of serine and glycine converting enzymes.

Remarkably, growth rates of syntrophic cultures with amino acids are among the highest observed for *T. phaeum*. Proteome analysis and enzyme assays showed that serine converting enzymes and GCS enzymes were highly produced and activated mainly during growth with amino acids. In contrast, only minor amounts of these proteins were produced, and almost no activity could be observed during growth with non-amino acid substrates. Serine dehydratase was active during growth with serine, as judged by in vitro enzyme assays. Surprisingly, the abundance of this enzyme was always below the average in the proteome.

Although *T. phaeum* oxidizes acetate by reversing the WLP when cooperating with a methanogenic partner, the acetate produced from amino acid degradation cannot be further utilized, indicating that *T. phaeum* cannot switch to the thermodynamically unfavorable acetate oxidation pathway immediately after amino acids are consumed. A modified thermophilic degradation pathway linking glycine and serine degradation to the WLP is proposed.

L8

Effect of magnetite nanoparticles on recovery of continuous anaerobic digestion from propionic and acetic acids accumulations at varying pH conditions

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Anaerobic digestion (AD) is a widely used biological process for converting organic waste into methane. Its performance depends on close syntrophic cooperation between fermentative bacteria and methanogens. However, this balance can be disrupted under organic overloading, leading to volatile fatty acid (VFA) accumulation–particularly propionic and acetic acids–causing pH reduction and process instability. These inhibitory effects intensify under low pH due to increased undissociated acid forms, which suppress microbial activity and methane production. Thus, strategies to enhance process resilience under acid stress are essential. Magnetite nanoparticles have shown potential to enhance direct interspecies electron transfer (DIET), promoting syntrophic interactions. However, their effectiveness in continuous AD systems under acidic conditions remains underexplored. This study evaluated the impact of magnetite supplementation on process stability and recovery during propionic and acetic acid shock loads in continuous AD systems. Four mesophilic reactors (PCp, PMp, PCa, PMa) were operated at 30-day hydraulic retention times. Magnetite (20 mM) was added to PMp and PMa. Propionic acid shocks (8–12 g COD/L) were applied to PCp and PMp; acetic acid shocks to PCa and PMa at pH 7.5 and 7.0. Methane production, VFA accumulation, and microbial community dynamics (via 16S rRNA sequencing) were monitored. Magnetite-amended reactors showed faster recovery and significantly higher methane production compared to the controls. Under pH 7.5, methane production rates in PMp and PMa was 55–155 % and 1–28 % higher, respectively. Under pH 7.0, where acid inhibition was more severe and recovery was delayed, PMp and PMa still achieved 15–120% and 1–47 % higher methane production rates, respectively. These performance improvements were consistent with faster removal of accumulated VFAs and were correlated with shifts in distinct microbial communities. These findings highlight the potential of magnetite particles to mitigate acid-induced inhibition, promote faster recovery from the VFA accumulations, and enhance robustness of the continuous AD processes. Further details will be presented at the conference.

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SESSION 3: Biogas Microbiology I

L9

Improved process stability and methanogen enrichment in magnetite-supplemented anaerobic sequencing batch reactor treating catechol wastewater

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Catechol, a phenolic compound commonly found in industrial effluents such as those from olive oil, wine, leather processing, and coal gasification industries, can reach concentrations up to 5300 mg/L. Its high organic load and toxicity pose significant challenges to biological treatment processes, particularly anaerobic digestion (AD), where methanogenesis is highly susceptible to inhibition. Magnetite, a conductive mineral, has been reported to promote direct interspecies electron transfer, potentially alleviating inhibitory effects and enhancing AD performance. This study investigated the effect of magnetite supplementation on the anaerobic treatment of catechol. Four lab-scale anaerobic sequencing batch reactors (AnSBRs) were operated using catechol (10 g COD/L) as the sole carbon source. Two reactors were supplemented with magnetite (CM), while the other two served as controls without magnetite (CO). Magnetite was added at 20 mM, and the organic loading rate (OLR) was gradually increased over 170 days. The CM reactors exhibited improved process stability, tolerating a 33% higher OLR (0.89 g COD/L/day) than the CO reactors (0.67 g COD/L/day), which failed due to instability. In addition, the CM reactors achieved a higher methane production rate (94 ± 1 mL CH_4/g VSS/day) compared to the CO reactors (80 ± 12 mL CH_4/g VSS/day). Anaerobic batch toxicity assays revealed that inocula from CM reactors (CMA) exhibited greater tolerance to catechol and shorter lag phases than those from CO reactors (CA). Microbial community analysis using 16S rRNA gene sequencing and quantitative PCR (qPCR) indicated distinct shifts in microbial composition and significantly higher methanogen abundance in the CM reactors. These findings suggest that magnetite addition enhances both reactor performance and microbial resilience under catechol stress, highlighting its potential as an effective strategy for treating phenolic wastewater in AD systems.

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This research was financially supported by the Korea Ministry of Environment as Waste to Energy-Recycling Human Resource Development Project (YL-WE-21-002). This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2024-00358944). This research was also supported by Global - Learning & Academic research institution for Master's·PhD students, and Postdocs (LAMP) Program of the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (No. RS-2023-00301702).

L10

Evaluating the Impact of Bio-Based Gasification Chars and Activated Carbon on Anaerobic Digestion in Wastewater Treatment

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Anaerobic digestion (AD) in wastewater treatment plants (WWTP) is a proven technology for bioenergy production. Continuous development of water treatment technologies in WWTPs is essential for water quality and environmental protection. EU directives require a fourth treatment stage to remove heavy metals and pharmaceutical residues from wastewater. Integrating bio-based gasification char (BGC) and activated carbon (AC) with high adsorption capacity is a novel approach to achieving this goal. After use as adsorbents for these pollutants, BGC and AC are transferred to anaerobic digesters via the sludge line, potentially increasing microbial activity and biogas yields. This study investigates the addition of BGC and AC in anaerobic digesters to improve process efficiency, purification efficiency, and waste management. Different chars were characterized and fed to AD at different concentrations to investigate their effect on methane production. BGC from a floating fixed bed gasification plant, carbon produced by chemical impregnation with $ZnCl_2$ from waste wood, carbon produced by thermochemical activation with CO_2 from BGC and commercial powdered AC were used for the experiments. Mesophilic AD batch tests with different concentrations of all chars were performed with digester sludge for 47 days. Volatile fatty acids (VFA) and biogas production and CH_4 concentrations were monitored. Concentrations below 1.0 $g_{char}\ L^{-1}$ did not result in significant effects on CH_4 and VFA production. However high concentrations of BGC and AC influenced both, the CH_4 yield and the growth kinetics. The impact of char characteristics on AD varied, showing both positive and negative trends in biogas yield and CH_4 production, depending on the production process of the absorbent. This study systematically evaluated char application in AD, predicting its applicability and effects. The findings identified suitable char types for WWTPs that even enhanced biogas production and efficiency.

L11

Impact of increasing ratio of sugar beet silage feed in laboratory scale biogas reactors' microbial community structure

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The green energy transition from fossil fuels to renewable energy sources places a significant reliance on technologies that are subject to seasonal and weather-related productivity fluctuations. Using Germany as an example, wind and solar energy produce the majority of renewable electricity, but also frequently experience low productivity times based on weather conditions. The ability of renewable energy infrastructure to adapt to unfavorable weather conditions and variable energy demand is critical.

Biogas from anaerobic digestion (AD) is a renewable energy source that is unaffected by seasonal and weather-related changes. Traditionally, AD plants are operated in steady-state conditions for based-load energy provision from biogas in combined heat and power units, which is not ideal in an energy grid with fluctuating electricity demand. Plant operators are therefore incentivized to provide demand-oriented electricity from biogas, which can be accomplished through expanding gas storage capacities or through flexible feeding to achieve short-term boosts in productivity. Although demand-oriented biogas production through flexible feeding has been demonstrated, the approach has not yet become established in practice, partly because the effects on microbial functions are poorly understood.

Our study investigated the impact of an increasing ratio of high-sugar feedstock, in this case sugar beet silage (SBS), on the microbial communities in two laboratory-scale biogas reactors operated in parallel. SBS is a favourable substrate for demand-oriented biogas production due to high degradation kinetics. Over six months, the reactors were fed an increasing proportion of SBS replacing corn silage at a constant organic loading rate, followed by a decrease in hydraulic retention time to induce process failure. Microbial community composition was monitored using amplicon sequencing of 16S rRNA and *mcrA* genes, revealing bacterial and methanogenic archaeal diversity. Gas production, volatile fatty acid (VFA) concentrations and hydrolytic enzyme activities were measured.

The feed change shifting to SBS resulted in a profound reorganization of the microbial communities, with the surviving microorganisms adapting to sugar beet digestion. Both reactor communities became enriched with sequence variants associated with *Dysgonomonadaceae*, *Mesotoga*, and *Proteiniphilum*. Ultimately, the AD process in both reactors broke down late in the experiment, cooccurring with reactor acidification due to an accumulation of organic acids. This acidification could have resulted from the washout of methanogens or syntrophic VFA degraders. Interestingly, the community composition in both reactors diverged towards the end of the experiment, although the operating and process parameters were the same. Ongoing metagenome analyses are attempting to explain the underlying functional mechanisms.

L12

Hydrolytic enzyme activities during increasing organic loading rate in laboratory scale biogas reactors

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With the increasing share of wind and solar energy in the power mix, flexibility options are needed to stabilize the electricity grid. Biogas can play a key role as a weather-independent, controllable, and storable energy source. To enhance the flexibility of biogas plants without requiring substantial investments in additional gas storage capacity, demand-oriented feeding offers a promising approach. However, this imposes significant demands on process stability and calls for a detailed understanding of anaerobic digestion (AD) under extreme operational conditions, particularly during increases in organic loading rate (OLR).

The hydrolysis stage, being the first and often rate-limiting step of AD, is typically not included in standard process monitoring and thus remains poorly understood in dynamic feeding scenarios. The aim of this study was therefore to investigate the behaviour of key hydrolytic enzymes under changing OLR during a long-term monitoring study to better understand their role.

Two laboratory scale biogas reactors were operated under identical conditions using the same feedstock, which was fed once per day, consisting of corn silage, coarse grain, and cow manure. However, coarse grain was only fed until a certain phase and not throughout the entire examined period. The OLR was gradually increased from 4.5 $g_{VS}/(L \cdot d)$ to 15 $g_{VS}/(L \cdot d)$ over several months until process failure occurred due to over-acidification. Hydrolytic enzyme activities - amylase, protease, and esterase - were monitored using colorimetric assays, alongside standard biogas process parameters such as pH, volatile fatty acid (VFA) concentrations, buffer capacity (VFA/TIC), gas production, and gas composition.

The activities of all three estimated hydrolytic enzymes closely followed the feeding regime. An increased proportion of corn silage in the substrate led to higher amylase activity due to the higher starch content fed, while a reduced share of protein-rich components of grain resulted in lower protease activity. Over-acidification occurred at an OLR of 12.5 $g_{VS}/(L \cdot d)$ in one reactor and 15 $g_{VS}/(L \cdot d)$ in the other. This offset revealed notable differences in enzyme activity patterns. In both cases, a sharp decrease in all estimated hydrolytic enzyme activities was observed during over-acidification.

The activities of the measured enzymes reflected substrate composition and process conditions, making them valuable indicators of functionality in AD. Their clear decline during acidification suggests their potential as early warning parameters for process instability. Long-term monitoring of hydrolytic enzyme activities deepened the understanding of the hydrolysis stage and could support the implementation of more flexible feeding strategies while improving operational reliability.

L13

Integrating microbial and physicochemical monitoring of two full-scale biomethane plants

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In the anaerobic digestion process, the efficiency of biogas production is influenced by the interaction among plant operating parameters, substrate composition, and the activity of the microbial community. Microbiome dynamics are typically monitored indirectly through parameters such as pH, volatile fatty acid and ammonia concentrations, and biogas composition. However, direct microbial monitoring as a management strategy remains challenging.

This project aims to characterize the microbiome dynamics in two biomethane plants located in Northern Italy, processing bovine and poultry waste, respectively, using metagenomic approaches and biochemical analyses. Integrating these data with operational parameters will provide insight into the mutual influence of these factors, with the goal of validating the potential of direct microbiological surveillance as a management tool.

Digestate samples are periodically collected from tanks dedicated to different process stages (digesters, post-digesters, and storage tanks) and subjected to shotgun sequencing for genome reconstruction of the microbial species present, followed by taxonomic assignment and abundance estimation across samples. Preliminary findings from the bovine waste-fed plant revealed a gradual shift in microbial composition across process stages. This observation may represent a starting point for defining a microbiological stability state and, consequently, detecting community imbalances.

In parallel, historical data on physicochemical parameters obtained from the same plants are being analysed to explore correlations and recurring patterns. Notable differences emerge between the two plants: the bovine waste-fed system exhibits greater stability and a certain degree of stratification across process stages, whereas the poultry waste-fed plant displays higher fluctuations in process parameters but a more homogeneous profile in all the tanks. This instability is likely linked to the elevated ammonia concentration typical of poultry waste.

Future efforts will involve collecting and integrating microbiological and physicochemical data over a period of one year. This comprehensive dataset will allow for a deeper investigation of the relationship between microbial dynamics and plant performances, contributing to a higher understanding of anaerobic digestion dynamics with the final goal of biomethane production systems optimization.

SESSION 4: Anaerobic Fungi

L14

Anaerobic gut fungi: the story of a unique research relationship

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This scientific recap tells the story of a research team focused on microbiological waste and wastewater management, with a particular emphasis on the anaerobic digestion process. A logical progression of this research was to compare engineered environments to natural systems, especially in the context of anaerobic digestion of lignocellulosic biomass (LCB), a substrate whose energetic potential is often underutilized. In nature, herbivores have developed a highly specialized microbiota for the efficient degradation of LCB. Among these microorganisms, anaerobic gut fungi (AGF) of the phylum *Neocallimastigomycota* stand out as key specialists, providing advanced mechanical and enzymatic disruption of plant biomass. Despite their importance, the environmental distribution, physiology and lifestyle of these fungi remain poorly understood.

Given their remarkable potential, there is growing interest in harnessing AGF for biogas production. However, challenges persist, particularly in the cultivation and preservation of AGF cultures, as only a limited number of research groups maintain active AGF culture collections, with many laboratories facing unexpected culture losses.

This résumé summarizes key findings from extensive, multi-year international collaborations focusing on the isolation, cultivation, and preservation of AGF and on the exploration of their environmental distribution. We also introduce new methodologies for detecting and categorizing AGF, incorporating both molecular and non-molecular approaches (e.g. near infrared spectroscopy, mass spectrometry and fluorescence *in situ* hybridization).

A critical analysis of the advantages and limitations of these methods is presented. Finally, we discuss the current state of AGF application in biomethanation, particularly regarding the direct use of AGF biomass or heterologously expressed enzymes as pretreatment strategies for LCB. We especially explore the potential for improving biogas production in the Alpine Rhine, Lake Constance and High Rhine region.

L15

The happy marriage of Methanogenic Archaea and Anaerobic Fungi

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Degradation of lignocellulose-rich biomass into biogas is an attractive alternative renewable strategy for growing energy demands and mitigate greenhouse gas emissions. Lignocellulose biomass is composed of interwoven polymeric carbohydrate cellulose and hemicellulose, glued together by lignin that is resilient to biological hydrolysis and microbial utilization. That is the main reason why bacteria and archaea in the biogas reactor are not sufficiently efficient in the disintegration of lignocellulose, leaving a considerable portion of the more easily convertible polymeric sugars untouched. Anaerobic fungi (AF) break down lignocellulose with high efficiency. AF belonging in the phylum *Neocallimastigomycota thrive* in the rumen and are of particular importance in the nutrition of herbivorous animals. AF anchor themselves to the plant material and crack the fibres mechanically by growth and expansion of their rhizoids or bulbous holdfasts as well as with their cellulosomes. To exploit their features, we need to understand what are the ideal conditions for them to function outside the herbivores and which microbes are their "preferred" partners.
We isolated AF and their natural, symbiotic, methanogenic partners from various herbivorous animals. Under controlled conditions we examined the lignocellulose degradation efficiencies using pure AF culture, AF enzymes, and enriched AF – methanogenic archaea (MA) co-cultures.
Pre-treatment of lignocellulosic substrate with AF-MA co-cultures for 14 days, followed by standard biochemical methane potential (BMP) tests indicated biogas production improvement and effective lignocellulose degradation at mesophilic temperatures. The AF-MA pre-treatment yielded 100-300% increase in specific biogas production using various lignocellulosic substrates. The results correlated well with the volatile fatty acid concentrations measured by HPLC and with the participating enzyme activities. Taken together, we propose to involve AF-MA syntrophic, naturally enriched co-cultures in biogas production communities treating lignocellulose-rich substrates. Studies to incorporate the AF-MA teams sustainably into the diverse biogas communities for permanent cohabitation are in progress.

L16

Physiological and metabolic analysis of six anaerobic gut fungi

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Anaerobic fungi, with a unique diversity of carbohydrate-active enzymes and the ability to produce hydrogen, have great potential in the utilisation of lignocellulosic materials. Despite the first classification as anaerobic fungi nearly 50 years ago and the re-categorisation into the phylum *Neocallimastigomycota* in 2007, growth conditions and metabolism remain largely unknown. While many genetic analyses have been performed, physiological data is scarce. The work presented here describes the metabolic response of the organisms *Aestipascuomyces dupliciliberans*, *Caecomyces churrovis*, *Khyollomyces ramosus*, *Orpinomyces joyonii*, *Pecoramyces ruminantium* and *Neocallimastix cameroonii* at different growth temperatures, straw particle sizes, different alternative C-Sources, different soluble carbon sources and their concentrations. In addition, a comparison of batch and fed-batch cultivation was carried out.

After inoculation all tested conditions were incubated for 7 days at 39°C if not changed for the specific experiment and afterwards analysed with a manometer, µGC and HPLC.

While growth is no longer possible at temperatures under 33°C and over 42°C, the highest amount of metabolites was detected at 39°C. The highest amount of hydrogen was detected in three out of six strains at 41 °C. The straw particle size experiment showed that larger particles could be utilised for further cultivation, while the highest yield of hydrogen was found for particles with a size of 2 mm to 3 mm. The analysis of soluble carbon sources showed a shift in metabolism with increasing concentrations and an indication of a bifurcating hydrogenase that was previously postulated in literature. Batch and fed-batch cultivations did not lead to increased metabolite formation, showing the limitations of bottle fermentations in production. The cultivation on alternative C-sources like cucumber peel, carrot peel and potato peels showed the potential in biowaste utilisation.

This study delivers on its premise on providing physiological growth data on anaerobic fungi while analysing metabolite production in different conditions. While process engineering will lead to maximum metabolite production, the observed behaviours with different straw particle sizes show huge potential in reducing costs for biomass preparations in big scale applications. The analysis of the effect of temperature improves the understanding of handling anaerobic fungi.

L17

Gut Feeling: Isolation of Anaerobic Gut Fungi from Unconventional Hosts

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Anaerobic gut fungi (AGF, phylum Neocallimastigomycota) are known for their unique aptitude to degrade recalcitrant plant matter within the digestive system of herbivorous animals. Their enzymatic systems have attracted attention for their potential in biotechnological applications, particularly in improving the anaerobic degradation of plant biomass in biogas reactors. Hence, the search for novel AGF strains that could expand this enzymatic repertoire is still ongoing. Recent global analyses of herbivorous mycobiomes uncovered over 50 novel AGF genera through expanded host sampling, revealing substantial unexplored diversity tied to host phylogeny and gut morphology.

This study explores host animal characteristics influencing AGF colonization and presents isolation efforts across three anatomically distinct herbivores: alpine chamois (*Rupicapra rupicapra*), degu (*Octodon degus*), as well as four alpine grouse/rock chicken species (tribes Tetraonini/Coturnicini). Standard anaerobic isolation protocols for AGF were performed from fecal samples.

We successfully isolated novel AGF strains from chamois (LGB2) and degu (SD2E), but isolation efforts from birds were unsuccessful. Phylogenetic assignment and monitoring of specific enzyme activities of the novel strains, including substrate utilization profiling, are in progress. Furthermore, ITS/LSU sequencing of feces will clarify fungal community structures, while sequencing of the V4 region of the SSU gene will map the prokaryotic co-microbiome.

We want to discuss the importance of expanding AGF diversity screenings through isolation from previously underrepresented or novel AGF hosts. We further discuss the value of investigating co-habitation of AGF and prokaryotes through amplicon sequencing to elucidate potential syntrophies and antagonisms that could guide isolation efforts. These endeavors establish the foundation for targeted AGF isolation from a wider array of host animals, potentially leading to the discovery of novel enzymes with industrial relevance for anaerobic lignocellulose degradation.

L18

Anaerobic fungi *Neocallimastix, Caecomyces and Piromyces* each have a distinct role in the degradation of plant biomass

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Lignocellulosic biomass is a promising feedstock for renewables-based biotechnology, but complete degradation of this material remains challenging. We investigate anaerobic gut fungi (AGF, phylum *Neocallimastigomycota*) to provide potential solutions. These fungi have distinct degradation mechanisms compared to the aerobic fungi usually employed as enzyme producers in biotechnology: they employ plant-biomass penetrating hyphae and leverage an arsenal of enzyme complexes that are unique among fungi. Despite lacking oxygen dependent lignin-degrading machinery or LPMO's, these AGF are highly effective in degrading raw lignocellulose. However, how distinct AGF species leverage their enzymatic arsenal to degrade plant material, and what the result of these activities is on plant material, is largely unknown.

Here we demonstrate how the degradative activity of three AGF isolates from genera *Neocallimastix, Caecomyces and Piromyces*, has distinct effects on plant biomass composition and architecture, via integration of distinct lignocellulose characterization techniques. Growth experiments indicated the fungi also had distinct capacities for metabolism of simple sugars that are derived from lignocellulose. These findings suggest that each species has unique roles and degradation capabilities. To further explore this, we are now using RNA-seq and untargeted proteomics to compare how our *Neocallimastix* and *Caecomyces* isolates leverage their degradative machinery during wheat straw fermentation.

L19

Neocallimastix frontalis effectively degrades starch via multiple amylases; an initial look into the diversity of starch metabolic capability between genera of anaerobic gut fungi

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Anaerobic Gut Fungi (Phylum *Neocallimastigomycota*) are primary degraders of plant cell wall materials within the specialised digestive system of ruminant herbivores, including agriculturally important animals such as cattle. This important role in ruminant digestion makes them a promising target for microbiome modulation.

High starch-containing grains are an important component in livestock diets due to their high calorific content. However, in animals fed these high-starch diets, some Anaerobic Gut Fungi increase in abundance while others are impaired. The reasons for this difference, and the roles Anaerobic Gut Fungi play in host starch metabolism, are unclear.

Here, we uncovered how distinct metabolic and degradative activities underpin differences in starch degradation abilities in fungi from three genera: *Neocallimastix*, *Caecomyces* and *Piromyces*. Fungal growth was measured alongside the rate of starch degradation and product formation during *in-vitro* culture.

