Poster Abstracts

On-site poster session 2

10th May 12:40-2:00 pm

Biofilm application

Tools and Modelling

On-site

Poster session: Biofilm applications

Prophages in Pseudomonas aeruginosa contribute to resistance to phages in biofilms

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Background: Cystic fibrosis (CF) is a genetic disorder that is associated with difficult-to-treat infections by Pseudomonas aeruginosa (PA). Lytic phages could be a promising alternative therapy to antibiosis. The biggest challenge in CF is the formation of biofilms and resistance development rendering antimicrobial therapy ineffective. Moreover, Pf1-like prophages are abundant in the genome of PA enhancing the pathogenesis of the bacteria. We hypothesized that prophages could contribute to antibiotic resistance and bacteriophage resistance, both in a planktonic and biofilm state. Here we evaluated the effect of induced prophage on PA biofilms, both in reference strains and clinical strains collected from CF patients.

Methods: To determine the presence of different prophages (Pf1, Pf4, and Pf5), PCR was performed on laboratory strains and clinical isolates from different cohorts (CF and non-CF specimens). The Pf1-like positive strains were cultured in a microtiter plate as a biofilm over a maximum of 6 days with daily medium changing. Subsequently, daily the supernatant was collected and filtered, the biofilm was scrapped off to perform CFU determination and filtered for induced prophage detection. Afterward, the collected colonies were subjected to phage resistance assay and antibiotic resistance was determined using micro-dilution methods.

Results: Results indicate that the induction of prophages happens spontaneously after 3 days in the case of reference strain PAO1, the spontaneous induction was followed by the emergence of bacteriophage-resistant clones in 80% of the cases, these bacteriophage resistant clones also led to enhanced antibiotic resistance in selected clones. In