The amylolytic machinery of *N. frontalis* was biochemically characterised. By analysing product formation after hygromycin inactivation mid-growth, we confirmed that starch is completely degraded to glucose extracellularly. Amylases were purified over two chromatography steps, their activities characterised using colourimetric assays, and their end-products identified via thin layer chromatography.

Three distinct enzymes were identified: one glucose-producing exo-amylase and two endo- amylases; one produces an oligosaccharide with 5 degrees of polymerisation (DP); the second produces oligosaccharides of DP8 and longer. We have also confirmed the presence of a biomass- bound maltose-cleaving α-glucosidase.

These results provide insight into the different metabolic and degradative abilities of AGF, which may enable better prediction of their activities and importance *in vivo*.

SESSION 5: Biogas Microbiology II

L20

Unravelling the influence of pH and alkalinity in haloalkaliphilic methanogenic community dynamics

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CO_2 accumulation in the atmosphere has been linked to climate change, highlighting the need for effective CO_2 capture and utilization. Hydrogenotrophic methanogens offer a potential solution by converting CO_2 and H_2 into methane. In anaerobic digestion, the acetoclastic methanogenesis pathway, which converts acetate into methane and CO_2, is typically reported as dominant. However, a shift to hydrogenotrophic methanogenesis is often observed under high-pH conditions (pH 8-9.5). This shift is both observed in nature (soda lakes) and in engineered systems (anaerobic digestion). At higher pH, CO_2 is speciated towards bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), which contribute to alkalinity, the system's buffering capacity. This suggests that this shift may not only be driven by pH but also by CO_2 speciation, and pH stability. This study aims to investigate the shift in community composition from acetoclastic to hydrogenotrophic at different pH values, and explore the role of alkalinity in influencing this shift.

An haloalkaliphilic anaerobic community was cultured in triplicate batch tests across a pH range (8.0, 8.5, 9.0, 9.2, 9.5, 10.0) at three alkalinities (0.1, 0.6, 1.2 eq/L). Each batch was fed 15 mM of acetate and operated until acetate depletion. The inoculum originated from an alkaline anaerobic digestion treating complex substrate at pH 8.7 and 0.6 eq/L alkalinity. This digester was initially inoculated with soda lake sediments. In this inoculum, the methanogenic community was dominated by *Ca. Methanocrinis* (acetoclastic) and *Methanocalculus* (hydrogenotrophic). To monitor the relative abundance of these genera throughout the batch tests, qPCR probes were developed. 16S rRNA gene amplicon profiling was used to identify microbial community composition.

Results showed that the relative abundance of *Methanocalculus* increased with the pH increase for all alkalinities. At low alkalinity (0.1 eq/L), the relative abundance doubled from 5% to 10% (pH 8.7-9.8); at moderate alkalinity (0.6 eq/L), it increased from 3% to 53% (pH 8.6-9.9); and at high alkalinity (1.2 eq/L), it increased from 12% to 61% (pH 8.4-9.6). In addition, the community profiling confirmed that *Methanocalculus* and *Methanocrinis* remained the main genera throughout all batch tests. These results suggest that pH increase promotes a shift from acetoclastic to hydrogenotrophic methanogens, with a higher alkalinity (and salinity) causing this shift to occur more significantly and at a lower pH.

In conclusion, promoting the dominance of hydrogenotrophic methanogens depends not only on increasing pH but also on considering CO_2 availability through alkalinity. Therefore, methanogenic community shifts cannot be predicted by pH alone.

L21

Longitudinal multiomics analysis of anaerobic microbial community to a pulse salinity disturbance

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The stability of anaerobic digesters is a critical industrial concern, rooted in the fragile equilibrium of complex microbial communities responsible for anaerobic degradation. Enhancing process resilience requires a deep understanding of microbial assembly and adaptation mechanisms, particularly under environmental stress. In this longitudinal study, we investigated the impact of a pulse salinity perturbation – an abrupt 30 g/L NaCl addition – on microbial dynamics in anaerobic digesters through a multi-omics approach.

Two independent sets of triplicate lab-scale digesters (5 L, continuously stirred) were subjected to the salt shock, followed by gradual dilution of the inhibitor. A third triplicate set served as an undisturbed control. Microbial and functional changes were monitored at nine timepoints using 16S rRNA gene sequencing (Illumina), long-read shotgun metagenomics (Oxford Nanopore) with MAG reconstruction, metabolomics via MALDI-FTICR high-resolution mass spectrometry on solid samples, and biogas isotopic analysis via IRMS (81 samples total).

The salt addition caused a transient decline in digester performance – characterized by reduced methane production and volatile fatty acid accumulation – followed by recovery into an alternative stable state, indicating a functional shift in the microbial ecosystem. This shift was corroborated by stable isotope analyses, which revealed changes in methanogenesis pathways. Initial exploration of each omics dataset using Common Component Analysis (CCA) characterized distinct time-resolved dynamics. Metataxonomic and metagenomic profiles showed similar temporal trajectories, whereas metabolic responses were more delayed. This suggests that community compositional shifts precede metabolic reorganization. Strong reproducibility across replicates underscored the robustness of these patterns. Multiblock integration of the omics layers revealed coordinated, staggered responses and identified microbial taxa and metabolites associated with resilience or susceptibility to salt stress.

By applying a multi-layered, integrative framework, this study provides ecological insights into how anaerobic microbial communities reorganize in response to acute disturbances. The emergence of an alternative stable state highlights the potential for microbial ecosystems to adapt through reassembly rather than returning to a prior equilibrium.

L22

Comparative meta-omics analysis of antibiotic resistance in anaerobic systems treating agricultural biomass or municipal wastewater

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Antimicrobial resistance has ancient roots, originating from natural microbial competition and the evolution of resistance genes long before the use of synthetic antibiotics. Today, human activities have amplified this issue, making processes like anaerobic digestion essential for managing organic waste streams that are often rich in resistance genes. This study explores the occurrence and activity of antibiotic resistance within microbial communities from two types of industrial-scale anaerobic digesters: those treating agricultural biomass and those processing municipal wastewater sludge. By integrating deep sequencing with a machine-learning guided genome-centric approach, we analyzed both metagenomic and metatranscriptomic data to understand the diversity, abundance, and expression of resistance genes at the population level.

Across both systems, a substantial portion of microbial genomes (57%) contained antibiotic resistance genes. These genes were primarily located on bacterial chromosomes, although plasmid-associated genes tended to show higher levels of expression. Resistance categories were diverse, including multidrug resistance, beta-lactam resistance, glycopeptides, peptides, tetracyclines, and macrolide-lincosamide-streptogramin types.

The results suggested that anaerobic treatment effectively reduces resistant pathogens; however, certain populations, such as Staphylococcus spp., showed resistance to the process. Risk assessments revealed that many resistance genes, identified in the anaerobic digesters, could pose a significant threat to human health. However, the lower expression of these genes in pathogenic bacteria suggests that actual risk may be reduced, albeit their presence. Differential expression analysis consistently highlighted antibiotic resistance genes linked to potential pathogenicity, emphasizing the importance of assessing gene activity using metatranscriptomic data alongside gene presence revealed by metagenomic data.

These findings highlight the complex role of anaerobic digestion in agricultural and wastewater treatment processes in shaping microbial resistance profiles. Anaerobic systems are effective at both reducing pathogen abundance and expression of some resistance genes. Nevertheless, persistent microbial populations and their evolving resistance mechanisms remain a concern.

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L23

Impact of chemical and biological additives on microbial community and methane emissions in pig slurry storage

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In intensive pig farming, where slurry-based manure management is common, over 90% of farm-level greenhouse gas emissions are methane (CH_4), with 70-80% of it is produced during slurry storage. While chemical additives like sulfuric acid (H_2SO_4) are effective in suppressing these emissions, they raise concerns about environmental toxicity, biogas inhibition, and soil acidification. Therefore, alternative strategies particularly biological treatments are urgently needed to mitigate CH_4 emissions during pig slurry storage.

The effects of chemical additives (sodium dodecyl sulfate (SDS), H_2SO_4) and biological additive (*Limosilactobacillus reuteri* with glycerol) on CH_4 emissions and microbial community dynamics in residual slurry from fattening pig farms were investigated. Residual slurry which was left over in the manure pits after flushing was collected, as it is rich in methanogens and responsible to CH_4 emissions from manure pits. Two experiments were conducted to evaluate the effects of SDS, H_2SO_4 and *L. reuteri* on CH_4 emissions and methanogenic microbial communities. In exp 1, a combination of weekly removal of slurry and treatment of residual slurry was simulated in laboratory scale with SDS and H_2SO_4 treatments for a period of 7 weeks. In exp 2, *L. reuteri* treatment was applied to residual slurry and incubated for about 4 weeks. In both the studies, emissions from the treated and control reactors were measured continuously with a gas analyser. Cumulative CH_4 emissions and methanogenic microbial communities were determined at the end of the study to evaluate the treatment effectiveness.

SDS (1.5 g/kg) and H_2SO_4 (pH 4.7-6) treatments were observed to reduce CH_4 emissions by about 90% and 66%, respectively in Exp 1. SDS showed substantial alterations in dominant microbial groups such as *Clostridia*, *Actinobacteria*, *Methanobacteria* and *Gammaproteobacteria*. The relative abundance of *Methanobacteriales* was higher (6.8%) in SDS treatment compared to untreated sample (4.3%), yet CH_4 emissions were significantly reduced. This suggests that SDS treatment likely inhibit methanogenic activity without eliminating methanogen populations, possibly by disrupting syntrophic interactions or metabolic pathways. In contrast, H_2SO_4 treatment reduced *Methanobacteriales* abundance (3.4%) but with lesser impact on CH_4 emissions. In Exp 2, *L. reuteri* + glycerol treatment achieved about 80% reduction in CH_4 emissions, and also lowered relative abundance of *Methanomicrobiaceae*, *Methanobacteriaceae* and *Methanomethylophilaceae*.

Although both SDS and *L. reuteri* treatments reduced CH_4 emissions from pig slurry, their effect on microbial communities varied markedly. The above findings highlight that CH_4 mitigation is not solely dependent on reducing methanogen abundance but also on altering microbial functionality and interactions.

L24

Microbial Community Dynamics Triggered by In Situ Biomethanation

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This study explored *in situ* biomethanation as a biogas upgrading strategy by injecting hydrogen (H_2) into anaerobic fed-batch reactors treating wastewater from the pulp and paper industry. Granular sludge was used as inoculum, and H_2 was supplied at two pressures (0.6 and 0.9 bar) to evaluate its impact on methane (CH_4) production and microbial community dynamics. This approach aims to increase the calorific value of biogas while retaining the treatment efficiency of industrial effluents.

Nine stirred fed-batch reactors (500 mL) were set up with 50 mL of granular sludge and 50 mL of wastewater from a full-scale internal circulation reactor at a paper recycling plant. After an adaptation phase (three cycles per week, with feeding every 48 hours with wastewater), three triplicate sets were established: a control group without additional H_2 (CA1–CA3), and two groups with 0.6 bar (CB1– CB3) and 0.9 bar (CC1–CC3) H_2 overpressure. The evaluation phase consisted of seven fed-batch cycles, under identical operating conditions. Physicochemical parameters (e.g., gas composition, pressure, pH, COD) and microbial community compositions were monitored, the latter through amplicon sequencing of 16S rRNA and *mcrA* genes.

A rise in pH, especially in CB and CC, was due to microbial utilization of CO_2 and disruption of the carbonate buffer system. High H_2 partial pressure contributed to the accumulation of propionic acid, which inhibited CH_4 production by disrupting acetic acid formation and reducing methanogenic activity. Additionally, competition for H_2 between homoacetogenic bacteria and hydrogenotrophic methanogens was observed. Microbial community structure changed first as a function of new stirred reactor conditions and later according to H_2 addition resulting in three clearly separated groups.

The family Syntrophobacteraceae responsible for propionate degradation declined in all reactors due to operational changes and following microbial succession. In control reactors it was out-competed by members of Geobacteraceae and Desulfobulbaceae. In CB and CC assays, Ethanoligenenaceae, Bacillaceae, Kosmotogaceae, Anaerolineaceae, and Anaerolineaceae families were enriched as a result of H_2 supplementation. *Methanothrix* was predominant in all batch reactors and it remained abundant in the control group, aligning with its role as a strict acetoclastic methanogen. Upon H_2 addition a microbial shift favored hydrogenotrophic *Methanobacterium*, which became predominant in later cycles of CB and CC. Despite this shift, both genera coexisted throughout the experiments, suggesting that multiple metabolic pathways (homoacetogenesis, acetoclastic and hydrogenotrophic methanogenesis) contributed to CH_4 production under H_2-enriched conditions.

Although the process demonstrated potential for simultaneous biogas upgrade and wastewater treatment, the overall performance was negatively influenced by increased H_2 pressure. This highlights that proper H_2 dosing and microbial monitoring are critical to ensure process stability for *in situ* biomethanation systems. Considering the fragile balance of the investigated wastewater treatment process an *ex situ* upgrade using a separate reactor is recommended.

SESSION 6: Anaerobic Sulfur Cycle

L25

Functional Generalists and Key Degraders: Genome-Resolved Niche Analysis in a Long-Term Sulfate-Reducing Benzene-Degrading Consortium

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Defining microbial niches using genome-resolved approaches is essential for understanding microbial community structure, function, and ecological roles in anaerobic constructed and natural ecosystems. We investigated a model sulfate-reducing, benzene-degrading microbial community maintained as an enrichment culture for over 15 years. Using genome-resolved metagenomics, we developed an approach to defining niches based on functional potential.

We recovered 127 metagenome-assembled genomes (MAGs) (120 Bacteria, 7 Archaea) spanning 22 bacterial and 4 archaeal phyla, including several unclassified groups using MuDoGeR; these MAGs were dereplicated into 52 species-level Operational Taxonomic Units (OTUs). Six presence-absence datasets were constructed to capture functional diversity based on domain-specific experts (e.g., genes involved in aromatic degradation), KEGG modules and reactions, genome-scale metabolic reconstructions (GSMRs), and general annotations from PROKKA. Two clustering algorithms were applied to define MAG groups with similar functional traits. Statistical filtering and Spearman correlation were used to determine statistically significant correlations ($p < 0.05$; correlation ≥ 0.5) between functional features and clusters. Significant associations were visualized as undirected, weighted networks in Cytoscape, where nodes represented clusters or features (i.e., genes or KEGG Modules), and edges reflected statistically significant correlations. Network and clustering analyses identified at least 12 distinct functional niches with extensive functional overlap among taxa. Most MAGs were associated with multiple niches, suggesting generalist roles within the community. Niches with functional potential encompassed by a smaller number of species may represent key niches within a community. For example, our analyses indicated that only two species (belonging to the phylum Desulfobacterota) showed the genetic potential for activating benzoate to benzoyl-CoA, indicating this niche is key for the aromatic degradation of benzene in our sulfate-reducing model community.

Most MAGs belong to multiple niches, indicating they perform generalist roles in the community. In contrast, niches with only a few MAGs encoding for a particular functional potential were rare (e.g., activation of benzoate to benzoyl-CoA). Our findings highlight that functional redundancy is widespread in our model microbial community with (just a few) key aromatic degraders under sulfate-reducing conditions. Future improvements, including increased sample diversity, broader data integration, and semi-automated workflows, could enhance niche resolution and deepen ecological insights.

L26

Evaluating the biotechnological potential of anoxygenic phototrophic bacteria for desulfurization

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There are various phototrophic organisms among anaerobic bacteria, most notably purple and green sulfur bacteria (PSB and GSB). Both of these ecological groups perform so-called anoxygenic photosynthesis: instead of photooxidation of water to elemental oxygen, known from cyanobacteria and eukaryotic phototrophs, they oxidize other electron donors, especially H_2S, which they transform either to elemental sulfur or to sulfate. This property could potentially be applied in biotechnology, for example, in biogas desulfurization.

Enriched cultures of PSB and GSB can be obtained from anoxic sediments using a Winogradsky column. Isolating pure cultures is difficult and may decrease viability due to lack of interaction with other species. Therefore, we prefer working with enriched cultures. Culture media for PSB and GSB include basic minerals, H_2S, $NaHCO_3$ as a source of carbon and cyanocobalamin as a precursor of bacteriochlorophylls. For faster growth, organic compounds such as acetate can be added. We use classical light bulb as light source. From its continuous light spectrum, the bacteria can absorb whichever wavelength they prefer. We culture PSB and GSB in fed-batch mode with regular additions of H_2S and $NaHCO_3$ solutions. The cultivation takes place in 120 mL vials with 50 mL medium and nitrogen atmosphere, sealed with butyl septa to maintain anoxic conditions.

Our goal was to study photosynthetic activity of several cultures enriched with PSB or GSB. We measured optical density and H_2S concentration in the cultures over time. All of the cultures were grown for at least 14 days. When hydrogen sulfide concentration dropped to zero, the culture was fed with H_2S and $NaHCO_3$ solutions. The most active cultures with PSB *Allochromatium* sp. or *Thiocapsa* sp. as the dominant species, had to be fed every day since the beginning of the cultivation. When we measured in shorter intervals, we found that H_2S concentration dropped to zero in 6 hours in both of these cultures. The cultures enriched with GSB *Chlorobium limicola* had slower growth and H_2S uptake.

Our results show that anoxygenic phototrophic bacteria can effectively remove H_2S from the media, confirming their potential for biogas desulfurization. Such a technology could be a more environmentally friendly alternative to current chemical desulfurization processes and would make biogas, an important biofuel, even more sustainable. The excess biomass, rich in sulfur, bacteriochlorophylls and glycogen, could also be utilized, for example as a biofertilizer. In the next step, we are going to set up a laboratory-scale photobioreactor for biogas desulfurization.

SESSION 7: Enzymes of Anaerobes

L27

Tungsten-dependent aldehyde oxidoreductases: reaction mechanism and applications

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Aldehyde oxidoreductases (AOR) are obligately tungsten-dependent enzymes carrying a W-bis-metallopterin cofactor (W-Co) in their active center. These enzymes occur in many strictly or faculatively anaerobic Bacteria and Archaea and are the only known biocatalysts capable of reducing non-activated organic acids to the corresponding aldehydes in addition to aldehyde oxidation reactions. The known enzymes vary widely in their substrate specificity, ranging from highly specific towards only one substrate (e.g. glyceraldehyde-3-phosphate) to highly promiscuous, accepting a wide range of aldehydes or acids. The best studied examples are the enzymes from hyperthermophilic Archaea, e.g. *Pyrococcus furiosus* (AOR_{Pf}) and from the mesophilic Betaproteobacterium *Aromatoleum aromaticum* (AOR_{Aa}). The former enzyme is a homodimer of the catalytic subunit, which is only active at elevated temperatures and highly sensitive against oxygen. In contrast, AOR_{Aa} is much more stable against oxygen and consists of three different subunits, which assemble to a filamentous quaternary structure carrying "nanowires" of Fe_4S_4 clusters connecting the reactive centers of the complex. The subunits containing the W-Co are highly similar to the AOR_{Pf} subunits, but AOR_{Aa} is additionally equipped with polyferredoxin subunits providing most of the Fe_4S_4 clusters of the nanowires and an FAD-containing subunit at the base of the filaments, which is capable of transferring the electrons from aldehyde oxidation to NAD. The reaction can be directed to either or acid reduction, depending on the redox potentials of the used resp. donors. In addition to NAD, viologen dyes (MV, BV), methylene blue and tetrazolium dyes act as electron acceptors for aldehyde oxidation, while compounds with very low redox potentials such as Ti(III) or Eu(II) complexes,tetra- or hexamethyl viologen can serve as electron donors for acid reduction. AOR_{Aa} was also shown to use molecular hydrogen as electron donor for either acid or NAD reduction.

We present some insight into the biochemistry of AOR_{Aa}, such as the conditions required for acid reduction vs. aldehyde oxidation, the surprising ability to use hydrogen as electron donor, and a proposal for its reaction mechanism in both directions. In addition, we show some proof-of-concept experiments for making the enzyme applicable in biotechnology. We have been able to produce it recombinantly with high specific activity and have used AOR_{Aa} to establish coupled enzyme reactions for either producing alcohols from acids or ATP from electricity.

L28

Cobalt complexes enable anodic cultivation of *Dehalococcoides mccartyi* strain CBDB1 in a bioelectrochemical cell

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Extracellular electron transfer (EET) enables microbial respiration to be coupled with external electron acceptors, such as electrodes, allowing microorganisms to be cultivated using electrons from an external circuit. A notable example for EET is *Dehalococcoides mccartyi* strain CBDB1, an anaerobic, organohalide-respiring bacterium. Its unique respiratory system, the organohalide respiration (OHR) complex, mediates exergonic electron transfer coupled to proton translocation across the membrane – independent of quinones and cytochromes, where electrons from hydrogen oxidation are transferred *via* multiple metallocofactors to reductively dehalogenate polyhalogenated organic compounds. Notably, all active centres of the OHR complex face the outer side of the membrane, making them accessible to electrodes.

We began our investigation by screening potential anode mediators using a high-throughput activity assay, with reduced methyl viologen serving as an artificial electron donor, where we identified cobalt-containing metal complexes, referred to as cobalt complexes, as functional substitutes for toxic organohalides (terminal electron acceptors). Among the tested candidates, cobalt complexes such as methyl cob(III)alamin and methyl cobaloxime(III) were effectively reduced by *D. mccartyi* strain CBDB1. These compounds were further evaluated in cyclic voltammetry experiments with different working electrode materials, including indium tin oxide and gold, which demonstrated that both cobalt complexes can exchange electrons with an electrode – particularly when using gold. Subsequently, we tested the identified cobalt complexes as electron acceptors in an activity assay using hydrogen as the electron donor. Monitoring absorbance changes associated with the reduction of Co^{3+} to Co^{2+} confirmed that the electron transfer was catalysed by the OHR complex. To determine whether this transfer occurs specifically at the reductive dehalogenase (RdhA), we mimicked its cobalamin active centre by reducing cob(III)alamin to cob(I)alamin, which in turn successfully reduced the cobalt complexes. The involvement of RdhA in the reduction process was further supported by bioinformatic analysis, which predicted binding of methyl cobaloxime(III) to the RdhA active site with an estimated binding energy of -28 $kJ \cdot mol^{-1}$.

In summary, we identified cobalt complexes as promising anode mediators capable of replacing toxic halogenated compounds during the cultivation of *D. mccartyi* strain CBDB1. The next step will be to establish a bioelectrochemical system demonstrating anodic cultivation of strain CBDB1 *via* these cobalt complexes, linking the OHR complex to an electrode. Thus, our work lays the foundation for a novel, electrode-based cultivation strategy for anaerobic organohalide-respiring bacteria, with significant potential for *in situ* bioremediation applications.

L29

Anaerobic fermentation of dichloromethane in *Dehalobacterium formicoaceticum* strain EZ94 is driven by methyltransferases

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Chlorinated organic compounds, such as dichloromethane (DCM), are among the most widespread groundwater contaminants and are classified as pollutants of high concern due to their persistence and toxicity. While anaerobic bioremediation offers a promising solution, the microbial mechanisms driving the dechlorination of DCM in bacteria remain poorly understood.

In this study, we identified and characterized the key enzymes involved in the anaerobic fermentation of DCM in *Dehalobacterium formicoaceticum* strain EZ94 as a model organism using a combination of biochemical techniques. First, we performed blue native polyacrylamide gel electrophoresis (BN- PAGE) on cell-free protein extracts from strain EZ94, followed by enzymatic assays to detect DCM- transforming activity. Protein mass spectrometry analysis of the gel slices with the highest activity revealed that the methyltransferase MecC was the most abundant protein followed by other methylases from the *mec* cassette. To further explore the roles of these methyltransferases in DCM transformation, we expressed the three candidate methyltransferases—MecC, MecE, and MecB— heterologously in *Escherichia coli* BL21 (DE3). Enzymatic assays were then performed with the recombinant proteins, both individually and in combinations. Complete DCM transformation *in vitro* occurred only when all three enzymes and a methyl group acceptor were present, indicating a cooperative action between the methyltransferases in the dechlorination process *in vivo*. Mass spectrometry analysis confirmed that during DCM transformation *in vitro*, the methyl group from DCM was transferred to either acetate or coenzyme M used as methyl group acceptors. This suggests that the methyltransferases facilitate the transfer of the methyl group from the contaminant to intermediates involved in the bacterium's metabolism. Finally, computational analyses, including protein structure predictions and cofactor binding site identification, helped to predict the specific functions of each enzyme in the dechlorination process. Our data demonstrates that MecE and MecC are zinc-dependent methyltransferases, catalyzing DCM demethylation and re-methylation to acceptors *in vitro*, respectively. MecB, on the other hand, acts as a cobalamin-dependent shuttle protein, transferring the methyl group between MecE and MecC. These findings provide the first biochemical evidence for a complex anaerobic dechlorination system mediated by three methyltransferases in *Dehalobacterium formicoaceticum*.

This research not only elucidates the enzymatic machinery behind DCM bioremediation but also offers insights into potential applications for monitoring and optimizing anaerobic bioremediation processes in contaminated environments. By characterizing these enzymes, we provide a foundation for the development of biological markers that could enhance the efficiency of bioremediation strategies in the field.

L30

The ATP-dependent methyl-coenzyme M reductase activation complex from *Methanococcus maripaludis*

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Methyl-coenzyme M reductase (MCR) is the enzyme responsible for nearly all biologically generated methane (CH_4). Its active site comprises coenzyme F_{430}, a porphyrin-based cofactor with a central nickel ion that is active exclusively in the Ni(I) state. How methanogenic archaea perform the reductive activation of F_{430} represents a major gap in understanding one of the most ancient bioenergetic systems in nature. Here, we purified and characterized the MCR activation complex from *Methanococcus maripaludis*. McrC, a small subunit encoded in the *mcr* operon, co-purifies with the methanogenic marker proteins Mmp7, Mmp17, Mmp3 and the A2 component. We demonstrate that this complex can activate MCR *in vitro* in a strictly ATP-dependent manner, enabling the formation of CH_4. In addition, we determined the cryoEM structure of the MCR activation complex exhibiting different functional states with local resolutions reaching 1.8 to 2.1 Å, showing important rearrangements driven by ATP hydrolysis. Strikingly, our data reveal three complex iron-sulfur clusters that form an electron transfer pathway toward F_{430}. Topology and EPR spectroscopy analyses indicate that these clusters are similar to the [8Fe-9S-C] cluster, a maturation intermediate of the catalytic cofactor in nitrogenase. Our phylogenetic results imply that the [8Fe-9S-C] cluster was first used for MCR's activation and later incorporated into nitrogenases and nitrogenase-like systems Altogether, our findings offer insights into the activation mechanism of MCR and prospects on the early evolution of nitrogenase.

L31

Cofactor F_{420}-polyglutamate-chain-length profiles in methanogens

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Anaerobic digestion is a multi-stage biochemical process driven by a wide variety of microorganisms that convert complex organic substrates into biogas, primarily composed of methane and carbon dioxide. The microorganisms involved in anaerobic degradation possess a large array of coenzymes and cofactors that are involved in the redox reactions essential to methanogenesis. Among these, the cofactor F_{420} plays an important role as an electron carrier, catalysing the terminal biochemical step in methane production under thermodynamically constrained conditions. While the cofactor F_{420} is primarily associated with methanogenic *Archaea*, its biosynthesis has also been observed in *Bacteria* and some *Eukaryotes*. Structurally, the cofactor comprises of a deazaflavin core linked to a polyglutamate chain of variable length. Furthermore, it has been demonstrated that the length of the expressed polyglutamate residues can differ among methanogenic species.

This work aimed at investigating the distribution of the F_{420}-polyglutamate-chain-length in various methanogenic cultures with regard to methanogenic pathways, growth dynamics, and electron donor availability. A range of different cultivation based, chemical analytical, and molecular biological methods were applied to investigate the distribution and role of the F_{420}-polyglutamate-chain-length profiles. For this purpose, batch reactors containing inoculum consisting of a methanogenic culture and different substrates were cultivated anaerobically and set up on a laboratory scale. The growth of the organisms was determined by measuring their gas production using a gas chromatograph and their substrate consumption using a high performance liquid chromatograph. Following a heat-pressure treatment leading to cell disruption, the cofactor F_{420} was extracted and purified via solid phase extraction. The cofactor F_{420} was then analysed quantitatively and qualitatively via ion-pair-reversed-phase high performance liquid chromatography equipped with a UV/Vis and a fluorescence detector. The results obtained revealed differences in methane production, substrate consumption, and expressed glutamyl residues.

SESSION 8: Microbes in Anoxic Environments

L32

Microbial communities influencing methane cycling in temperate Swedish peatlands

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Northern peatlands are significant organic carbon pools, naturally acting as carbon dioxide (CO_2) sinks but emitting methane (CH_4) as a product of the anoxic decomposition of organic matter driven by complex microbial communities in soil. This study investigates the unexplored microbial communities of pristine peatlands in Värmland, central Sweden, (Björsmossen [BM], Norra Romyren [NR], Lungsmossen [LM] and Havsjömossen [HM]) with a particular focus on microorganisms involved in CH_4 cycling. Peat samples up to 60 cm depth were subjected to biogeochemical measurements and 16S rRNA amplicon sequencing to compare the biogeochemistry and microbial diversity across the sites. Additional deep samples up to 700 cm depth were retrieved and subjected to amplicon sequencing to shed light on the microbial communities across the entire peat layer. This revealed similar biogeochemical conditions (temperature, acidic pH, high CO_2 to CH_4 ratios) and some consistent dominant microbial phyla across sites that may play a fundamental role across peatland locations. BM and NR had the highest species diversity (alpha diversity), which generally decreased with soil depth. Among the deep samples those from BM and LM, collected at 540 and 700 cm of depth respectively, were dominated by archaea, mainly *Crenarchaeota*, comprising many uncultivated lineages (e.g., *Bathyarchaeia* and *Methanomethylicia*). Seven microbial taxa were identified as potentially involved in CH_4 cycling, comprising aerobic methanotrophic bacteria and methanogenic archaea, but no anaerobic methanotrophic archaea. Potential methanotrophs made up around 20% of the microbial community in the top
10 cm across sites but significantly decreased in abundance below 30 cm sampling depth. Potential methanogens belonging to *Halobacterota* were found at all depths but were most abundant at 30 cm (LM) with 68% of relative abundance. Additionally, elevated CO_2 to CH_4 ratios measured at three sites at these depths suggest that there is an unidentified microbial process causing the low CH_4 levels relative to CO_2 in these layers. These findings provide unprecedented insights into the taxonomic composition and potential metabolism of microbial communities inhabiting temperate northern peatlands of the Värmland region, highlighting their correlation with peat biogeochemistry. Upcoming metagenomic and metatranscriptomic data will help clarify their metabolic roles and involvement in CH_4 cycling.

L33

How to handle the lignin in absence of oxygen: a bacterial lesson

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The industrial utilization of the plant biomass, earth's most abundant renewable raw material and a credible alternative to the fossil resource's utilization is hampered by the high structural intrinsic stability of lignocellulose. Fortunately, (ligno-)cellulolytic microbes have evolved performant systems to deconstruct this huge and recalcitrant reservoir of carbon we can build on to design sustainable applications. Contrary to the well-studied (hemi-)cellulolysis that is integrated in plant polysaccharide bioconversion-based processes (i.e. biofuels), delignification constitutes a major conundrum to solve since lignin precludes the access to the polysaccharides and its aromatic derivates inhibit cellulolytic enzymes and are toxic upon accumulation. Its microbial aerobic uncontrolled oxidation (via free-oxygen-radical reactions and metalloproteins as laccases and peroxidases) in compounds poorly usable in industry is well documented. In contrast, very little is known about how lignin is handled in an oxygen-free environment where plant material accumulates. Only the anaerobic fungal lignin deconstruction was proposed in 2023 but without identification of actors.

Before this recent publication, we hypothesized anaerobic bacteria are likely to have developed, as described for the cellulolysis, still unidentified and sophisticated ways to deal with lignin without the use of reactive oxygen to avoid the oxidative stress and consequently in a more specific and controlled manner. Then, we applied an explorative *in vivo* approach by deep analysis of *Ruminiclostridium cellulolyticum* transcriptome evolution during growth on lignified substrates or not (cellobiose to insoluble wheat straw) and correlation with the substrate composition evolution. We 1) showed that modification of a lignified substrate in the absence of oxygen occurs and 2) identified some of the potential actors. We then performed biochemical characterization of their enzymatic activity and combined it with a genetic approach to confirm their function *in vivo*. This allowed us to propose a never reported role as acting on lignocellulosic assemblies for several enzymes.

L34

Deciphering microbial strategies for lignocellulose degradation under denitrifying conditions

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Excessive use of fertilizers in agriculture may lead to nitrate leaching into the surrounding areas, and eventually, eutrophication and increased greenhouse gas emissions. To counteract this issue, farmers are installing woodchip bioreactors where excess nitrate is converted to nitrogen gases by microorganisms, a process powered by the breakdown of lignocellulose in the woodchips. Degradation of lignocellulose under denitrifying conditions is only scarcely studied and may hold significant potential for discovering new lignocellulose-degrading enzyme systems. Obtaining a deeper understanding of this process and the microbial activity driving it could enhance our ability to mitigate greenhouse gases as well as expand our knowledge regarding their potential utilization in biotechnological applications.

To achieve this, we aimed at finding organisms that degrade lignocellulose under denitrifying conditions from a woodchip bioreactor. Using a mixture of woodchips, soil and water from the bioreactor, anaerobic enrichment cultures were set up for multiple months, followed by anaerobic isolation through multiple rounds of growth using cellulose as the sole carbon source and nitrate as the sole electron acceptor. Whole genome sequencing was conducted on the isolates, and promising strains were investigated further with gas kinetics and proteomics to assess the denitrification potential and enzymes involved in the degradation of different lignocellulosic components.

An interesting isolate was *Pseudomonas veronii*, which possessed the genes for the complete denitrification pathway as well as multiple CAZymes and lignin-active enzymes. Phenotyping of the strain using gas kinetics was performed on glucose, which confirmed the expression of the full denitrification pathway without accumulation of N_2O, suggesting its potential as a sink for N_2O. Additionally, we assessed the growth and performed proteomics after growth on acetylated xylan and alkali lignin under denitrifying conditions, examining both soluble and insoluble fractions of lignin. Small aromatic compounds derived from lignin were analysed using LC-MS to get a more detailed understanding of the metabolization of lignin.

The findings were intriguing as the organism appears to be capable of extracting and utilizing the acetyl groups of xylan and the monomers of lignin. Especially interesting is the potential degradation of aromatic compounds from lignin under denitrifying conditions, as this is normally achieved by enzymes requiring oxygen to be present. Deciphering these mechanisms in could offer new insight into how complex carbon sources are degraded in anoxic natural environments and offer potential solutions to exploiting natural resources such as plant waste material.

L35

Anaerobic metabolization of ^{13}C-labeled *Pseudomonas alloputila* cells by thermophilic microorganisms from aquifer sediments

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High-Temperature Aquifer Thermal Energy Storage (HT-ATES) is a promising low-carbon energy system with high energy storage capacity based on the injection and extraction of heated (≥ 40°C) and cooled water in shallow aquifers. However, sediment microbial responses to thermal disturbances due to HT-ATES operations are poorly understood; such knowledge is however essential for evaluating biogeochemical effects and potential environmental risks associated to HT-ATES. Recent data from laboratory and field experiments indicate that thermophilic organotrophic endospore-forming bacteria, generally known for their resilience and metabolic versatility, rapidly develop in heated aquifer sediments, thus being crucial for degradation of organic matter at elevated temperatures. We hypothesize that cells of the indigenous mesophilic or psychrophilic aquifer community may be metabolized by aquifer thermophiles. In this study, we applied stable isotope probing (SIP) combined with nanoSIMS to investigate the anaerobic mineralization of the ^{13}C-labeled mesophilic model strain *Pseudomonas alloputila* mt-2 KT2440 by indigenous thermophiles in pristine aquifer sediments at three temperatures (60, 70, and 80°C). Six labeled replicates were prepared for each temperature treatment. Significant $\delta^{13}CO_2$ production was observed in all replicates at 60°C and 70°C. At 80°C, only three replicates (e.g., 80-03) exhibited notable δ ^{13}C mineralization. NanoSIMS analysis of sample 80-03 revealed high ^{13}C enrichment (~80%) in microbial cells, with preferential assimilation into nitrogenous compounds (e.g., peptides and nucleic acids) rather than lipids. 16S rRNA gene sequencing indicated that endospore-forming taxa affiliated with the phylum Bacillota dominated the thermophilic community and were primarily responsible for microbial biomass degradation. Our findings highlight the potential of thermophilic endospore-forming bacteria to anaerobically assimilate carbon from dead cells under high-temperature conditions and provide new insights into microbial metabolic activity and ecosystem function in HT-ATES.

Keywords: high-temperature aquifer thermal energy storage (HT-ATES); thermophiles; endospore-forming microorganisms; organic carbon mineralization; Bacillota; nanosecondary ion mass spectroscopy (nanoSIMS); ^{13}C-labeling

L36

Quorum-Sensing-mediated biofilm formation in anaerobic microbial communities

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Biofilms are the predominant form of collective life in the microbial world, featuring a much higher level of organization than planktonic microbial populations. Biofilms are now known to dominate all natural habitats of the Earth, accounting for 40%-80% of all bacterial and archaeal cells. In the health sector and food industries, biofilms are generally regarded as a threat due to the risk of infection and the increased resistance of pathogenic bacteria growing in biofilms. However, biofilms represent an opportunity for attached-growth bioreactor systems, where biofilm traits such as nutrient sharing, toxicant resistance, and increased biomass density can be harnessed for boosting the bioproduction of e.g. biofuels and commodity chemicals.

Microbial communities, whether composed by multi- or single-species populations, result from a complex interaction network dependent on cell-to-cell communication facilitated by Quorum Sensing (QS) signalling. In fact, QS has been found to modulate a wide range of phenotypical changes across all domains of microbial life, including biofilm formation and cell density, among many others. Nevertheless, understanding the regulation of biofilm assembly in anaerobic microbiomes poses a major challenge due to the diverse and complex interplay between QS mechanisms, microbial interactions and population dynamics prevailing in biofilm communities.

This study investigates the role of QS molecules, specifically N-acyl homoserine lactones (AHLs), in modulating the intricate dynamics of biofilm formation in methanogenic microbial communities. A series of high-throughput experimental methods and flow-cell systems were run to study the impact of different AHLs on the growth and development of anaerobic biofilms. Initial screening experiments in microtiter plates with anaerobic cultures provided preliminary insights on the effects of different AHLs on biofilm formation and their growth rates. Further experimentation using flow-cell systems and confocal laser scanning microscopy on a subset of AHLs allowed to accurately quantify the biofilm formation and visualize its structure, as well as to study changes in population dynamics and gene expression profiles triggered by the addition of these AHLs.

The results of the initial screening indicated that most of the AHLs had a positive impact on the biofilm growth rate during the initial growth phase. Further experimenting with C6-HSL and C10-HSL using flow-cells confirmed prior observations and revealed a much higher thickness and complex structure compared to the control after 7 days. Overall, this work provides valuable insights on the role of AHLs in governing biofilm formation and population dynamics in anaerobic microbial communities.

L37

Reactor-Based Enrichment of an Autotrophic *Paracoccus*-Dominated Culture Using Hydrogen and Nitric Oxide

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Nitric oxide is a highly reactive and toxic molecule, yet it plays a key role as an intermediate in the denitrification pathway from nitrate or nitrite to dinitrogen gas. Because of its toxicity, nitric oxide can only be introduced into microbial cultures in very small amounts during cultivation, necessitating repeated dosing. This limitation has likely contributed to the absence of prior demonstrations of microbial growth directly on nitric oxide. In this study, a digitally controlled reactor system was employed to periodically dose small quantities of nitric oxide into the reactor headspace, aiming to enrich microorganisms capable of using it. Hydrogen served as the electron donor, and bicarbonate was provided as the sole carbon source. The concentration of nitric oxide in the headspace was carefully maintained below 0.2% (v/v), corresponding to an equilibrium aqueous concentration of 3.8 µM. Over the course of the enrichment, increase in cell density was observed, indicating microbial growth. Both hydrogen and nitric oxide were consumed, although not in a stoichiometric manner. Metaproteomic analysis using *de novo* peptide sequencing and taxonomic classification with Unipept identified *Paracoccus versutus* as the dominant species. Metagenomic sequencing produced 60 high-quality metagenome-assembled genomes (MAGs), including that of *P. versutus*. Notably, apart from nitric oxide reductase which was expected to be present, nitrate reductase, nitrite reductase, and nitrous oxide reductase of *P. versutus* were also abundant in the proteome. Collectively, these findings provide the first evidence of autotrophic microbial growth on hydrogen and nitric oxide, although the precise mechanism – whether nitric oxide reduction or dismutation – remains to be determined.

SESSION 9: Biotechnological Applications of Anaerobes

L38

Eco-engineering strategies to target propionic acid production from food waste fermentation

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The valorisation of food-waste (FW) through anaerobic fermentation is a sustainable approach for waste management, by simultaneously producing valuable biochemicals such as volatile fatty acids (VFA). Among them, propionic acid (PA) holds significant industrial interest as a precursor of jet fuel production. This study explores different eco-engineering strategies to reach a selective production of PA from FW fermentation using mixed microbial cultures, where PA is generally a minor component within the VFA mixture.

Three distinct approaches were investigated to optimize PA production: 1) direct selection by applying different initial pH, temperature and FW concentration in batch, 2) successive selection, consisting in repeated fermentation cycles of batch incubations with increased FW concentration (11.7-20 $gVS \cdot L^{-1}$); and 3) bioaugmentation in a two-stage fermentation process; which aimed to convert FW to lactate in acidic conditions and then lactate to propionate by adding *A. acidipropionici*. The microbial communities involved in the process were analysed.

The first strategy (initial pH 9, T=35°C and FW=7.8 $gVS \cdot L^{-1}$) demonstrated a high substrate conversion (70.22 ± 9.56% $gCOD_{metabolites} \cdot gCOD_{introduced}^{-1}$), but exhibited poor PA selectivity (20.76 ± 10.43% $gCOD_{PA} \cdot gCOD_{metabolites}^{-1}$), as multiple microbial families coexisted without a clear dominance of PA-producing strains. The successive batch selection resulted in a higher selectivity for PA (59.46 ± 13.21% $gCOD_{PA} \cdot gCOD_{metabolites}^{-1}$), but lower VFA yield due to methane production (9.56 ± 3.64% $gCOD_{metabolites} \cdot gCOD_{introduced}^{-1}$). In contrast, the two-stage fermentation process showed quite promising results. In the first stage, a high lactate selectivity was achieved (53.89 ± 2.96% $gCOD_{lactate} \cdot gCOD_{metabolites}^{-1}$), together with a low substrate conversion yield (39.90 ± 4.55% $gCOD_{metabolites} \cdot gCOD_{introduced}^{-1}$). In the second stage, the introduction of *A. acidipropionici* to the system resulted in complete lactate conversion into PA and acetate, without inhibition nor side-product formation. However, the global conversion from FW showed a low efficiency due to the first stage (25.63 ± 15.33 % $gCOD_{metabolites} \cdot gCOD_{introduced}^{-1}$). The dominance of PA formation from lactate enhanced the PA selectivity (37.90 ± 11.82% $gCOD_{PA} \cdot gCOD_{metabolites}^{-1}$) with a high yield in the second stage (65.34 ± 24.39% $g_{PA} \cdot g_{Lactate}$).

These findings demonstrate the effectiveness of bioaugmentation in a two-stage fermentation process for enhancing PA production from FW with high selectivity, while single-stage fermentation results in a high conversion of FW to mixed VFA. Future research should focus on the role of indigenous microorganisms and their interactions with bioaugmented bacteria, studying the factors influencing the maintenance of *A. acidipropionici* within the community.

L39

BIOcubed: Biosynthesis of Green Hydrogen Using a Newly Discovered Microorganism

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In order to achieve the objective of climate neutrality by 2040, the Austrian government is placing an increasing emphasis on the expansion of renewable energy sources. Hydrogen (H_2) plays a pivotal role in this strategy as a clean energy carrier with the potential to replace fossil fuels and facilitate the decarbonization of various sectors. Among the methods for biological H_2 production, dark fermentation using anaerobic bacteria has gained significant attention. Recently, *Thermoactinomyces mirandus*, a thermophilic bacterium first isolated in 2015 from a biogas plant in Tyrol, Austria, has shown promise for H_2 production under anaerobic conditions. To explore the potential of *T. mirandus* in H_2 production, the BIOcubed project was launched to develop an efficient and economical process for generating H_2 from lactose-containing biomass and other renewable resources by leveraging the bacterium's unique properties.

The primary objective of the present study was to optimize H_2 biosynthesis by *T. mirandus* in small-scale systems, focusing on cultivation conditions to maximize H_2 yield. Standard anaerobic media preparation and cultivation were performed in serum bottles (50 mL working volume) under controlled conditions: pH 7.2 ± 0.1, temperature 51.4 ± 0.2 °C, and defined nutrient concentrations with lactose as the carbon source (0.8 g C/L) and yeast extract as the nitrogen source (0.04 g N/L).

Experimental results revealed that yeast extract combined with fructose or xylose produced significantly higher H_2 yields compared with yeast extract and lactose. Further optimization of the carbon-to-nitrogen (C:N) ratio demonstrated that reduced carbon concentrations (0.8 g C/L) improved substrate utilization and enhanced H_2 production. For each carbon source and concentration, a specific C:N ratio optimum was determined. Additionally, the influence of initial pH and temperature on H_2 yield was systematically evaluated, with optimal conditions established for maximizing H_2 production. Metabolite analysis using HPLC-RID revealed succinate, lactate, acetate, and ethanol as key fermentation (by)products, aligning with mixed acid fermentation pathways characteristic of H_2-producing bacteria. This study further investigated potential inhibitory factors affecting H_2 production, such as acidification of the medium and elevated H_2 partial pressure. The findings demonstrated that pH regulation and gas-phase sparging with nitrogen significantly improved H_2 yields.

L40

Investigating resilience of biological methanation to prolonged hydrogen starvation

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Biological methanation presents a promising route for CO_2 utilization by converting CO_2 into CH_4 through the metabolism of hydrogenotrophic methanogens. When H_2 is sourced from water electrolysis fueled by renewable energy, the process becomes sustainable. However, the intermittent nature of renewable energy poses challenges for continuous H_2 generation. While biological methanation has been extensively studied, its robustness under fluctuating and prolonged H_2 starvation remains poorly explored. The current work investigated the biomethanation process under intermitted feeding, with starvation periods extending up to five weeks.

Trickle bed reactors (TBR) were used to investigate the impact of two packing materials with different compositions and surface areas: activated carbon pellets (TBR1) and high-density polyethylene Raschig rings (TBR2) on biological methanation. Enriched hydrogenotrophic inoculum and nutrient broth consisted of the liquid phase. The gas phase, serving as the feedstock for the methanogens, was a 4:1 H_2/CO_2 gas mixture. The experiment had five starvation periods, each followed by an equal operation period. Starvation and operation durations progressively increased from one up to five weeks. Moreover, genomic samples from reactors' liquid phase were collected, aiming to correlate community shifts with variations in gas supply across the experimental cycles.

The study demonstrated that biological methanation under intermittent H_2/CO_2 feeding can maintain high CH_4 yields. In general, TBR1 exhibited remarkable adaptability, consistently recovering quickly and achieving methane concentrations above 95% after each starvation period. In contrast, TBR2 needed more time to recover from the short-duration starvation phases but showed improved performance in the longer starvation periods. This difference is attributed to the higher specific surface area of the activated carbon, enhancing biofilm formation and mass transfer. Microbial analysis revealed that TBR1 was dominated by *Methanothermobacter* spp., supporting its stable performance, whereas TBR2 showed greater bacterial diversity and slower methanogen recovery. Starvation periods led to increased bacterial abundance, particularly *Coprothermobacter* for TBR1 and Firmicutes for TBR2, suggesting a shift in community function toward VFA degradation and acetate production.

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L41

Unlocking the Potential of Lignocellulosic Waste: Alkali Pre-treatment to Sustainable Chemicals in the Circular Bio-economy Context

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Lignocellulosic agricultural residues, such as sunflower stalks and heads, are abundant bioresources with high organic content. However, their complex structure hinders biodegradability and anaerobic digestion (AD) efficiency. This study explores alkali pre-treatment with potassium hydroxide (KOH) to improve substrate solubilization and enhance volatile fatty acid (VFA) production over methane, given VFAs' greater industrial and economic value. Pre-treatment performance was assessed via soluble chemical oxygen demand (sCOD), VFA yield, total solids (TS) and volatile solids (VS) reduction, microbial community shifts, and cost-effectiveness. The findings contribute to advancing sustainable waste valorization and bioenergy recovery, supporting circular economy principles and environmental sustainability goals.

Sunflower stalk and head residues were subjected to KOH pre-treatment at concentrations of 0%, 6%, 8%, and 10% (w/v), using a solid-to-liquid ratio of 100 g TS/L for 24 hours at ambient temperature. Following pre-treatment, the solid and liquid fractions were separated using sieves and coffee filters. Anaerobic digestion was conducted in batch reactors at 37°C for 30 days, with an inoculum to substrate ratio of 2:1 and a controlled pH of 5.5. Microbial community analysis was examined using Oxford Nanopore MinION sequencing targeting the 16S and 18S rRNA genes at days 0 and 30. VFA concentrations were quantified via gas chromatography, and cost analysis was performed based on market prices of VFAs.

Results demonstrated that pre-treatment with 8% KOH significantly enhanced AD performance. sCOD levels increased from 13,200 mg/L in the untreated control to 22,200 mg/L, indicating improved solubilization of organic matter. Cumulative VFA production in the 8% KOH treatment reached 15,738 mg/L—more than double that of the control (7,396 mg/L)—with propionic acid (1,601.55 mg/L) and butyric acid (1,513.90 mg/L) as the dominant products.

Improvements in TS and VS removal efficiencies (28% and 29%, respectively) reflected enhanced substrate degradation. Microbial community analysis revealed a shift from a diverse initial community (Day 0: *Bacteroidales*, 8.9%) to a dominance of *Romboutsia* sp. (30.26%) by Day 30. The suppression of methanogenic populations and the enrichment of fermentative bacteria under acidic conditions supported the observed accumulation of VFAs.

The 8% KOH dosage offered an optimal balance between performance enhancement and cost-effectiveness, whereas the 10% KOH treatment resulted in diminished yields, indicating potential inhibitory effects due to over-treatment. These findings confirm the potential of alkali pre-treatment to facilitate the conversion of lignocellulosic residues into high-value bioenergy intermediates. Future research should focus on process scale-up and the long-term stability of microbial community structures.

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SESSION 10: Gas Fermentation

L42

Unlocking Sustainable Bio-Hexanol Production with *Clostridium carboxidivorans* through Adaptive Evolution and Process Optimization

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The shift from a fossil-based to a circular bioeconomy requires sustainable microbial technologies to valorize carbon-rich waste. Gas fermentation with acetogens offers a promising route to convert CO_2 into high-value chemicals, such as 1-hexanol. This study focuses on developing a H_2-CO_2-based gas fermentation process using *Clostridium carboxidivorans* P7, with the specific aim of producing bio- hexanol, a compound with extensive industrial applications.

The study aimed to enhance the growth and production performance of *C. carboxidivorans* P7 when utilizing CO_2 as the carbon source and H_2 as the electron donor. Adaptive Laboratory Evolution (ALE) was implemented to promote improved phenotypes, resulting in an evolved strain, designated *C. carboxidivorans*_hex21. Genomic analysis of the new strain revealed a conserved frameshift mutation in the catalytic subunit of a hexameric hydrogenase gene, which likely facilitated a shift toward alternative hydrogenases, thereby improving H_2-based growth. Compared to the wild-type, the evolved strain exhibited a reduced lag phase and increased biomass production. Notably, hexanol emerged as the primary fermentation product, with the process achieving a carbon selectivity of 45% and a maximum specific productivity of 0.74 $g_{HexOH}\ g_{CDW}^{-1}\ day^{-1}$, the highest reported to date for gas fermentation. Subsequently, the obtained strain was evaluated in elevated-pressure stirred-tank reactors operated in a liquid batch configuration. Critical process parameters, including CO_2 and H_2 partial pressures, gas feeding strategies, and H_2 to CO_2 gas ratio, were systematically investigated. Continuous gas feeding enhanced acetogenic and solventogenic metabolisms, while gas-limited conditions favored chain elongation toward caproic acid, a precursor of hexanol. Achieving high caproate concentrations during the initial fermentation phase was crucial for improving subsequent hexanol production. An integrated process combining an initial gas-limited step followed by continuous gas feeding resultedin a maximum cell-specific productivity of 1 $g_{HexOH}\ g_{CD\ W}^{-1} day^{-1}$. Furthermore, the implementation of in situ product extraction increased hexanol carbon selectivity to an unprecedented 60%. Overall, these findings demonstrate the feasibility of scaling up a H_2-CO_2-fed fermentation process from laboratory-scale serum bottles to elevated-pressure stirred-tank reactors. The successful production of hexanol marks a significant advancement in expanding the range of chemicals obtainable through gas fermentation processes beyond acetate and ethanol. A comprehensive understanding of the metabolic responses to various process parameters proved to be a successful strategy for channeling the strain's efforts toward hexanol production. This work represents a significant advancement in the sustainable production of biochemicals, providing a promising framework for future research and industrial application in circular bioeconomy.

L43

Quantification of an anaerobic synthetic co-culture containing *Acetobacterium woodii* and *Clostridium drakei*

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Rising global warming has intensified into a worldwide environmental crisis. The increasing emissions of greenhouse gases such as CO_2 drive this development. Nonetheless, the use of fossil fuels is increasing constantly. Climate-friendly alternatives are needed to stop this trend. Microbial gas fermentation is a sustainable approach for generating platform chemicals, such as hexanoate and butyrate. We are establishing a synthetic co-culture containing the acetogens *Acetobacterium woodii* and *Clostridium drakei* to produce hexanoate and butyrate via gas fermentation.

A. woodii is known for its capability to grow autotrophically, while rapidly utilising H_2 + CO_2. Its main product is acetate. The product spectrum was extended to lactate by establishing a plasmid-borne expression of a D-lactate dehydrogenase from *Leuconostoc mesenteroides*. *A. woodii´*s native genes encoding the bifurcating lactate dehydrogenase complex were knocked out to avoid reutilization of the produced lactate. The lactate provided by the recombinant *A. woodii* is used by *C. drakei* as a substrate. *C. drakei* is capable of producing butyrate and hexanoate via reverse-β-oxidation.

One challenge in cultivating a synthetic co-culture is to establish a suitable inoculation strategy to improve product concentrations and to ensure that none of the species is overgrown. Therefore, the monitoring of each species during co-cultivation is necessary.

Quantitative PCR (qPCR) is an option for quantifying the respective species by amplifying the specific genes encoding a formate dehydrogenase subunit (*fdhH*) of *A. woodii* and phosphate butyryltransferase (*ptb*) of *C. drakei*. Our approach is to define a correlation between respective c_q values and OD_{600} values. Primers from both strains were designed to amplify a fragment of *fdhH* from *A. woodii* and of *ptb* from *C. drakei*, which are unique in each genome. In several measurements, two characteristic exponential growth phases of *C. drakei* were identified during autotrophic growth experiments. A strong correlation was found between c_q and OD_{600} values in both growth phases (first exponential growth phase $R^2 = 0.89$, second exponential growth phase $R^2 = 0.87$).

Next, an autotrophic *A. woodii* growth experiment will be performed to define a correlation between the c_q value and the OD_{600} value. Synthetic *A. woodii* and *C. drakei* mixtures with known OD_{600} ratios will be quantified via qPCR to validate the method.

L44

A new point of view: studying syngas biomethanation biofilms in flow-chambers

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Biofilms are ubiquitous in the environment, and as often as they are problems to be dealt with, they can also be beneficial. Their usefulness in a broad range of biotechnological applications is becoming increasingly apparent, such as in gas fermentation, where a widely used bioreactor type is the trickle bed reactor, where microbes grow forming biofilm. Current process optimisation overlooks the behaviour of biofilm, and little is understood of the spatial distribution of the microbes within it. This raises the need for detailed biofilm studies using specialised methods. One such method is the use of flow-chambers and confocal scanning laser microscopy (CSLM) for *in-situ* inspection of biofilms. In this study, a syngas biomethanation biofilm was studied in a flow-chamber system to elucidate the distribution of its main microbial groups. Syngas is a mixture of hydrogen, carbon monoxide and carbon dioxide, and is generally considered a waste gas. Its conversion to methane allows the storage its energy into a stable and versatile energy carrier that can be applied as a renewable substitute for natural gas. The conversion of syngas to methane takes place through a series of interconnected metabolic reactions carried out by different microbial groups. One main reaction is the conversion of carbon monoxide to hydrogen and carbon dioxide, and another is the conversion of hydrogen and carbon dioxide to methane. These reactions are carried out by members of the Firmicutes phylum and the Methanobacteriales order, respectively. Fluorescent in-situ hybridisation (FISH) targeting these two groups was applied to observe their spatial distribution. Crucially, this method maintains the 3-dimensional structure of the biofilm, which can then be reconstructed using image processing software. This allows the observation of the relative distribution of the two groups in the biofilm, and sheds further light on their relationship. Stratification of the biofilm was observed with Firmicutes layering on top of Methanobacteriales. Firmicutes consume CO and provide hydrogen and carbon dioxide to Methanobacteriales, and they may also offer the latter protection from CO toxicity. Additionally, this work expands the existing image analysis toolbox by adding additional quantification of the spatial relationship between microbial groups and the possibility to visualise it. This resulted in a more direct and biologically meaningful visualisation of the relative spatial distribution of Methanobacteriales and Firmicutes. Notably, this analysis can be extended to a variety of applications with the aim of utilizing the full potential of 3-dimensional confocal scanning laser microscopy images.

SESSION 11: Power-to-Gas

L45

From methanogen physiology to a robust power-to-gas process: addressing intermittent substrate supply and oxygen tolerance

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Power-to-methane presents a promising technology for the long-term storage of surplus renewable energy in the form of CH_4. The biocatalytic conversion of H_2 and CO_2 to CH_4 and water is carried out by hydrogenotrophic methanogens. Successful application of this technology can be enhanced by a better understanding of methanogen physiology, specifically the metabolic response of methanogenesis to fluctuations in substrate supply, including H_2 or CO_2 limitations and intermittencies.

Cultivating *M. maripaludis* in chemostats under either H_2 or CO_2 limitation did not affect yield or methanogenesis activity. However, differences were observed when *M. maripaludis* was completely starved in the presence of either excess CO_2 or H_2. *M. maripaludis* exhibited a higher tolerance to H_2 starvation compared to CO_2 starvation, resulting in shorter lag times and greater methanogenesis activity upon revival with replenished substrates. CO_2-starved cells exhibited higher F420 reduced/oxidized ratio as well as NADH/NAD+ ratio, and were more susceptible to O_2 inactivation, indicating reductive stress from the accumulation of reducing equivalents. These insights can help stabilize the operation of power-to-methane processes by adjusting the ratio of H_2 to CO_2 feed in case of intermittent operation or shutdowns.

In a follow-up study, we have preliminary data showing that an aerobic methane-oxidizing bacteria (methanotroph) isolated from a marine environment can form a co-culture with methanogen *M. maripaludis* for an oxygen-tolerant methanogenesis process.

L46

Microbiology of the mesophilic *ex-situ* biological methanation: influence of operating parameters such as inoculum and scaling up

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Many microbial processes are not yet well characterized, and a better knowledge of the involved actors and environmental parameters influencing them would lead to process yield improvements and reduction of cost. Biological methanation (BM) is one interesting solution for energy supply: electricity from intermittent renewable sources such as wind and solar power can be stored under the form of methane with a high storage capacity and a long discharge time. Indeed, BM allows to convert CO_2 from biogas or industries into CH_4 using H_2 as an electrically generated chemical reducer. However, this process still needs to be better understood at the microbial level when performed *ex-situ* and specifically at the mesophilic range. Indeed, achieving a biomethane purity over 95% *vol* with an adequate flow rate remains highly challenging at this temperature.

In this study, anaerobic digestion reactors (ADR) coupled to *ex-situ* mesophilic biological methanation reactors (BMR) at laboratory (5L working capacity) and pilot (800L) scales were used. Four coupled reactors were operated at the laboratory scale, while one couple of pilot scale reactors installed on a biogas plant was available. Each coupled laboratory reactors were inoculated with either sludge from an ADR of a wastewater treatment plant, sludges from ADRs treating a mixture of agricultural residues and bio-waste from two different biogas plants, and sludge from an on-farm ADR fed mainly with agricultural residues. The pilot scale coupled reactors were also inoculated with one of these inocula. ADRs were fed with dried sugar beet pulp. Biogas produced by the ADRs (or by the biogas plant for the pilot study) was injected by sparging in the BMRs, while H_2 was diffused using dense silicone tubing. Sludges samples were collected from each studied reactors to study physicochemical parameters such as *e.g.* pH and volatile fatty acid concentrations and the bacterial and archaeal community structure and diversity by 16S rRNA gene amplicon sequencing. Gas flows and composition were monitored online for each studied reactor.

Biogas could be upgraded in each BMRs with different success level. First results of the microbial ecology analysis indicate differences in terms of composition appearing between the ADRs and the BMRs and fluctuating over time. Analyses are currently in progress aiming to identify key involved microbial actors and if redundancies between the tested inocula or the laboratory *vs* pilot scale can be identified.

L47

Phage-driven microbial adjustments under different gas retention times in pilot-scale CO_2 biomethanation

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Keywords: biomethane, CO_2 biomethanation, anaerobic digestion, microbial community, bacteriophage
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Carbon capture and utilization (CCU) increasingly employs CO_2 biomethanation to convert hydrogen (H_2) and carbon dioxide (CO_2) into methane (CH_4). This study investigated a pilot-scale trickle-bed reactor (TBR) at 55°C, reducing gas retention time (GRT) from four hours to 50 minutes while maintaining CH_4 purity above 95%—until a mechanical malfunction compromised the trickling pump. Genome-centric metagenomics, metatranscriptomics, and metabolic modeling revealed a largely stable microbial consortium dominated by *Methanothermobacter thermautotrophicus*, alongside 503 uncultivated viral genomes. A "phage induction event" seemed to coincide with a surge in viral read depth at a one-hour GRT, correlating with the loss of specific microbial taxa and reduced CH_4 yield. Bioaugmentation with fresh methanogenic culture restored performance temporarily. Metabolic models indicate that cell lysis, triggered by phage activity, released nutrients that reshaped community dynamics. Single-nucleotide variant (SNV) analysis pointed to selective pressures on quorum-sensing mechanisms, defense genes, and hydrogenases critical for methanogenesis under reduced GRT. These findings highlight the intricate role of viruses, microbial adaptation, and mechanical stability in optimizing CO_2 biomethanation. By detailing phage-induced shifts and microbial resilience, this work provides insights for refining strategies to boost renewable energy production from carbon-based feedstocks.

POSTER PRESENTATIONS

P01

Syntrophies are steering the anaerobic microcosm

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Anaerobic digestion (AD) is carried out by well-structure microbial communities and has great potential for upcycling organic wastes and industrial/agricultural residual resources to achieve circular bioeconomy. AD efficiency and stability depend entirely on the concerted and syntrophic activity of the microorganisms. Pure culture fermentations are not suitable for complex substrates such as waste, which therefore require mixed microbial cultures. However, the inocula used for biological processes utilizing waste as a substrate are self-established random cultures. A deeper understanding of the microbial interactions of the anaerobic microbiome is essential for valorization and remediation of biowastes.

The overall aim of the ANAEROB ERC is to understand how to create "tailored microbial consortia" for specific bioengineering processes based on the genetic information of anaerobic microorganisms. The case of methane production was chosen here. The current objective of this study is, first, to identify and enrich anaerobic bacteria having a syntrophic relationship with our methanogens of interest in a mixed culture. Our hypothesis is that thanks to genomic information, we can select and enrich specific microorganisms in a mixed community.

Using enriched mixed cultures for which metagenome-assembled genomes (MAGs) were reconstructed, we first identified the main actors of methanogenesis, which are members of the genus *Methanosarcina*. Auxotrophies for carbon sources, amino acids and vitamins biosynthesis were determined for these MAGs. Three MAGs presumed to best complement the auxotrophies of *Methanosarcina* were selected based on their genomes. These MAGs belonged to the taxa: *Negativicutes*, *Advenella* and *Eubacteriales*. As these MAGs were too low in abundance to be isolated, incubations were conducted to enrich them. Based on their auxotrophies, the necessary amino acids and vitamins were added, as well as two carbon sources which they were able to use: butyrate or glucose. Methane production and substrate concentrations were monitored throughout the 3-month incubation period. The abundance of targeted MAGs was followed by qPCR.

Preliminary results show that of the nine combinations tested, three cultures show a clear syntrophic interaction with *Methanosarcina*, producing high amounts of methane and flocks specific to the archaea. While the main qPCR results will soon be obtained and will confirm or not the enrichment of the targeted MAGs, three incubations have already clearly enriched MAGs that benefit *Methanosarcina*.

The next step in this study will be to confirm the enrichment of the targeted MAGs and to isolate them using a single cell dispenser to try co-culture them with the isolated *Methanosarcina*.

P03

Improving anaerobic digestion of phenol wastewater under varying ammonia stress through magnetite addition and bioaugmentation

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Some industrial wastewaters from chemical, petrochemical, and pharmaceutical industries can contain high levels of phenol and ammonia, which severely hinder anaerobic digestion (AD) by inhibiting microbial activity and destabilizing process performance. Therefore, effective strategies are needed to overcome these challenges and ensure stable methane production from the AD of phenol- and ammonia-rich wastewaters. This study investigated the combined effects of magnetite supplementation and bioaugmentation on methane production and microbial community dynamics in continuous AD processes treating phenol-containing wastewater under increasing total ammonia nitrogen (TAN) concentrations (0.5–8 g/L). Two parallel reactors were operated for each treatment: control (C) and magnetite-supplemented (M). Magnetite particles (20 mmol/L) were continuously added to M reactors. Synthetic wastewater containing 10 g COD_{phenol}/L was fed every two days (OLR = 0.333 g COD/L/d), with a hydraulic retention time (HRT) of 30 days. In the C treatment, methane production was sustained up to 3 g TAN/L but failed at 4 g TAN/L, with phenol accumulation. In contrast, the M treatment maintained stable methane production up to 5 g TAN/L, with Syntrophus, Thermovirga, and Methanothrix as dominant taxa. Process inhibition occurred at 6–7 g TAN/L, which was successfully mitigated by bioaugmentation with magnetite supplementation using mixed liquor from the stable parallel reactor. Recovered reactors at 7 g TAN/L showed co-dominance of Syntrophus and Pelotomaculum, with Methanothrix supporting methanogenesis. However, all reactors failed at 8 g TAN/L, exhibiting reduced methane production and impaired phenol degradation. Overall, the results demonstrate that magnetite supplementation effectively enhances AD performance, process robustness, and microbial resilience under phenol- and ammonia-induced stress. Furthermore, bioaugmentation with acclimated AD sludge combined with magnetite particles was also validated as an effective strategy for recovering the failed digesters inhibited from such phenol- and ammonia-induced stress.

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P04

Functional convergence in anaerobic digestion communities driven by substrate and pH selection

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The anaerobic food chain describes the sequential degradation and utilization of biopolymers by a community of microorganisms in the absence of dioxygen. The anaerobic digestion process leverages these microbial degradation networks to reduce organic waste volume while producing biogas as a renewable energy source. Recent interest has emerged in applying anaerobic digestion to generate carboxylic acids as valuable precursors for repurposing organic waste. Previous research has characterized the various trophic layers and microbial metabolic groups involved in digestion, identifying key biological and operational factors that influence AD dynamics. However, designing a stable process that continuously produces targeted fatty acids with high productivity and purity by finely tuning the anaerobic digestion microbiota remains challenging.

To begin understanding the interactions among various factors and the dynamic feedback between microbial activity and the environment, we conducted enrichment experiments on microbiota sourced from three different anaerobic digestion reactors, which varied by feedstock, operational temperatures, and geographical locations. We utilized minimal media containing glucose, cellobiose, or no carbon substrates (as negative controls) across three different pH levels (5.5, 6.5, and 7.5). The production of metabolites, including short-chain carboxylic acids, H_2, CO_2, and CH_4, was monitored. Despite the differing inocula, we observed the functional convergence of the enrichment community based on substrate and pH conditions. Sequencing data is currently under analysis to further understand the microbial groups and functions present in the enrichments.

P05

The effect of pH on thermophilic anaerobic fermentation of polyhydroxybutyrate: solubilization, VFAs production and underlying anaerobic microbiology

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Polyhydroxybutyrate (PHB) is a biodegradable plastic with great potential to replace non-biodegradable plastics commonly used in single-use and packaging products. Anaerobic digestion (AD) could serve as a promising treatment solution for PHB wastes by enabling its degradation while generating biogas as a renewable energy source. However, the hydrolysis and acidogenesis of PHB are often the rate-limiting steps in AD, reducing its overall efficiency. Thermophilic anaerobic fermentation has potential as a biological pretreatment to enhance PHB degradation prior to AD, but its application has been rarely reported. This study investigated the effect of pH (4–7) on the thermophilic anaerobic fermentation of PHB. The results demonstrated that pH significantly influenced hydrolysis performance, with pH 7 providing the most favorable conditions. The highest soluble chemical oxygen demand (sCOD) reached 2.9 g/L at pH 7, while sCOD at pH 4 remained below 1 g/L throughout the batch experiment. Enzyme activity was also maximized at pH 7 (0.011 U/mL), approximately 21 times higher than at pH 4, indicating that neutral pH conditions could facilitate hydrolase production and accelerate polymer breakdown. Volatile fatty acids (VFAs) profiles further reflected pH-dependent metabolic shifts. Acetic acid, a key precursor for methanogenesis, was the dominant product at pH 6 and 7, whereas butyric acid was predominant at lower pH values, particularly at pH 5. Microbial community analysis revealed that Desulfovectis, Brucella, Alcaligenes, Acetomicrobium, and Fervidobacterium were selectively enriched at pH 6 and 7. Overall, thermophilic anaerobic fermentation at neutral pH (pH 7) was the most effective condition, highlighting its potential as a biological pretreatment strategy to enhance the subsequent AD of PHB.

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P06

Microbial communities for anaerobic digestion of phenolic compounds

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Phenolic wastewater is can be produced from petrochemical, pharmaceutical, and agro-industrial processes, and its improper discharge poses serious environmental risks due to the toxicity of phenolic compounds. Anaerobic digestion (AD) offers a cost-effective and sustainable treatment option, with the added benefit of biogas production. However, the AD of phenolic wastewater remains challenging due to the inherent toxicity of phenolics and the thermodynamic limitations of their anaerobic degradation. To achieve stable AD of phenolic compounds, close syntrophic relationships between bacteria and archaea are required, enabling the cooperative degradation of phenols and subsequent methane production. Therefore, understanding the microbial communities involved in the AD of phenol is essential to optimize AD performance and develop strategies to overcome process limitations. In this study, 16S rRNA gene-based metagenomic sequencing was conducted to analyze the microbial communities involved in the anaerobic digestion of various phenolic compounds. The dominant microbial taxa and their potential functional roles were identified, providing insights into the community characteristics and response patterns associated with phenol degradation under anaerobic conditions. These findings may contribute to a better understanding of the microbial ecology in phenols-degrading AD systems and offer valuable information for improving the stability and efficiency of AD processes for phenolic wastewater.

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P07

Microbial community adaptation to marine aquaculture sludge in biogas reactors

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As the world's largest producer of Atlantic salmon, Norway produces approximately 1.5 million tons salmon per year. Marine aquaculture sludge (feces and excess feed) represents a substantial sidestream from the aquaculture sector, which has traditionally been released at sea as most salmon production operates in open cages in the fjords. In 2022, 12 000 tons of phosphorous and 70 000 tons of nitrogen were released from salmon production in Norway, thus contributing to eutrophication of the sea environment and while also representing substantial nutrients loss. While anaerobic digestion of freshwater fish sludge has been studied to some extent, marine aquaculture sludge (MAS) as a potential substrate for biogas production remains largely underexplored. High saline and high sulfate substrates, such as MAS, are known to have pervasive effects on microbial community composition and can be inhibitory to methanogenic archaea. Continued advances in meta-omic approaches are increasing our understanding of microbial communities, key microbial divers and their metabolic mechanisms in a given environment. We explored the impact of MAS on continuous biogas process performance and microbial dynamics during gradually increased loading of MAS feedstock and finally through reducing hydraulic retention time (HRT).

The anaerobic digestion experiments were conducted in lab-scale continuous biogas reactors, and the amount of MAS, blended with cow manure, was increased from 20% volume to 100%, at a HRT of 28d. At 100% MAS loading, organic loading rate was further increased, by reducing HTR, from 28 to 12d. The process functioned successfully during the whole experimental phase (400 days), with maximum salt concentration reaching 25 g/L (12 g/L Na^+), substantially higher than reported inhibiting levels at ~8 g/L Na^+. Biogas production increased when MAS was increased, and specific biogas production at 25 and 12 d HRT remained relatively constant, at ~450 mL biogas/g tCOD. Initial microbial community analysis revealed a decrease in microbial species diversity with increased MAS load, yet no MAS-specific species emerged as key drivers in the recovered microbial community. Interestingly, the most abundant methanogenic archaea were associated with acetoclastic and halotolerant *Methanothrix* and *Methanosarcina*. Through 16S rRNA gene sequencing and metaproteomics, our study aims to provide further insight into the microbial populations and key metabolic pathways when subjected to high salinity and reduced HRT. Our study explores key microbial mechanisms during anaerobic digestion and biogas production from the novel MAS substrate, paving way for sustainable energy solutions, linking marine byproducts to renewable energy generation.

P08

Molecular monitoring of phytopathogens and the biogas microbiome during the co-digestion of reed canary grass and potato pulp under meso- and thermophilic conditions

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Co-digestion of potato pulp with reed canary grass (*Phalaris arundinacea*) for biogas production is a promising strategy for regions with high potato and low livestock production. In these regions, potato pulp cannot be used as animal feed. However, as potato pulp is sometimes not sanitized during starch production, it is important to assess the potential phytopathogenic risk. If phytopathogens, particularly those affecting potatoes, survive the fermentation process, they could spread through the fermentation residue, endangering local potato cultivation.

This project thus aimed to identify the pathogen spectrum in potato pulp and compare the thermophilic and mesophilic co-digestion of both substrates in a semi-continuous flow-through experiment.

Samples for molecular analyses were collected from potato pulp, potato pulp silage, and fermentation sludge. The potato pulp and silage were examined for phytopathogenic prokaryotes and eukaryotes using nanopore sequencing of the 16S (V1-V9) and 18S genes. The fermentation sludge samples were analyzed for bacterial (16S, V6-V8) and methanogenic archaeal (*mcr*A) presence and activity, with microbial composition assessed through sequencing. Overall, microbiological process monitoring was performed during mesophilic and thermophilic fermentation.

The potato pulp samples analyzed showed no phytopathogenic bacteria and only a very low presence of eukaryotic pathogens. In addition, examination of the ensiled potato pulp indicated that ensiling had a sanitizing effect, eliminating pathogens present in the untreated pulp. Overall, these results suggest that potato pulp is safe for biogas production from a phytosanitary perspective.

In the thermophilic and mesophilic bioreactors, the quantity and activity of methanogenic archaea were assessed. While the number of methanogens was lower under thermophilic conditions, their activity was higher. Switching substrates affected methanogen activity, with reed canary grass increasing both activity and specific biogas yield.

16S and *mcrA* sequence analyses revealed significant differences in the microbiome between thermophilic and mesophilic conditions. Thermophilic conditions were dominated by temperature-adapted methanogens (Ca. *Methanoculleus thermohydrogenotrophicum* and *Methanothermobacter wolfeii)*, while mesophilic conditions exhibited greater methanogen diversity. A similar trend was observed at the bacterial level. Non-metric multidimensional scaling analyses confirmed the differences in microbial composition over time and across various substrate compositions

Molecular biological investigations thus confirmed stable operation in both mesophilic and thermophilic ranges, demonstrating the successful use of reed canary grass and potato pulp as biogas substrates for energy production.

P09

Impact of Seasonal and Substrate Variability on Anaerobic Digestion of Sugar Beet Molasses in Single- and Two-Stage Systems

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Anaerobic digestion (AD) is a key technology for sustainably managing organic waste and producing biogas. Molasses, a sugar-refining by-product, is a promising AD substrate due to its high organic load and readily available fermentable sugars. While one-stage AD is widely used at biogas plants, two-stage AD, which separates acidogenic and methanogenic phases, is being researched for improved efficiency. Moreover, variations in molasses composition, stemming from environmental and processing factors, significantly affect microbial performance and biogas yield.

The aim of this work is to present an alternative method for managing sugar beet molasses at the place of its production - the sugar factory - for energy purposes in an innovative installation for the production of gaseous biofuels, biohydrogen and biomethane, as well as biofertilizer in the form of the final digestate. It also includes the verification of this concept through the use of molasses from successive sugar production campaigns.

Sugar beet molasses (20 g/L) was used as the substrate. In the one-stage anaerobic digestion (AD) system, all steps occurred in a 15 L upflow anaerobic sludge blanket (UASB) reactor. The two-stage system involved initial fermentation in a 3,5 L packed-bed reactor (PBR) producing biohydrogen and C2–C6 acids, followed by methanization in a 15 L UASB reactor. Microbial communities were selected from the IBB laboratory. Cultures were operated continuously, with monitoring of key parameters (e.g., pH, chemical oxygen demand). Elemental and organic composition of substrates and digestates were analysed.

Cultures using 2023 molasses ran for 198 days, and 2024 molasses has been running for 107 days so far. Results show that 2023 molasses was better for the AD process. Average biomethane amounts per 24 hours dropped from $5{,}84 \pm 2{,}00$ dm³CH_4 to $3{,}48 \pm 1{,}36$ dm³CH_4 in one-stage AD, and from $7{,}80 \pm 2{,}33$ to $5{,}05 \pm 1{,}76$ dm³CH_4 in two-stage. Biohydrogen production similarly declined. Process stability decreased, especially in hydrogen-producing cultures, with average pH dropping from $4{,}88 \pm 0{,}67$ to $4{,}28 \pm 1{,}39$. Energy recovery fell from $6{,}38 \pm 1{,}14$ kWh to $1{,}11 \pm 0{,}28$ kWh in one-stage and from $8{,}04 \pm 3{,}71$ kWh to $2{,}74 \pm 1{,}31$ kWh in two-stage system. Despite reduced gas yields, 2024 molasses-fed cultures showed higher substrate utilization, especially at 720 h HRT: 93% in two-stage AD (vs. 80%) and 92% in one-stage (vs. 83%). Overall, the two-stage system demonstrated greater resilience, maintaining better stability and higher utilization rates, ultimately supporting more efficient biogas production.

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P10

Illuminating *Limnochordia* Taxa in Biogas Microbiomes Through Genomic and Proteomic Lens

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The transition to renewable energy sources is a key aspect of global energy policy and microbial anaerobic digestion (AD) of biomass to produce methane is a critical component. This process uses microbiome management strategies to enhance biomass fermentation and boost methane production. A comprehensive approach integrating genome-centric metagenomics with transcriptomics and proteomics is essential to identify key microbial species that are both competitive and resilient under diverse and challenging operating conditions. This study focuses on the application of metagenome assembly and binning techniques to derive metagenomically assembled genomes (MAGs) from biogas microbiomes.

Specifically, our research investigated AD-microbiomes of more than 300 publicly available biogas studies through MAG-centric metagenomic analysis. This approach enabled the metabolic reconstruction of microbial genomes, highlighting their roles and niches within the biogas fermentation process. Particular emphasis was placed on MAGs belonging to the class *Limnochordia*, which previously were shown to be resilient and abundant in different digesters, regardless of the process conditions. The metabolic versatility of *Limnochordia*, particularly in fermenting carbohydrates and amino acids, suggests their potential to enhance the robustness of the biogas process.
The main scientific objectives of this study were (i) to differentiate obtained *Limnochordia* MAGs based on phylogenetic analysis, (ii) determine the prevalence and abundance of *Limnochordia* taxa across different biogas process conditions, (iii) assign distinct metabolic functions to specific *Limnochordia* clades, (iv) compare protein expression profiles of *Limnochordia* MAGs under varying operational conditions, and (v) predict microbiome members that engage in synergistic associations with *Limnochordia* species. Preliminary results indicate that certain *Limnochordia* members, owing to their competitive and robust nature under fluctuating conditions, are promising candidates for developing inocula aimed at refining biogas production processes. This increased focus on *Limnochordia* underscores the need for their detailed genomic and metabolic characterization to implement effective microbiome management strategies in biogas systems.

P11

Biogas potential of acid whey residues and microbiome dynamics during anaerobic digestion

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The substantial growth of the cheese industry, driven by increased dairy production, has resulted in the generation of large volumes of acid whey as a by-product. Acid whey, a liquid effluent produced during the manufacture of fermented dairy products, poses significant environmental challenges due to its high organic content and low pH. However, it also represents a valuable resource for the production of renewable energy and bio-based products. In recent years, advanced biotechnological approaches have increasingly been applied to extract bioactive proteins from acid whey, thereby reducing waste while creating functional food ingredients. Even after protein extraction, acid whey residuals contain considerable organic matter, making them a promising substrate for anaerobic digestion and biogas production.

This study aimed to evaluate the biomethane potential (BMP) of acid whey obtained from fresh curd cheese production at Celeia Dairy (Slovenia), following partial protein removal via CIM® monolithic chromatography and subsequent ultrafiltration of the residual whey. BMP assays were conducted in 1,5 L bioreactors with a working volume of 1 L under mesophilic conditions (38 °C) for up to 93 days. The inoculum concentration was 4 g TVS/L, with acid whey loaded at 0,5 g $COD_{\_whey}$ per g $TVS_{\_inoculum}$. Permeate was added at concentrations of 0,3 and 0,5 g $COD_{\_permeate}$ per g $TVS_{inoculum}$. Substrates were fed into the bioreactors at three-day intervals.

Throughout the experiment, biogas production, pH, and short-chain fatty acids (SCFAs) concentrations were monitored, while biogas composition was analysed using gas chromatography. To track microbiome dynamics during the fed-batch experiment, samples were collected at four time points (days 0, 27, 57, and 93) and DNA sequenced on the Illumina MiSeq platform using standard V3–V4 primers.

The sequential addition of acid whey increased BMP to 10,5 L CH_4/g VS over 93 days. In contrast, methane yield was 18,7 % lower when using the higher concentration of added permeate. The lower concentration of permeate resulted in 20 % greater methane production, indicating that substrate loading plays a critical role in process efficiency. Neither acid whey nor permeate significantly affected the biogas composition or SCFA levels, and no inhibition of methanogenesis was observed. Changes in both bacterial and archaeal part of microbiome were detected throughout the fed-batch experiment.

These results demonstrate the strong potential of acid whey and its residuals after extraction of bioactive proteins as feedstocks for biogas production, contributing to the development of a sustainable circular economy within the dairy industry.

P12

Development of microbial consortia for biohydrogen and biomethane production from complex by-products

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This study explores the development and optimization of microbial consortia to convert "hard-to-degrade" organic by-products, such as Organic Fraction of Municipal Solid Waste (OFMSW), into renewable energy carriers - specifically biohydrogen (via Dark Fermentation) and biomethane (via Anaerobic Digestion). Through next-generation sequencing (NGS) methods, the microbial composition of the consortium was profiled, revealing high biodiversity, and, consequently a strong adaptive potential to different substrates and conditions. Selective enrichment experiments using milk and starch as carbon source were performed to promote the selection of distinct microbial communities. Pre-activation strategies, such as thermal shock, low pH and chemical inhibitors were applied to suppress methanogens and other competing microorganisms, while favouring hydrogen-producing taxa such as Clostridium. Initial trials showed promising hydrogen yields. Once optimal conditions were selected, fed-batch cultures were established to assess community stability over time. Samples were periodically collected and sequenced, revealing a gradual simplification of the microbial community, with dominance of hydrogen-producing bacteria, confirming the effectiveness of the selection process. Starting from these enriched hydrogen-producing consortia, adaptation trials were initiated by progressively substituting the conventional growth substrates with OFMSW.

A complementary work focuses on developing a methanogenic consortium tolerant to high ammonia levels, one of the major inhibitory factors in anaerobic digestion. The microbial consortium, once adapted to OFMSW as growth substrate, was subjected to a stepwise increase of ammonium salt. Over six months, the system successfully adapted to ammonia levels seven times higher than the baseline concentration in untreated OFMSW, while maintaining stable methane production.

Overall, this multidisciplinary approach, integrating molecular biology and environmental biotechnology, demonstrates the potential of developing tailored microbial consortia for transforming complex organic wastes into sustainable energy. Further efforts will focus on evaluating the performance of these enriched consortia in comparison to conventional and pre- adapted inocula for biohydrogen and biomethane production from OFMSW. Additionally, strategies for inoculum preservation and scale-up will be explored, for pilot application of these communities.

P13

Optimizing coagulant combinations and dosages for sustainable sludge treatment: trade-offs between sludge settling and anaerobic digestion

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Effective sludge management remains a critical challenge in wastewater treatment, particularly under increasing demands for sustainability and operational cost reduction. Chemical coagulation is commonly applied to enhance sludge settling, reduce sludge volume, and improve solid–liquid separation. These improvements can benefit subsequent anaerobic digestion (AD) by increasing organic concentration, thereby minimizing hydraulic loading and facilitating enhanced biodegradability and biogas production. However, excessive or inappropriate use of coagulants may adversely affect methane production by inhibiting key anaerobic microorganisms through residual metal ions, densified floc structures, or osmotic stress, posing a trade-off between settling efficiency and AD performance. This study aimed to optimize coagulant combinations and dosages to improve sludge settling while minimizing adverse impacts on AD performance. Additionally, 16S metagenomic sequencing was conducted to elucidate the underlying anaerobic microbial community dynamics. Various polymeric coagulants, including polyaluminum chloride (PAC), polyferric sulfate (PFS), and polyacrylamide (PAM), were evaluated in jar tests and AD tests using primary sludge (PS) and waste activated sludge (WAS). Results showed that PAC and PFS effectively improved supernatant quality in PS, achieving over 90% removal of turbidity and total suspended solids at 100 mg/L. PAM exhibited the highest performance in improving sludge settleability, reducing SVI by up to 60% in WAS, although higher doses negatively affected supernatant quality. AD tests revealed that PFS caused the strongest inhibition of methane production (up to 59% reduction in PS), while PAC exhibited moderate inhibition, and PAM showed dose-dependent effects, primarily at higher concentrations. Response surface methodology identified the optimal coagulant combination as PAM and PAC (each at 100 mg/L) without PFS, achieving highest sludge settling and supernatant quality with minimal inhibition of methane production. Microbial analysis further revealed distinct shifts in community structure depending on the coagulant treatment. This study provides practical insights into coagulant dosing strategies that optimize sludge settling while maintaining higher AD performance, supporting improved sludge management and biogas production in wastewater treatment processes.

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P14

Temp4AD – A high-resolution temperature assay to fire up anaerobic digestion

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Anaerobic digestion (AD) is a promising technology for biogas production, providing a more stable energy output compared to other renewables such as wind and solar power. As such, it represents a vital energy source to mitigate greenhouse gas emissions and meet global climate goals. However, biogas plants face a number of challenges, including rising biomass costs, fluctuating feed-in tariffs, and legal restrictions on digestate application, underscoring the need to enhance their competitiveness by maximizing methane yields, reducing operation costs and minimizing digestate production. A critical factor in regulating the AD process is fermentation temperature, which can be optimized to improve methane yields and process efficiency. However, most studies dealing with the effect of temperature on AD focused on classical mesophilic (30-40°C) and thermophilic AD (50-60°C), while the range in between was largely neglected.

The goal of the project Temp4AD is to meticulously assess the effects of temperature on a fed-batch lab-scale AD system across a broad temperature range (34°C to 61°C) for three different inocula from large-scale digestion plants operated at different temperatures. Three main experiments will illuminate various aspects of temperature impact on AD. First, the influence of temperature will be assessed by operating them at different temperatures in 3°C steps. Next, the robustness of AD systems against unstable or extreme organic loading rates will be assessed for the 5 most promising temperatures, and finally, the resilience of AD systems to temperature fluctuations will be studied to account for real-world conditions where precise temperature control is challenging. Reactor performance within all experiments will be evaluated by monitoring biogas quantity and quality, measuring crucial physico-chemical parameters and hygienization potentials, and investigating microbial community structures via intracellular DNA-based amplicon sequencing, ddPCR and meta-proteomics.

We hypothesize that the temperature yielding highest methane yields and process stabilities lies between 40°C and 50°C, and that different inocula will exhibit varying temperature optima with higher resilience to shocks in organic loading rates and temperature fluctuations.

By providing insights into the optimal temperature strategies for AD, the project will contribute to the establishment of AD as a central pillar in future renewable energy systems, thereby supporting the transition to a post-fossil era.

P18

Hydrogenotrophic Methanation in a Plug Flow Reactor

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To counteract the climate crisis, new technologies are needed to defossilize and create carbon cycles. Therefore, sustainable fuel and chemical production through gas fermentation is gaining attention, particularly when using hydrogen and carbon dioxide as renewable substrates. One such Power-to- Gas technology is hydrogenotrophic methanogenesis, a microbiological process carried out by various archaea. This process requires CO2, which could be utilized from defossilization by using carbon dioxide, e.g. from carbon capture. Additionally, methane formation is driven by hydrogen, which can be produced by electrolysis. If the entire process can be operated flexibly, the system could also be used to regulate the electricity grid. The process product methane has many applications, ranging from energy storage to the production of chemical products.

Challenges remaining for hydrogenotrophic methanogenesis include the efficient mass transfer of poorly soluble gases, achieving high methane production rates, and ensuring the process is flexible. In our poster, we present a novel 20-liter Plug Flow Reactor designed to enhance gas-liquid mass transfer and enable flexible operating modes suitable for fluctuating substrate availability. The process operates at 55°C. The system integrates a continuous liquid circulation loop, a gas dispersion unit, and a tubular reaction zone that facilitates mass transfer. Temperature control, nutrient dosing, and process monitoring are automated to ensure stable and reproducible operation.

Initial operation of the reactor demonstrated a stable methane production rate of up to 2 L/(L*d) after 48 d and efficient conversion of hydrogen and carbon dioxide into methane under laboratory conditions. We will present results from long-term and flexible operation. Flexible operation could increase the economic efficiency of the process, making it a promising candidate for integration into future decentralized, renewable, energy-based organic chemistry processes and energy storage systems.

P19

Integrating anaerobic fermentation with urban mining: a novel indirect bioleaching strategy for metal extraction from EoL mobile phone PCBs

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The rapid digital transformation has intensified the production of Electrical and Electronic Equipment, leading to growing volumes of end-of-life (EoL) devices. Among these, mobile phones represent a significant portion of electronic waste (e-waste) containing hazardous elements that require controlled disposal, but also valuable quantities of Critical Raw Materials, rare earth elements, and base metals such as copper (Cu), iron (Fe), nickel (Ni), zinc (Zn) and tin (Sn), often at concentrations higher than primary ores. As mining costs and environmental concerns grow, e-waste recycling offers a viable and necessary solution. Bio-hydrometallurgy, a biotechnology involving microorganisms or their metabolites, is emerging as promising alternative among energy intensive and resource demanding technology such as pyrometallurgy and hydrometallurgy.

This study explores the application of anaerobic fermentation effluents as bioleaching agents to recover metals from printed circuit boards (PCBs) of EoL mobile phones. The effluents, produced during the anaerobic digestion of a mixed matrix of municipal organic waste and dairy wastewater, are rich in low-molecular-weight organic acids, particularly lactate, acetate, and butyrate. Our approach integrates two key steps:1) processing industrial effluents through anaerobic fermentation to produce energy as biomethane, 2) valorisation of fermentation effluents rich in biogenic organic acids for bioleaching applications. In this system, fermentation effluents replace synthetic chemicals and are used to extract metals from shredded and pulverized smartphone PCBs.

After manual dismantling of mobile phones, PCBs were shredded and analysed via Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), X-ray Diffraction (XRD), and Fourier-Transform Infrared Spectroscopy (FTIR). Effluents were characterized by High-Performance Liquid Chromatography (HPLC). Bioleaching experiments were performed under laboratory conditions using a 10% (w/v) pulp density, at 60 °C and 200 rpm, without the presence of living cells (indirect bioleaching).

Within five hours, metal analysis show that our process successfully extracted Cu, Fe, Ni, Sn and Zn, with recovery efficiencies ranging from 30% to 90%, depending on the elements and organic acids composition.

This study highlights the potential of industrial waste-derived microbial consortia and waste streams as sustainable bioleaching agents, aligning with circular economy principles. Future research will explore process optimization to enhance organic acids production and scalability for broader e-waste recycling.

P20

Deciphering the Anaerobic Microbiome for Constructing Robust Microbial Consortia to Produce Medium-Chain Fatty Acids

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Anaerobic fermentation represents a pivotal sector in biotechnology, contributing significantly to the green transition. Utilizing anaerobic microorganisms is particularly advantageous due to their ability to produce secondary metabolites in substantial quantities, which are extensively employed in industrial applications. An effective approach for producing high-value chemicals, such as medium-chain fatty acids, through microbial chain elongation is the utilization of mixed-species cultures, comprising microbial species that engage in synergistic interactions. These mixed cultures exhibit superior adaptability to environments characterized by extreme thermodynamic conditions.

Microbial chain elongation is a process where an electron donor, like ethanol, elongates the carboxylic chain of a short-chain fatty acid, such as acetic acid, to form medium-chain fatty acids. Medium-chain fatty acids are important chemicals with numerous applications. Currently, they are derived through the catalytic conversion of fatty acids. These fatty acids are mainly produced from coconut or palm oil, contributing to the devastating effects of tropical deforestation.

This project aims to develop a comprehensive model of the metabolic reactions occurring within mixed-species cultures of anaerobic microorganisms, specifically focusing on taxa involved in microbial chain elongation.

This research will involve the development of a laboratory protocol for the microbial chain elongation of short-chain fatty acids, investigation of microbial consortia interactions, and the generation of community genome-scale metabolic models. The objective is to establish synthetic mixed microbial cultures capable of efficiently producing medium-chain fatty acids. This work will advance the field of microbial community dynamics and contribute to the development of sustainable high-value chemicals.

P21

Development of a Cascading Biorefinery Concept for Medium-Chain Fatty Acid Production from Sugar Beet Pulp

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Achieving net-zero CO_2 emissions requires innovative strategies that valorise existing resources more effectively. Agricultural by-products, particularly from the sugar industry, offer untapped potential for generating high-value products. This research proposes a cascading biorefinery approach using sugar beet pulp as a feedstock to produce medium-chain fatty acids (MCFA) – in particular caproic and caprylic acids – as local and sustainable alternatives to products currently derived from tropical oils such as palm and coconut.

Germany, as the largest sugar producer in the EU, generates substantial volumes of sugar beet pulp (~ 2.78 Mt/year) and molasses (~ 0.88 Mt/year) as co-products. Currently, sugar beet pulp is primarily used as animal feed, while future strategies outlined in the German sugar industry's roadmap aim to valorise it for energy production via biomethane or solid biofuels. However, these applications represent low-value endpoints and do not fully exploit the resource's potential. Our proposed cascade utilization framework seeks to increase value creation through microbial fermentation for MCFA production, followed by nutrient recovery and, finally, energy generation.

Chain elongation using naturally enriched microbial consortia enables the conversion of sugar beet-derived short-chain fatty acids into MCFA. Electron donors such as ethanol and lactate are formed naturally during the fermentation process, eliminating the need for external supplementation. A key challenge in this process is product inhibition caused by the accumulation of MCFA, which can negatively affect microbial activity and limit yields. To address this, an in-line extraction and purification strategy will be implemented to enhance product recovery and potentially improve process stability. The effects of this approach on microbial activity, product yields, and overall process dynamics will be systematically investigated. The study will also examine how different bases used for pH regulation affect microbial activity, product yields, and the stability of the chain elongation process. Local MCFA production from sugar beet pulp could significantly reduce the environmental impacts associated with tropical oil-based routes, including deforestation, biodiversity loss, and long-distance transport emissions.

The research is structured into four work packages: (1) investigation of fermentation yields and parameters for MCFA production from sugar beet pulp at lab and pilot scale; (2) development of an in-line product recovery method to improve fermentation efficiency and stability; (3) analysis of extract impurities and identification of contaminants or valuable by-products; and (4) conversion of the post-extraction fermentation sludge, in combination with phosphorus-rich sludge from the Bio-P process in wastewater treatment, into a high-value recycled fertilizer, with final energy recovery via biogas. The project aims to close carbon and nutrient loops within a regional circular bioeconomy, offering a scalable and sustainable model for industrial transformation in the sugar sector.

P23

Applying recombinant enzymes from anaerobic fungi for improved biogas production from lignocellulosic biomass

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Anaerobic Fungi (Neocallimastigomycota) are ubiquitous in the digestive tracts of herbivores, where they aid the animal in digesting plant biomass. This ability has made them a topic of interest for the pretreatment of lignocellulosic biomass that is hard to degrade in anaerobic digestion and usually requires extended retention times. Apart from the mechanical degradation of plant biomass via their filamentous structures, anaerobic fungi have a vast array of carbohydrate active enzymes at their disposal. Most of these enzymes are not yet characterized, making these fungi a potential source of novel, more efficient enzymes.

We selected 30 enzyme sequences from existing genome data according to selection criteria that make them promising candidates for simple lignocellulosic biomass pretreatment cocktails. These enzymes were then recombinantly expressed in *Escherichia coli* BL21 (DE3) using the pET vector, a well-established and robust expression system. Without further optimization, ten of the selected enzymes were expressed in a soluble state and displayed their bioinformatically predicted activity.

In a next step, we will apply a mixture of selected crude cell extracts to wheat straw. This pretreatment is expected to improve biogas yield in batch tests, and findings of this proof of concept study will be discussed.

In case of significant improvement of biomethanation, the simple and synergistic crude extract cocktail of recombinant anaerobic fungal enzymes may act as a promising alternative to commercially available enzyme mixes. Optimization strategies of the recombinant expression system are also a focus of this study and may further help to decrease product cost.

P25

Exploring the Enzymatic Potential of Anaerobic Gut Fungi (Neocallimastigomycota) - A Tailored DNS Assay

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Anaerobic gut fungi (AGF) are a unique fungal group with a vast repertoire of lignocellulolytic enzymes, making them valuable candidates for biotechnological applications, in the context of anoxic degradation of lignocellulose. A key challenge for advancing their application is understanding their enzymatic capabilities when grown on different substrates, which can be assessed using the 3,5-dinitrosalicylic acid (DNS) assay. This method detects reducing sugars by reaction of DNS with the free carbonyl (C=O) groups and photometric measurement of the product.

While the handling of the DNS assay is straightforward and does not require specialized equipment, it is still error-prone due to chemical interferences from other sugars. Additionally, variations in the buffer systems and incubation times throughout the literature lead to varying results and a lack of comparability. Despite these limitations, we were able to establish a well-adapted protocol for DNS assays and characterized the enzymatic potential of several AGF strains.

The data presented include results from three strains: *Anaeromyces mucronatus* (filamentous, polycentric), *Caecomyces communis* (bulbous, monocentric), and a newly isolated, novel AGF strain SD2E (filamentous, monocentric) from degu feces (*Octodon degus*). These strains were tested for xylanase, avicelase, and carboxymethyl cellulase activity. Xylanase and carboxymethyl cellulase activities were detectable and varied considerably among strains, but avicelase was below the detection limit for all tested strains.

Further analyses of AGF are necessary to fully understand their enzymatic diversity and -activity. Nevertheless, the DNS assay served as valuable tool for quick screening of strains for biotechnologically relevant enzyme potentials.

P26

Isolation and characterization of cellulolytic microbes from the intestinal tract of the Eurasian Beaver (*Castor fiber*)

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Effectiveness of biogas production can be enhanced by improving digestion of woody lignocellulose. These substrates are the natural food sources for the Eurasion beaver (*Castor fiber*). Therefore, the approach is to isolate, characterize and identify microorganisms and enzymes in the beaver's digestive system that are responsible for the degradation of these materials in the gut. The bacterial community composition in different gut compartments of the beaver was analysed by Pratama et al., 2019[1] using 16S rRNA gene amplicon analysis. The predominant cellulolytic microbes in the digestive tract of these animals were identified as Firmicutes and Actinobacteria. These analyses solely identified the gut microbiome but the isolation and cultivation of these specific bacteria is still pending.

Therefore, in close collaboration with the beaver population management (GEDO) in the Oder (river) region, Germany and in accordance with statutory German and European regulations, intact digestive tracts from animals taken by authorized hunters were examined. Chymus samples from different gut compartments (stomach, small intestine, front cecum, back cecum, colon) have been characterized (pH, feed analysis). Subsequently, dilutions of direct samples were cultivated on selective agar media[2] to isolate enzyme-active microbes using anaerobic digester jars or aerobic cultures. The following carbon sources were chosen for the selection: cellulose, xylan, starch and pectin, with only one source per selection. A total of 73 isolated strains were picked and examined, focusing on facultative anaerobic microbes. After isolation the enzymatic activities of the strains were determined either qualitatively by substrate clearing zones around strain colonies or by selective staining. In total, ten strains showed at least three enzyme activities on selective agar media. Six strains were then selected for liquid cultivation to quantify the secreted enzyme activity (media composition: 10 g/L LB + 10 g/L carbon source, either xylan, starch, CM-cellulose or pectin). The highest enzyme activities measured were 118 U/L pectinase (strain A isolated from colon), 439 U/L xylanase (strain B isolated from colon) and 126 U/L cellulase as well as 460 U/L amylase (strain A isolated from back cecum). The next steps will involve strain identification by 16S rRNA amplicon analysis and initial experiments by adding a complete culture of one strain as well as a mixture of several strains to continuous biogas trials in laboratory fermenters and analysis of effects by rheology and methane production rates.

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P27

The dynamics of single stranded, positive sense RNA bacteriophages in the rumen virome

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While ruminal bacteria, archaea, protozoa, and, to a lesser extent, fungi have been extensively studied, ruminal viruses remain poorly characterized. Any role for such viruses in shaping or maintaining the rumen microbiome is sparsely documented. Emerging evidence, however, suggests the presence of a highly diverse rumen virome that may likely play a role in maintaining rumen homeostasis and host health and productivity. Obligate lytic bacteriophages, e.g. single-stranded RNA (ssRNA) phages of the class *Leviviricetes*, may be of particular interest due to their selective predation on Gram-negative bacteria, including symbionts, commensals, and pathogens present in high abundance in the rumen.

Here, we analysed 88 metatranscriptome samples collected from 12 high-yielding dairy cows to identify *Leviviricetes* phages residing in the rumen environment and to study their potential dynamics. Rumen grab samples were subjected to metatranscriptomics (~24 mill PE reads of 150 nucleotides in length per sample after removal of adapters and low-quality reads) were collected sequentially with intervals of a few hours and up to 1-4 weeks. In 8 cows, rumen samples were further separated into solid and liquid fractions to compare phage distribution.

We assembled and curated 52 *Leviviricetes* phage contigs (~ 3-5 kb) based on high protein homology to characteristic *Leviviricetes* proteins. Our findings show that these phages are enriched in the rumen liquid fraction, with abundance patterns varying across cows and time points, consistent with dynamic replication bursts and clearance cycles. Some phages persisted over 8 weeks, indicating they are part of a resident but fluctuating virome, while others appeared transient, likely requiring external reintroduction.

Together, these findings highlight active predator-prey dynamics between ssRNA bacteriophages and their bacterial hosts in the rumen, supporting a role for the virome in shaping the microbial ecosystem of dairy cows.

P28

EVALUATION OF DIFFERENT ARTIFICAL SALIVA FORMULATIONS AS BUFFERING MEDIA FOR *IN VITRO* RUMEN SIMULATION

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In vitro rumen fermentation is commonly modelled using batch systems that incorporate artificial saliva, an anaerobic, phosphate-bicarbonate buffered media, mixed with rumen fluid, and a selected diet. These cultures are incubated for up to 48 hours at 39 °C under continuous shaking to simulate *in vivo* conditions. The buffering agents play an essential role in stabilizing pH, which is critical for maintaining a rumen-like environment. Essential electrolytes and minerals found in natural ruminant saliva, such as sodium, potassium, magnesium, calcium, phosphate, bicarbonate and chloride are included in these artificial formulations to support microbial activity and overall system homeostasis. However, the composition of these artificial salivae varies across different research protocols and this variability may influence fermentation parameters and microbial dynamics.

To evaluate this, we utilized the Gas Endeavour bioreactor system to investigate five distinct artificial saliva recipes. We measured their impact on fermentation characteristics, including total gas output and specific gases (CO_2, H_2, CH_4), as well as pH stability, redox potential, production of short-chain fatty acids (SCFAs), and microbial community composition using 16S rRNA gene sequencing and beta diversity analysis. For comparison, fermentations were also carried out using the general-purpose rumen medium M2, which differs substantially in formulation.

Across all artificial saliva types tested, fermentation outcomes remained broadly consistent. There were no notable differences in gas or methane production, nor in the overall microbial composition, which remained similar to the initial inoculum over the incubation period. However, experiments conducted in M2 medium yielded significantly higher gas production and showed notable changes in the microbiome, which diverged from the original community structure.

These findings support the suitability of artificial saliva for rumen batch *in vitro* culture experiments, with different formulations yielding largely comparable results. While variations in recipe exist, their impact appears minimal relative to more divergent media such as M2. Establishing a standardized formulation for artificial saliva could enhance reproducibility and data comparability in rumen microbiology research.

P29

Effect of plant protection products on the metaproteome and metabolome of human gut microbiome

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The gut microbiota plays a crucial role in maintaining human health and a great impact on metabolic health. Growing evidence links microbiome dysbiosis to various diseases and metabolic conditions, including obesity, which has become a global health epidemic. Simultaneously, there is increasing concern over the effects of environmental contaminants, such as plant protection products (PPPs), on our ecosystems. While these agrochemicals are tested for their toxicity on life and environment, but their potential impact on the human gut microbiota is often overlooked. We aim to explore how gut microbiota from obese and lean phenotypes respond differently to exposure to plant protection product, using metaproteomics and metabolomics.

In this study, we examine the short-term effects of relevant PPPs on gut microbial communities derived from obese (BMI > 35) and lean (BMI < 25) male human donors. Faecal derived microbiota were cultured under anaerobic conditions for 24 hours in complex intestinal media and exposed to 6 concentrations of 10 common pesticides and herbicides. Following incubation, samples were analysed with liquid chromatography-tandem mass spectrometry to identify effects on the metaproteome, which will give insight on the taxonomic composition and the functional profile of the microbial community. Additionally, untargeted metabolomics as well as targeted quantification of amino acids and short-chain fatty acids (SCFAs) will be performed, as SCFAs are key indicators of microbiome metabolic activity, and altered SCFA profiles have been linked to various diseases, including obesity.

Together, these analyses are designed to capture both taxonomic-dependent functional shifts and metabolic profile in response to chemical exposure. These effects could give new insights on how phenotype-specific microbiomes respond to environmental stressors and if future assessments of agrochemicals should consider their effect on the human gut microbiome.

P30

Gut microbiome shifts as the results of the *Helicobacter pylori* antibiotic eradication

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Helicobacter pylori as a recognized factor for gastric cancer is already the subject of eradication programs in some countries. Eradication therapies based on a mixture of antibiotics effectively eliminate *H. pylori*, but at the same time lead to significant changes in the composition of not only the gastric but also the gut microbiome and the resistome. We enrolled 72 patients who were randomly assigned to two groups receiving three- or four-line antibiotic treatment (group 1: 14-day treatment with esomeprazole, amoxicillin and clarithromycin; group 2: 14-day treatment with esomeprazole, amoxicillin, metronidazole and bismuth colloidal subcitrate). Stool and blood samples were taken from the patients before administration of the antibiotics and two months and one year after eradication. Short-chain fatty acid content was determined in the fecal samples, and total microbial DNA was isolated and sequenced by amplicon 16S rRNA NGS sequencing and metagenomic shotgun sequencing for resistome analysis. Bioinformatic analysis of the sequences showed a significant decrease in alpha diversity indices two months after antibiotic administration, although no significant differences were observed between the three- and four-line antibiotic regimens used. After one year, the alpha diversity indices increased, but not quite to the level before antibiotic administration. The same was true for beta diversity. The composition of the microbiome was significantly altered two months after administration of the microbiome, and after one year it was still not the same as after the start of therapy. Using the DESeq 2 software tool, we were able to show that after two months of antibiotic administration, the number of sequences from the families *Lachnospiraceae* and *Bacteroidaceae* increased significantly, while the number of sequences from the unclassified order *Eubacteriales* decreased. The latter group remained at a lower level even after one year of antibiotic administration. We also analyzed changes in insulin resistance (HOMA-IR score), body mass index (BMI), serum levels of the hormones ghrelin and leptin as well as changes in the lipid profile and its subtypes (cardio-test INFA and NMR spectroscopy) and the level of the metabolite trimethylamine-N-oxide.

P31

Effect of hydrogen availability on the microbial community in anoxic marine sediments

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Hydrogen (H_2) is an important intermediate produced during anaerobic fermentation in marine sediments. As a key energy and electron donor in marine sediments, its concentration regulates the dominant terminal electron-accepting processes occurring in situ. Many microbes in sediments have the potential to utilize H_2 as an energy source. However, the competitive interactions between microbes and the influence of trace H_2 on microbial activity are still unknown.

To investigate autotrophic microorganisms involved in the utilization of H_2, we performed stable isotope probing on surface sediments of the Helgoland mud area. Active autotrophic members were labeled using ^{13}C-DIC. Hydrogen was supplied in headspace with varying concentrations from 5×10^4 ppmV to no extra H_2 addition. Our results show that *Sulfurimonas* and sulfate-reducing taxa such as *Desulfobacteraceae* and *Desulfoconvexum* were highly active with high H_2 concentrations (5×10^4 ppmV). At ~2800 ppmV and 100 ppmV, sulfate reducers were labelled to a lower degree, while *Sulfurimonas* remained active. At concentrations lower than 100 ppmV, the active microbial group shifted to *Gammaproteobacteria*, while *Sulfurimonas* became less active. In contrast, *Sedimenticolaceae* remained active under different H_2 concentration, including treatments without amendment of H_2.

These findings suggest that autotrophic sulfate reducers require sufficient H_2 concentrations, while *Sulfurimonas* acts as a strong H_2 competitor under limited H_2 conditions. Overall, H_2 availability plays a critical role in shaping the structure of the active microbial community, offering new insights into microbial interactions and energy flow in anoxic marine sediments.

P32

Hydrocarbon degradation potential of aquifer microbial communities at different electron acceptor conditions and temperatures

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The presence of hydrocarbons in the environment presents serious environmental and public health concerns, especially in areas impacted by industrial operations or oil spills, resulting in long-term accumulation of pollutants. Research in the last 45 years has shown that besides aerobic biodegradation, anaerobic hydrocarbon transformation and mineralization reactions are crucial natural processes in oxygen limited environments such as underground aquifers. However, in temperate zones, low groundwater temperatures may seriously limit hydrocarbon biodegradation. This study aims to enrich hydrocarbon-degrading microbial communities from hydrocarbon-contaminated aquifer sediments under different redox conditions and temperatures. Sediment microcosms amended with various aromatics (benzene, toluene, ethylbenzene, m-xylene, naphthalene) or aliphatics (C14 to C16 alkanes) were established and divided into four treatment groups: sulfate reducing, nitrate-reducing, methanogenic and aerobic conditions (supplemented with 10% oxygen). Incubations were carried out at three different temperatures (12°C, 28°C, and 50°C) to simulate psychrophilic, mesophilic and thermophilic conditions. The ongoing enrichment seeks to promote the growth of hydrocarbon-degrading microorganisms under the selected conditions. Future monitoring will assess mineralization of distinct hydrocarbons and associated shifts in microbial community composition to evaluate the potentials for biodegradation under the selected environmental settings and the associated key organisms.

P33

Distribution of N_2O-Reducing Bacteria with Urbanization Gradients and Their Emission Reduction Potential

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Nitrous oxide (N_2O), a potent greenhouse gas, plays a significant role in climate change and the depletion of stratospheric ozone. Although molecular biological studies indicate the widespread presence of N_2O-reducing microorganisms in the environment, the distribution patterns of their encoding genes (*nosZ* Ⅰ and *nosZ* Ⅱ) along urbanization gradients remain unclear, and the successful isolation and cultivation of N_2O-reducing strains are still limited. Additionally, the impact of inoculating N_2O-reducing strains on soil N_2O emissions and their colonization dynamics require further investigation. In this study, we focused on N_2O-reducing bacteria by first analyzing the composition and diversity of *nosZ* Ⅰ and *nosZ* Ⅱ-type N_2O-reducing bacteria in soils along the urbanization gradient in the eastern coastal region of China using high-throughput sequencing. We then enriched and isolated N_2O-reducing bacteria through microbial pure culture techniques and validated their functional capabilities. Finally, microcosm experiments were conducted to explore the effects of N_2O-reducing bacterial inoculation on soil N_2O emissions, with specific primers designed to track strain colonization and the interactions of synthetic microbial communities, aiming to regulate and optimize microbial community functions. Our results demonstrate that urbanization significantly influences the composition and diversity of N_2O-reducing bacteria, with the *nosZ* Ⅱ clade being more sensitive to environmental changes, and pH being a key factor shaping community composition. Three N_2O-reducing strains were isolated, among which A66 exhibited the highest N_2O reduction capacity, while A74 and A92 were identified as novel species of *Brachymonas* and *Pseudomonas*, respectively. These strains showed varying N_2O reduction capabilities under different conditions. Inoculation with synthetic N_2O-reducing bacterial communities enhanced adaptation to soil environments, improved microbial interactions, and significantly reduced N_2O emissions by influencing specific microbial abundances and soil physicochemical properties. These findings reveal the critical role of N_2O-reducing bacteria in soil N_2O metabolism and provide valuable insights for developing environmentally friendly and efficient nitrogen removal biotechnologies, contributing to ecological sustainability and global warming mitigation. Future research will focus on optimizing the isolation and functionality of N_2O-reducing bacteria, designing *nosZ* Ⅱ-specific primers to elucidate their ecological roles, screening high-efficiency strains, and enhancing colonization rates through genetic engineering, thereby supporting the development of efficient nitrogen removal technologies and global warming mitigation efforts.

P34

Unraveling chloromethane conversion in the acetogen *Acetobacterium dehalogenans*

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Chloromethane (CM) is an abundant environmental pollutant that contributes significantly to ozone depletion. *Acetobacterium dehalogenans* is one of the few isolated anaerobic bacteria shown to utilize CM as its carbon and energy source. The enzyme system responsible for its CM demethylation/dehalogenation has not been identified so far. We aim to identify and characterize this system and to understand the metabolic changes of *A. dehalogenans* during growth on CM versus the methoxylated aromatic syringate.

We analyzed the growth and substrate conversion of *A. dehalogenans* grown on CM, syringate and both substrates and used comparative transcriptomics to analyze the gene expression pattern of *A. dehalogenans* grown under these conditions. We identified a corrinoid-dependent methyltransferase system with three adjacent genes cdmABC which were highly upregulated during growth on CM in comparison to syringate. We heterologously produced and purified the corresponding proteins in E. coli, subsequently performed activity assays to determine their substrate specificity, and conducted X-ray crystallography.

We discovered that this Cdm methyltransferase system demethylates methyl halides such as chloromethane and iodomethane. X-ray crystallography of the CdmB methyltransferase revealed a hydrophobic internal channelling system for methyl halides. Phylogenetic analysis showed that homologous proteins are encoded by several other anaerobic bacteria, especially Bacillota. Such organisms may also have the potential to convert methyl halides such as CM. The Cdm system is most closely related to the archaeal Mto system, which converts methoxylated aromatic compounds.

In summary, we identified and characterized a novel enzyme system used for methyl halide demethylation in anaerobic microorganisms.

P35

Expression of novel reductive dehalogenases from a dichloroaniline-contaminated site

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Anaerobic microbes are essential agents of both spontaneous and anthropogenic bioremediation. Of particular interest are the organohalide-respiring bacteria, which use reductive dehalogenase enzymes (RdhAs) to generate energy from the cleavage of halogen atoms from organohalides in the environment. RdhAs are highly conserved redox enzymes with activity on a wide range of halogenated substrates. Though they are well represented in metagenome data, few RdhAs have been empirically linked to their substrates. The study of these enzymes is complicated by their oxygen sensitivity, cofactor requirements, and the delicate needs of their native organisms. Previous work has successfully expressed active RdhAs in *E. coli* using enhanced cobalamin uptake and iron-sulfur cluster synthesis pathways. However, heterologous expression has yet to produce RdhAs with activity on aromatic organohalide substrates. In 2015, sediment and groundwater samples were collected from a retired industrial site in Camacari, Brazil, which had been impacted by dichloroanilines, monochloroanilines, and dichloronitrobenzenes through the past manufacture of herbicides. Anaerobic enrichment cultures were developed from site materials amended with chlorinated aromatics including dichloroanilines and chloroanilines. These cultures are capable of reductive dechlorination completely to aniline. Proteomic analysis of the cultures identified five potential RdhAs from *Dehalobacter* sp. and *Desulfitobacterium* sp. The sequences were cloned into *E. coli* and expressed anaerobically in medium supplemented with cobalamin. Substrates were predicted based on microcosm activity and the known substrates of close homologues, where possible. Experimentation is still in progress, but preliminary data shows that the heterologously expressed *Dehalobacter* RdhA, RdhA-Cam1, can reduce perchloroethene (PCE) to trichloroethene (TCE), activity which is consistent with known members of its ortholog group, OG 102. Heterologous expression and substrate identification of novel RdhAs will help to clarify the connection between sequence homology and substrate specificity within this important group of enzymes, as well as paving the way for future work exploring the structural differences which influence substrate preference among RdhAs.

P36

Purification and Structural Insights into the Organohalide Respiratory Complex of *Dehalococcoides mccartyi* strain CBDB1

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Dehalococcoides mccartyi is a strictly anaerobic bacterium that conserves energy by reducing persistent halogenated hydrocarbons by a membrane-integrated, multi-subunit organohalide respiratory (OHR) complex (~340 kDa). The OHR complex contains seven protein subunits and operates independently of quinones, cytochromes or canonical proton pumps. The key enzyme in the OHR is the reductive dehalogenase, which catalyses the final electron transfer to the substrate. Due to low biomass yield, the oxygen sensitivity, the membrane integration of the complex, and the lack of heterologous expression systems, its structure has not been solved experimentally.

In this study, a combined computational and experimental strategy was applied to investigate the architecture of the native OHR complex. A reproducible purification protocol from wild-type cells was developed. Special emphasis was placed on a detergent exchange step during chromatography, in which digitonin was replaced by lauryl maltose neopentyl glycol (LMNG), to improve stability and homogeneity of the isolated complex. In addition, a computational *in-silico* model of the entire stand-alone OHR complex was developed using AlphaFold2.

Several detergent-based extraction strategies were tested and optimised using a CBDB1 culture grown in a continuous-flow bioreactor, which allowed high cell densities and high protein expression rates. Further, various chromatographic purification strategies as well as sucrose density ultrafiltration were applied, tested and combined. The resulting purification protocol, a combination of anion-exchange and size-exclusion chromatography reliably yielded native protein preparations featuring *in vitro* dehalogenation activity and reproducible CN-PAGE banding patterns. Two prominent bands consistently appeared at ~300 kDa and ~480 kDa, corresponding to putative OHR assemblies. These complexes retained *in vitro* dehalogenase activity after purification and contained all seven expected subunits, as confirmed by shotgun proteomics. This purification strategy has been successfully upscaled and preliminary cryogenic electron microscopy results have now been obtained from the first vitrified samples. In parallel, AlphaFold2-based modelling was used and combined with experimental findings to develop a model of the OHR complex. The *in-silico* model suggests a potential integrated route for proton and electron transfer without quinones.

These findings represent an important advance toward resolving the structure of a functional, quinone-independent reductive dehalogenase complex. Beyond its relevance for understanding the energy metabolism of *D. mccartyi*, this work may have broader implications for microbial bioenergetics. It provides a blueprint for studying other genetically intractable, multi-subunit membrane complexes and opens new avenues for bioengineering and bioremediation applications involving organohalide respiration.

P37

New insights into anaerobic alkane oxidation in archaea revealed by metabolomics and physiological analyses

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Recent discoveries of novel clades of anaerobic archaea capable of oxidizing multicarbon alkanes – such as ethane, butane, and long-chain alkanes like hexadecane - have expanded our understanding of archaeal alkane degradation pathways. These pathways are initiated by alkyl-coenzyme M reductases (ACR), which activates alkanes to alkyl-coenzyme M. Metagenomics and metaproteomics analyses suggest that alkyl-CoMs are oxidized to acyl-CoA, but the enzymes and intermediates involved in these conversions remain unidentified.

Here we aim to resolve the anaerobic volatile alkane oxidation pathway, with a focus on the conversion of alkyl-CoM to acyl-CoA. As a model, we used thermophilic, butane-oxidizing enrichment cultures of *Candidatus* Syntrophoarchaeum butanivorans (Bu50), archaea which grow syntrophically with sulfate-reducing bacteria. Metabolomics showed that *Ca*. Syntrophoarchaeum produces both 1- and 2-butyl-CoM isomers, though their role in the pathway remains unclear.

To investigate potential intermediates and conversion steps, we applied a three-pronged approach: (1) physiology assays with candidate intermediates, (2) assays with crude cell lysate, and (3) comparative metabolomics. We tested culture responses to the exogeneous addition of 1- and 2-butanol and to the corresponding butanethiols. Neither butanol isomers affected sulfate reduction, while butanethiols inhibited sulfate reduction at concentrations of 1.5 mM even when co-supplied with butane. However, even the application of non-toxic buthanethiols concentrations failed to support growth. These results indicate that butanol and butanethiol isomers are unlikely to be pathway intermediates.

To test substrate utilization more directly 1- and 2-butyl-CoM were synthesized, purified and quantified by NMR, then added to Bu50 cultures at 0.5 mM. Neither isomer was consumed nor toxic, suggesting they are not transported into the cells. To overcome this limitation, crude cell lysates were supplied with butyl-CoMs, and their concentrations were followed over time via LC-MS. Unexpectedly, we observed fluctuations of both butyl-CoM concentrations, suggesting dynamic production and consumption.

For comparative metabolomics, cultures were grown with unlabelled and D_6-labelled butane. Sulfide production confirmed oxidation of D_6-butane when provided as sole substrate or in mixtures with unlabelled butane. Metabolite extracts from time-resolved samples will be subjected to high resolution mass spectrometry to identify labelled intermediates and clarify the conversion of alkyl-CoM to acyl-CoA.

P38

Unraveling Archaeal Alkane Oxidation: Understanding the interspecies energetic interactions

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The volatile alkanes ethane, propane, and butane are the main components of natural gas. In anoxic marine sediments, they are oxidized by anaerobic microorganisms, including newly discovered clades of Archaea. The reducing equivalents released during alkane oxidation to CO_2 are transferred to partner sulfate-reducing bacteria (SRB). We investigated the mechanism of electron transfer in cultures of ethane-oxidizing archaea of the clade *Candidatus* Argoarchaeum and their partner SRB (Chen et al., 2019).

In this culture, both partners grow as free cells, excluding a direct interspecies electron transfer, as proposed elsewhere. We propose a transfer of electrons mediated by diffusible reduced compounds, with reduced sulfur compounds as likely candidates. To test this hypothesis, we tracked the flow of sulfur between archaea and their SRB partners by performing assays with ^{34}S-labeled sulfate, aiming to distinguish sulfur uptake by each organism. Correlative imaging using fluorescence microscopy and single-cell chemical imaging by nano-scale secondary ion mass spectrometry (nanoSIMS) revealed that archaeal cells became enriched in sulfur faster than their SRB partners.

These findings suggest that archaea may harbor a non-canonical sulfate reduction pathway, reducing exogeneous sulfate to insoluble or poorly soluble intermediates that accumulate in the cells. Yet-unidentified mechanisms govern the excretion of these intermediates by archaea and their uptake by the SRB. The SRB likely disproportionate the reduced sulfur to sulfate and sulfide, the latter as a final metabolic product. This metabolic model, which highlights an unexpected role of archaea, remains to be validated by identifying the corresponding archaeal genes and enzymes.

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P39

Methane-cycling microbial communities and biogeochemistry in Swiss peatlands

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Peatlands naturally store organic carbon while also emitting methane. Native microbial communities, including methane-producing and methane-consuming bacteria and archaea, regulate carbon dynamics in peatlands. Yet the factors governing the balance between carbon storage and emission are not fully understood. In this work, we examine the microbial community composition via 16S amplicon sequencing, the biogeochemistry via analytical methods, and historical land use of six peatlands across the pre-Alps region of Switzerland.

Biogeochemical measurements revealed significant differences in pH (pH 4.1 – 8.2), conductivity (52 – 924 µS/cm), and ion concentrations (µM to mM range) across sites and depths ($P < 0.05$), while propionate and acetate concentrations remained consistent (~0.8 mM). While keystone microbial species were observed across all sites, some were exclusive to the top (10 cm) or bottom (40 cm), and overall microbial composition seemed to be influenced specifically by pH, calcium, sodium, and manganese ($P < 0.05$). 16S amplicon based functional predictions (PICRUSt2) revealed different abundances of key metabolic pathways for carbon and nitrogen cycling across sites, including a ~11-fold increase in acetoclastic methanogenesis between the sites with the lowest and highest ion concentrations.

We detected a large diversity of methane-cycling microorganisms, including 9 genera of methanotrophic bacteria (MOB) and 8 of methanogenic archaea (methanogens). MOB generally dominated the top layer (10 cm), with *Methylocapsa* being the dominant MOB genus (<0.1 – 2.7% of the community). Methanogens were more abundant in deep layers (25 and 40 cm), where Rice Cluster II, a putative hydrogenotrophic methanogen, was the dominant methanogen (<0.1 – 13.9% of the community). Interestingly, "*Candidatus* Methanoperedens", a methane-consuming anaerobic archaeon, was detected at 40 cm in a site that has recently undergone rewetting. Initial coring suggests that the peatland exceeds 540 cm in depth, and subsequent analyses will evaluate the microbial community along a vertical depth profile.

P40

Investigating the gene regulation and physiology of methoxydotrophic archaea

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Lignin, one of Earth's most abundant organic polymers, is a significant source of methoxylated aromatic compounds (MACs). While bacteria-mediated MAC conversion is well-documented, archaeal degradation of MACs has only recently been discovered. *Methermicoccus shengliensis*, a methanogenic archaeon, is the first archaeon identified to convert MACs, a process termed methoxydotrophy. Recent studies reveal that *M. shengliensis* uses an *O*-demethylase system for MAC conversion, resembling methyltransferase systems of acetogenic bacteria. Comparative transcriptomics identified an operon, the *mto operon*, within *M. shengliensis* that encodes essential MAC-converting proteins, with homologous genes found across diverse archaeal taxa. While the roles of most *mto* proteins in *M. shengliensis* have been elucidated, two hypothetical proteins with DNA-binding structural motifs encoded within this operon remain uncharacterized. We aim to elucidate *mto* gene regulation and characterize these putative DNA-binding proteins by confirming their DNA-binding abilities through EMSA (Electrophoretic Mobility Shift Assay) and performing a promoter pull-down assay with *M. shengliensis* cell extract, followed by MALDI-TOF analysis.

Additionally, we seek to characterize the *O*-demethylase system of further archaea and understand their role within the metabolism of these organisms. *Methanolacinia petrolearia* is a hydrogenotrophic methanogen that primarily uses H_2 and CO_2 for CH_4 production and encodes the Mto proteins, but no MAC transporters. Growth experiments with *M. petrolearia* revealed tolerance to toxic methyl halides (e.g., chloromethane), suggesting these compounds might be converted/detoxified by the Mto system. We will determine the substrate spectrum of this Mto system by conducting enzyme activity assays while using methyl halides such as chloromethane and various MACs as substrates. Additionally, gene expression analysis (RT-qPCR) will be conducted to assess *mto* gene expression in response to chloromethane or MAC exposure. This research enhances our understanding of archaeal methoxydotrophy by studying the regulation of the *mto* genes and by characterizing Mto systems of further archaea alongside their role within the metabolism.

P41

When anaerobes are unwanted: survival and activity of methanogens in a process developed for aerobic hydrogen-oxidizing bacteria

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There are accumulating evidences that methanogens can survive and be active in many oxygenated or partially oxic habitats. This can be problematic when H_2 and CO_2 are utilized in a microbial protein production process inoculated with complex microbial community and methanogens compete with the target hydrogen-oxidizing bacteria (HOB) for electron donor and carbon.

This competition was investigated in fed-batch reactors inoculated with complex microbiota under varying conditions including different stirring intensities (350 and 700 rpm, no stirring) and gas compositions in headspace including addition of ethylene as inhibitor of methanogenesis. The gas composition of the headspace and the dissolved oxygen levels were monitored during three transfers and were compared to abiotic controls. The community structure of bacteria and methanogens were assessed by amplicon sequencing of the 16S rRNA and *mcrA* genes, respectively. The relative abundance of methanogens was further investigated by qPCR quantification of these two genes.

Stirred reactors demonstrated more effective gas utilization compared to the unstirred ones. Increased stirring speed notably improved gas uptake, with decreased CH_4 production due to enhanced O_2 availability; however, CH_4 production was not completely eliminated. Reactors with lower headspace O_2 concentration exhibited the highest CH_4 production and the lowest protein yield, while protein production in unstirred reactors was comparable to stirred ones. The dissolved oxygen levels showed that the efficient O_2 scavenging of HOBs can create an oxygen-deficient environment favourable for methanogens to compete for H_2 and CO_2. The ethylene addition had limited inhibitory effect on methanogenesis or enhancement of protein production.

The qPCR analysis showed significantly higher relative abundance of *mcrA* genes in reactors with lower oxygen levels but methanogens were detected even in highly stirred and oxygenated reactors after three transfers. Most of the *mcrA* sequences were affiliated to the genus *Methanobacterium*. Community analyses revealed increased abundance of families Coprothermobacteraceae, Sporomusaceae and Comamonadaceae in oxygen limited reactors, while in highly oxic reactors the predominance of Pseudomonadaceae and Mycobacteriaceae increased.

The major aim of microbial protein production is to provide a high quality, nutrient rich protein alternative, and the quality should be prioritized. The persistence of methanogens, even under oxic conditions, alongside the presence of other microbial community members revealed that HOB enrichment cultures are probably less effective for protein production than using pure cultures or mixture of few pure cultures. Oxygen-tolerant methanogens might have potential applications in biomethanation reactors with temporal oxic conditions.

P42

Methane emissions from a pulp and paper mill landfill

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Pulp and paper mills in North America landfill much of their wastes, which results in methane emissions. Mills in the USA report methane emissions using the US-EPA industrial landfill emissions model, which is the IPCC model with custom default degradable organic carbon (DOC) and degradation rate values (*k*) for industry wastes, including pulp mill wastewater sludge and boiler ash. The methane production of mill wastes in landfills has not been widely investigated. The default values may be inaccurate, because many pulp mill wastes include organics that are more difficult to degrade anaerobically than model assumptions. Additionally, inhibitors may decrease methane production. An American pulp mill expressed interest in determining whether their landfill's passive vent system should be converted to flaring.

Tested waste streams were wastewater sludge (mixed and primary sludge from two mills), boiler ash, digester knots and rejects, and lime mud. Total and volatile solids were determined. Wastes with a high volatile solids content were used in a biochemical methane potential (BMP) test using the gas density method. Results were compared against the US-EPA model default values. Methane emissions from the passive vents were measured using a flowmeter and by collecting landfill gas samples for measurement by gas chromatography in January 2024 and 2025, and August 2025.

BMP results suggest that the model overestimates emissions from both mills. Pulp mill sludge and digester rejects are less degradable than model assumptions. Ash, even after neutralising its highly alkaline pH, inhibits methane production during BMPs. Rather than a typical inhibited-methane-production curve, the pattern suggested the ash samples have a lower DOC than controls. Landfill gas flow was not detected from the landfill vents during two sampling campaigns in winter. Deep- vent landfill gas concentrations measured 18.3% v/v methane at one vent, and very low (0.0–1.5% v/v) for remaining vents, suggesting low methane production from these wastes.

Follow-up tests are being conducted to determine the cause of inhibition from ash. The ash had a high total zinc content, so a soluble nutrients extraction was performed on ash at neutral pH to determine the bioavailable nutrient concentration. Another hypothesis is that black carbon in the ash sequesters electrons, so the ash was heated to 600 °C for removal of black carbon, and the residue was used in an anaerobic toxicity assay and compared to unmodified ash. Results of these tests will be reported in the presentation.

P43

An insight into *Methanosarcina* substrate affinity and gene expression modulation for methane production in bioreactors

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Methane is an efficient energy carrier and a major component of natural gas that can be used to produce higher fuels such as methanol or butanol. Renewable methane can be produced biologically by methanogenic archaea during anaerobic digestion (AD). The efficiency and stability of this process depend entirely on the concerted and syntrophic activity of the microbial community. Among archaea, the genus *Methanosarcina* is known as a versatile methanogen capable of using both acetoclastic and hydrogenotrophic pathways of methanogenesis, making it one of the most robust actors in AD. However, the environmental pressures leading *Methanosarcina* to choose one pathway or the other and the velocity and flexibility of its metabolic shift remain little studied and poorly understood.

Our overall aim is to optimise methane production during AD in bioreactors, by identifying the key species for methane production and the necessary syntrophic interactions. We chose to focus on *Methanosarcina* as it is the most robust and main methanogen in our bioreactors. The objective of this study is therefore to better understand the functioning of *Methanosarcina*-bacteria syntrophies. More specifically the effect of the substrate affinity of *Methanosarcina* that leads to the transition from acetoclastic to hydrogenotrophic methanogenesis was investigated.

Firstly, controlled incubations with two enriched cultures displaying apparent syntrophic interactions with *Methanosarcina* were performed by adding different combinations and concentrations of substrate (H_2/CO_2 or acetate). Metagenome-assembled genomes (MAGs) had already been obtained for *Methanosarcina* and the microbial community in the original enrichments. RNA has been extracted from the cultures exposed to new substrates and metatranscriptomics will be performed to observe how gene expression is modulated in *Methanosarcina* during the switch in methanogenesis pathway.

Second, a database of *Methanosarcina* genomes was constructed from the NCBI RefSeq database and our new MAGs to perform a comparative genomic analysis, known as pangenomics. This analysis provides a context for our MAGs regarding genomic organisation and differences between *Methanosarcina* species. The metatranscriptomic data obtained from enriched cultures will be mapped to our MAGs. Our preliminary observations show a relatively small core genome and a wide variety of accessory genes, most of them species-specific. Core genes are mainly related to central metabolisms, as translation, and ribosomal structures, while defence mechanisms, membrane biogenesis, and signal transduction mechanisms are more represented among accessory genes.

Together, these two approaches will contribute to the overall aim of optimizing the use of this archaea in bioreactors and improving control of the end product.

P44

Characterizing archaeal methyltransferases utilizing methanol as a substrate for circular biocatalysis

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Methylation reactions are widely used in the chemical and pharmaceutical industries as key diversification reaction. Traditional chemical methylation typically requires strong bases, metal catalysts, and toxic methyl donors. In contrast, enzymatic methylation offers high chemo- and regioselectivity under mild conditions. Methyltransferases (MTases) present a biological alternative, using low-molecular-weight methyl donors such as methanol, acetyl-CoA, or cofactors like methyl-tetrahydrofolate, *S*-adenosylmethionine (SAM), and methyl cobalamin. Among the various enzymatic systems, B_{12}-dependent methylation systems remain largely unexplored. The B_{12}-dependent methylation machinery consists of: (i) an MTase I that demethylates the methyl group donor, (ii) an MTase II that transfers the methyl group to an acceptor molecule, and (iii) a B_{12}-shuttle protein that carries B_{12} as a prosthetic group, enabling reversible methyl group transfer between MTase I and MTase II.

My project focuses on the investigation and characterization of recombinant archaeal MTase I enzymes that utilize methanol and other C1 compounds, which can be sustainably derived *via* electrochemical CO_2 reduction, as methyl donors. To facilitate their biotechnological application, expression vectors have been constructed for heterologous expression in *E. coli*, and various conditions have been screened to optimize protein production. Following expression, the candidate MTase I enzymes will be purified, and activity assays will be conducted under anaerobic conditions, and V_{MAX}, K_M as well as the k_{cat} will be determined for different substrates. Moreover, recombinant enzymes will be further characterized, including metal cofactor analysis using ICP-QQQ-MS. In addition, structural analysis of the MTase I together with the B12-shuttle protein and MTase II will be conducted followed by multiple sequence alignment to identify conserved residues for mutagenesis, aiming to enhance enzyme activity, stability, and substrate affinity.

Overall, this research aims to establish a sustainable, circular biological methylation platform using C1 compounds as renewable methyl group donors, providing a CO_2-neutral alternative to conventional methylation with potential applications in pharmaceuticals and biofuels.

P45

Development of an information and eLearning portal for stable isotope applications

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The Laboratories for Stable Isotopes (LSI) are an association of UFZ research laboratories that build on a long-standing expertise in the analysis of stable isotopes (e.g., H, C, N, O, S, Cl), respective concepts and applications, and thus forming a unique center of competence in isotope analytics. Innovative, advanced instrumentation and laboratory facilities with expert guidance and support are provided and the spectrum of available analytical techniques allows the bridging of scales from molecular levels up to catchment areas. In the context of anaerobic microbial processes, these methods are applied, for e.g. monitoring of biogeochemical element cycles in relation to climate change adaptation, energy storage, and pollutants/nutrients transport and turnover in terrestrial and freshwater ecosystems or the characterization of pathways and quantification of biotransformation of organic groundwater pollutants. Although these stable isotope applications are established as scientifically valuable methods, their implementation falls far short of their expected potential, presumably due to their highly specialized nature and, more critically, the lack of effective knowledge transfer to potential key stakeholders, such as regional authorities, environmental agencies and industry professionals but also academia. To fully utilize the potential of stable isotope methods, a diverse range of users must thus develop a basic understanding of those methods and their benefits. To bridge this gap and unlock the full potential of stable isotope applications, we intend to build an open-access, centralized web platform providing user-customized knowledge on stable isotope applications. This platform is proposed to provide tailored information, training, and resources for two key target groups: Stakeholders i.e. commercial users, and local and regional authorities, to support decision-making and facilitate real-world implementation as well as the academic community, incl. students, who represent the next generation of stable isotope experts and exploiters.

Here, we will present our general approach for building the concept which starts with the identification of user needs and expectations. We invite you to share your views, experiences and requirements, reflecting on questions such as i) what do you know about stable isotopes, ii) what information would help you better understand and apply stable isotope techniques, and iii) what are your preferred learning and information formats? Your feedback will help us to create an accessible, user-adapted, web-based information and eLearning portal.

P46

The Laboratory of Anaerobic Microorganisms

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The Laboratory of Anaerobic Microorganisms (LAM) focuses on the exploration of microbial communities thriving in oxygen-free environments and their applications in biotechnology and environmental engineering. The laboratory specializes in the isolation, cultivation, and molecular characterization of methanogenic archaea and anaerobic bacteria, particularly those involved in biogas production, microbiologically influenced corrosion, and greenhouse gas mitigation.

Key research activities include optimizing anaerobic digestion processes, investigating the interaction of microorganisms with metal surfaces in corrosive environments, and studying the role of microbial communities in energy and carbon cycles. In addition, the laboratory is actively involved in energy-related research, particularly in the fields of hydrogen storage and biological methanation, where microbial processes are harnessed to convert hydrogen and carbon dioxide into methane as a renewable fuel source.

On the other hand, a parallel line of research focuses on reducing methane emissions in livestock farming. This includes targeting specific microbial pathways to inhibit methanogenic archaea in the rumen without disturbing the overall microbial balance. The aim is to identify and develop molecules that selectively suppress methane production while preserving essential digestive functions.

The lab also contributes to applied research in collaboration with industrial partners, such as operators of biogas plants, wastewater treatment facilities, and energy storage systems, including underground hydrogen and methane storage.

Combining classical microbiological methods with advanced molecular biology and bioinformatics, LAM aims to deepen our understanding of microbial processes in anaerobic ecosystems and translate this knowledge into sustainable and innovative solutions for environmental and industrial challenges.

P47

Pyruvate:ferredoxin oxidoreductase (PFOR): a key metabolic enzyme in the anaerobic eukaryote *Giardia intestinalis*

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Giardia intestinalis, a unicellular eukaryote, has evolved distinct metabolic adaptations to support its anaerobic and parasitic lifestyle. One such adaptation involves pyruvate:ferredoxin oxidoreductase (PFOR), a cytosolic iron-sulphur (Fe-S) enzyme that catalyses the conversion of pyruvate to acetyl-CoA while reducing ferredoxin. To investigate its role, we generated full CRISPR/Cas9 knock-out cell lines for each of the two PFOR paralogs encoded in the genome. We found that PFOR is the dominant Fe-S protein, and, in line with observations in other organisms, both PFOR-1 and PFOR-2 form homodimers in *G. intestinalis*. However, our data show that the two do not interact with each other, and that PFOR-2 uniquely forms homotetramers. Previous hypotheses suggested that PFOR-2 might utilize a substrate other than pyruvate. Supporting this, our metabolomic profiling and enzyme activity assays indicate potential substrate divergence between the paralogs. Additionally, we assessed gene expression of both *pfor* genes, examined knock-out cells viability under microaerophilic conditions, and tested resistance to metronidazole, the primary drug used to treat giardiasis. Together, these results constitute the most comprehensive investigation of PFOR function in *G. intestinalis* in decades and provide new insight into its metabolic specialization and evolutionary diversification.

P48

Comparative Cultivation of *Methanobacterium formicicum* and Mixed Culture of *Methanobacterium formicicum* & *Methanosarcina barkeri* in Phosphate Buffer Medium (50 mM) Using Formate, Acetate, and H_2/CO_2 as Substrates Under Controlled Anaerobic Conditions

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Methanogenic archaea play a pivotal role in anaerobic carbon cycling and renewable energy generation through biogas production. This study presents a comparative investigation of the growth dynamics, substrate utilization, and methane production of *Methanobacterium formicicum* and a mixed culture of *M. formicicum* and *Methanosarcina barkeri* cultivated in 50 mM phosphate-buffer medium (PBS) under controlled anaerobic conditions.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) were used to monitor headspace gas evolution and substrate consumption over a 7 weeks incubation period. Using formate and H_2/CO_2 as substrates, *M. formicicum* exhibited rapid and complete formate consumption by day 6, accompanied by high methane production. Replenishment of sodium formate on day 28 further stimulated methanogenesis, confirming *M. formicicum*'s high metabolic efficiency. In contrast, *M. barkeri* displayed slower acetate metabolism, with a 56.7% reduction in acetate observed over 47 days in the mixed culture. The increase in methane concentration, coupled with the depletion of hydrogen and carbon dioxide in both *M. formicicum* and mixed cultures, highlights effective methanogenesis.

Notably, co-cultivation did not inhibit the metabolic activity of either strain; *M. formicicum* maintained robust formate-dependent methanogenesis, while *M. barkeri* slowly metabolized acetate. The compatibility and complementary substrate preferences of the two strains suggest their potential for integrated use in bioreactors processing mixed substrates.

These findings have practical implications for the design of synthetic microbial system and the optimization of anaerobic digestion systems for biomethane production. Future work should focus on continuous reactor systems, nutrient optimization, and omics-based analyses to further elucidate interspecies interactions and enhance bioprocess efficiency.

P49

High-throughput microbiome activity kinetics in response to chemical challenges

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Multivariate models improve diagnostic and prognostic accuracy by integrating multiple clinical and biological indicators, overcoming the limitations of single predictor approaches. These HPC-ready pipelines: GUMPP (General Unified Microbiome Profiling Pipeline), MetaBakery, MAGO (Metagenome Assembled Genomes Orchestra) use advanced statistical and machine learning techniques to synthesize complex data and improve the reliability of healthcare decisions to help physicians and individuals estimate probabilities and potentially guide their decision making. Especially in the case of (Non-Communicable Diseases) NCDs predictions can be utilized (i) to plan therapeutic decisions based on the risk for developing medical condition within specific timeframe; (ii) to stratify participants in intervention trials with direction of database generation for multiple clinical cohorts; and (iii) to develop preclinical early-warning tools in the near future.

The Gut Microbiome Response Chip (GMRC) concept enabled us to extend the 6 MetaBakery (approx. 20 million variables) human microbiome information layers (analysis of taxonomy, diversity, functional genes, enzymatic reactions, metabolic pathways, and metabolite datasets deconvoluted from WGS) with the real-time microbiome activity kinetics in response to chemical challenges, either single, in their mixtures, or in gradients. In the analytical example, the actual gut conditions of each individual healthy donor were amended with different dietary components (organic acids, sugars, fats, probiotics, nano-/microplastics) that caused different kinetics of microbiome response. Over 50,000 datapoints per test subject per experiment (in total 1,075,200 datapoints) were obtained for a small cohort of healthy individuals (n=5) as a proof of concept. In-house routines were prepared and utilized to deconvolute the data into biologically relevant descriptive summaries making them highly amenable for machine learning (numerous algorithms, hyperparameter search, reporting statistics, biomarker constellation search).

The effects of sample storage (time, temperature) on the real-time microbiome activity kinetics of five subjects were visualized using non-metric multidimensional scaling (Euclidean distance). The results show clear, reproducible and significant differences in microbiome responses to chemical challenges that are characteristic of individual subjects. Details of the multivariate relationships between the observed responses to chemical challenges show statistically significant differences between groups of samples from the same individual. The results of group significance tests utilizing NPMANOVA and ANOSIM showcase the highly individualized responses of human microbiomes making them highly amenable to exploration utilizing Machine Learning (ML) and big-data approaches. The GMRC enables database building for prospective studies and classification for multiple clinical cohorts of unknown patient samples into different disease types.

P51

Investigation of biofilm forming capabilities of the acetogen *Eubacterium callanderi* 'Marburg'

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The biofilm life cycle can be in principle divided into three phases. In the first phase, single cells attach to a surface. After the successful attachment, cells start to produce a matured biofilm through the formation of microcolonies. Afterwards, cells can detach and spread as planktonic cells. The production of biofilm and extra polymeric substances (EPS) offers severed advantages for bacterial cells such as a lower susceptibility towards changes in temperature, pH or the presence of harmful substances. Additionally, EPS serves as a diffusion barrier e.g. regarding produced enzymes which can lead to an increased substrate degradation, also it confers mechanically stability. Those advantages can be used during the cultivation in bioreactors as it is the overall goal of this project. Therefore, this work aims the identification of potential biofilm related genes in *Eubacterium callanderi* 'Marburg' to increase the production of C4 compounds.

In the close relative *E. limosum* ATCC 8486, an operon which consists of five genes was recently identified which gene products might be involved in biofilm production. Knockout strains in which the whole operon was deleted showed in contrast to wildtype cells no biofilm formation (Sanford et al., 2023, doi: 10.1093/femsle/fnad030). The respective genes are also present in the genome of *E. callanderi* 'Marburg' and are arranged in the same order as in the genome of *E. limosum*. Thus, the biofilm forming properties of *E. callanderi* are going to be investigated. Therefore, cell numbers and biofilm thickness of bacterial cells will be analyzed of cells grown on polypropylene (PP) or polydimethylsiloxane (PDMS) disks attached on a stainless-steel wire. Staining fixed cells with DAPI (4',6-Diamidino-2-Phenylindole) will enable the determination of total cell numbers using flow cytometry. The structure and cell densities of biofilms of *E. callanderi* will be investigated through confocal laser scanning microscopy and the thickness of fixed samples using light microscopy.

P52

Microbial interactions in a high-performance tubular foam-bed reactor for biomethanation

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Biomethanation can be used to convert excess electrical energy into storable biomethane. Recently, a lab-scale high-performance tubular foam-bed reactor (TFBR) was developed to increase gas transfer which is a major limitation of this process. The addition of foam-stabilizing detergents to a microbial community (MC) consisting of methanogenic archaea and associated bacteria is thought to induce stress and trigger adaptation within the MC.

To investigate this adaptation, the MC of the TFBR was analyzed, focusing on both structural and functional characteristics. Combined metagenomics and metaproteomics were applied to analyse the MC of TFBR.

The MC consisted of three archaeal MAGs assigned to *Methanobactericeae* (40%) and 60% bacterial MAGs, predominantly *Thiopseudomonas* (12%), *Alkaligenes* (5%), *Natronincolacaea* (5%), *Massilibacterium* (4%), *Petrimonas* (3%). The presence of bacteria, such as *Petrimonas*, feeding on archaeal biomass was previously observed in the stirred tank reactor for biomethanation and was further supported by the detection of extracellular hydrolases and proteases. However, the high abundance of *Thiopseudomonas* and *Alkaligenes* was surprising. Highly expressed proteins involved in oxidative phosphorylation, including complex IV of respiratory chain, implement metabolism using O_2 as electron acceptor. The unexpected presence of O_2 was confirmed by high expression of enzymes responsible for detoxifying reactive oxygen species (ROS) in most of the members of the MC. Although H_2 produced by PEM electrolysis is considered to be very pure, the high abundance *Thiopseudomonas* and *Alkaligenes* clearly indicated a continuous contamination with O_2. The oxidative metabolism *Thiopseudomonas* and *Alkaligenes* scavenged most O_2 protecting the strict anaerobic archaea from damage by ROS.

The oxidative metabolism of *Thiopseudomonas* and *Alkaligenes* protected the methanogenic archaea from damage by ROS by scavenging O_2 from H_2 supply. This process might be crucial for process stability when operating biomethanation as an industrial process.

P53

Biomethane from syngas by mixed anaerobic community

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Syngas fermentation is a promising biotechnological process that enables the microbial conversion of gas mixtures - primarily composed of CO, CO_2, and H_2 - generated from biomass gasification to carboxylates, alcohols or CH_4. The process relies on obligate anaerobic, acetogenic and/or methanogenic microorganisms capable of metabolizing these gases. The technology offers a sustainable alternative to fossil-based chemical production, contributing to carbon recycling following the principle of a circular economy. The universally accepted principle in dissecting microbiological events yielding organic products is that process should be done by microbiologically pure cultures. Sustained anaerobic fermentation by pure cultures is costly, mixed communities may offer unique benefits. The main element in our working hypothesis is that the selection pressure by the uncommon substrates, which are not suitable to drive the metabolism of most microbes, may lead to the development of enriched mixed microbial communities through directed evolution.

Our main goal was to uncover the biomethane formation in anaerobic fermentation of various H_2 and CO gas mixes injected into the headspace filled with ultrapure N_2. The fermentations were done in fed-batch systems inoculated with a mesophilic biogas reactor effluent, which produced residual biogas (1:1.2 volumetric mixture of CH_4 and CO_2) throughout the experiment, making up about 10% of the head space in each feeding cycle. The performance of the microbial community was followed by measuring the metabolic products with gas chromatography and HPLC, the evolved communities were characterized by metagenomic analysis. The pH, organic acid profile, other important analytical parameters were also monitored.

The long term fed-batch supplementation of the gases in various ratios produced distinct responses. The injection of a H_2:CO in 4:1% volumetric ratio triggered vigorous initial CH_4 production. However, the dissolved, residual CO_2 was quickly consumed by the hydrogenotrophic methanogens, which lead to drastic elevation in pH (>9) and diminishing CH_4 production. The 1:4 mixture of H_2:CO injection resulted in intensive initial as well as sustained long term CH_4 production. The high CO supply apparently suited the carboxydotrophic microbes and balanced the CO_2 depleting effect of the hydrogenotrophic methanogenesis. Under these conditions, the cumulative CH_4 production increased to 245% while the 4:1 H_2:CO mixture yielded 141% CH_4 production improvement relative to the control, i.e. the residual CH_4 production from the biogas effluent.

Overall this study confirmed that a mixed anaerobic microbial community can generate biomethane from syngas with good efficiency.

P54

Utilizing *Methanothermobacter thermautotrophicus* ΔH as an expression host for recombinant enzymes

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Due to the increasing global temperature, research across various fields to counteract climate change remains an ever-present necessity. Reducing the amount of carbon dioxide in the atmosphere through carbon capture at industrial sites of emission and converting it into methane shows promise as one avenue to become independent from fossil fuels. Using *Methanothermobacter spp.* as biocatalysts in the power-to-gas platform to convert hydrogen produced from renewable electricity and captured carbon dioxide into methane piqued industrial interest. Another area of interest is taking the platform one step further and using *Methanothermobacter spp.* to produce more valuable chemicals from the same renewable sources. Recent research from our lab developed the fundamental tools for *Methanothermobacter thermautotrophicus* ΔH genetic research, such as functional conjugation and electroporation protocols, two selective markers in a shuttle vector system, and a thermostable Cas9 for genome editing. The immediate use of *M. thermautotrophicus* ΔH as a model microbe is to produce key homologous and heterologous recombinant enzymes for isolation and purification. We will present on the initial findings and methodology in the expression of recombinant enzymes, as well as further optimization of the electroporation protocol. Utilizing *M. thermautotrophicus* ΔH as a production host will provide key insight into the gene expression and role of these enzymes in methanogenesis and these developments would push *M. thermautotrophicus* ΔH forward as a model methanogen for fundamental research and industrial application.

P55

Methane-free biohydrogen conversion from syngas fermentation: effect of pH and sludge concentration

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The search for a biological process to produce hydrogen is a promising alternative to fossil fuels. Synthesis (syngas) fermentation is a possible technology where hydrogen can be generated by a biological water-gas shift reaction conversion. The use of natural consortia has been gaining attention as an easier solution against pure cultures regarding the ease of operational requirements and overall cost associated with fewer requirements for the facility and equipment. However, challenges such as methanogen activity often hinder stable yields without chemical inhibitors. This study investigates an alternative approach by evaluating the impact of volatile suspended solids (VSS) concentration and initial pH on hydrogen production while avoiding chemical methanogen suppression methods. Experiments were conducted at five VSS levels (0.2, 10, 20, 30, and 40 g/L) with two initial pH conditions (6 and 9), using disrupted sludge granules from wastewater treatment to assess their influence on methane inhibition. Results demonstrated that granule disruption, combined with heat shock pretreatment, effectively suppressed methane production at a VSS concentration of 20 g/L and an initial pH of 9, enabling exclusive hydrogen conversion with a faster carbon monoxide consumption. However, at pH 9 with a higher VSS level (40 g/L), methane was detected, suggesting reduced heat treatment efficacy under these conditions. These findings indicate that both pH and VSS concentration critically influence the success of physical methanogen inhibition methods. The study highlights the potential of optimizing operational parameters, such as pH and VSS levels, to achieve stable hydrogen conversion without chemical additives. Further research should explore the utilization of the proposed technique in a continuous mode to evaluate the duration of the methanogen's inhibition and hydrogen conversion stability. Moreover, additional chemical-free pretreatment strategies to enhance methane suppression techniques are needed to advance sustainable biohydrogen production.

P56

Developing a syngas fermentation process with *Clostridium ljungdahlii* for cyanophycin production

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Cyanophycin is a biopolymer consisting of a poly aspartate chain with arginine bound to the side groups of the aspartate chain. The biosynthesis of cyanophycin requires only a single enzyme: cyanophycin synthetase (encoded by *cphA*). It was first discovered in cyanobacteria, and later it was found that many other bacteria, including Clostridia, possess the genetic capability to produce cyanophycin. Cyanophycin has many potential applications, such as the use as bioplastic in medical approaches. This triggered the development of biotechnological production routes with cyanobacteria and E*scherichia coli*, both of which have certain limitations, such as difficult scalability as well as low quantity and/or quality of the biopolymer. The objective of this study is to establish *Clostridium ljungdahlii*, a model microbe for syngas fermentation, as an alternative production platform *via* genetic engineering.

To achieve efficient cyanophycin production from syngas with *C. ljungdahlii*, several cyanophycin synthetases from various microbes will be investigated under different growth conditions. Additionally, metabolic engineering to enhance aspartate and arginine production from syngas will be performed. The resulting strains will be transferred to bioreactors and the production process further optimized to achieve larger amounts of cyanophycin with a high quality, subsequently enabling research to enhance the properties of the biopolymer for use in different applications. The planned strategies for the genetic modification of *C. ljungdahlii* and first results will be presented.

P57

Engineering *Clostridium ljungdahlii* for sustainable acetone production

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For a sustainable future, the reliance on fossil fuels must be further reduced. While significant progress has been made in replacing fossil fuels in electricity production, most industrial production of commodity chemicals still relies on fossil raw materials. Decarbonizing the chemical industry, therefore, requires implementing sustainable production routes. Acetone is a key chemical as a solvent and an essential resource in the production of various specialty chemicals, but it is currently primarily sourced from fossil raw materials.

Clostridia spp. have a long history in industrial bioprocesses. The solventogenic bacterium *Clostridium acetobutylicum* was already used in the early 20th century to produce acetone, butanol, and ethanol from starch. While this process is sustainable, its reliance on carbohydrates as substrate puts it in direct competition with food production. *Clostridium ljungdahlii*, on the other hand, can grow with hydrogen and carbon dioxide as sole electron and carbon sources, which makes it attractive for the sustainable bioproduction of chemical commodities. However, wild-type *C. ljungdahlii* only produces ethanol, acetate, and small amounts of 2,3-butanediol as metabolic end products.

In the project BETA (bioethanol to acetone), we aim to provide an efficient process for the sustainable production of the base chemical acetone from bioethanol. Here, we present the progress on the development of a microbial biocatalyst within the overall process. We illustrate ways to produce acetone with *C. ljungdahlii* and aim to optimize the production through pathway engineering and metabolic modeling.

P58

Expanding the Genetic Toolbox for *Acetobacterium wieringae*

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Over the last 25 years, scientists have developed various molecular tools, expressed non-native pathways, and enabled recombinant production in a handful of different gas fermenting acetogenic bacteria. While some acetogens, such as the commercialized *Clostridium autoethanogenum*, possess a well-developed toolbox, others have only poor or no genetic system available. One of such promising but understudied acetogens is *Acetobacterium wieringae* JM, a carboxydotrophic acetogen that efficiently converts CO via the Wood-Ljungdahl pathway to produce acetate and ethanol. Unlike other ethanol-producing carboxydotrophic acetogens, *A. wieringae* operates at pH 7, making it an ideal co-culture partner to produce chain-elongated products.

In the present work, we expanded the genetic toolbox of *A. wieringae* by implementing various promoters, a fluorescent reporter protein, and a potent gene-knockout system. We constructed a small promoter library consisting of seven promoters from different acetogenic and solventogenic *Clostridia*. Promoter activity was assessed using the fluorescence-activating an absorption-shifting tag (FAST) as a reporter protein. All tested promoters exhibited activity at varying strengths determined by FAST mediated fluorescence.

Moreover, we implemented the theophylline-inducible CRISPR-Cas-based genome engineering system SIBR-Cas for targeted gene knockouts in *A. wieringae*. Given that deletion of aldehyde ferredoxin oxidoreductases (AOR) in *C. autoethanogenum* influenced ethanol production, we targeted two homologous AOR genes in *A. wieringae* using SIBR-Cas. While the successful deletion of AOR1 did not impact ethanol production when the strain *A. wieringae* ΔAOR1 was cultivated with CO, knockout of AOR2 is still in progress.

Our findings substantially advance the genetic engineering capabilities of *A. wieringae*, paving the way for its use in co-cultures for the production of valuable industrial platform chemicals.

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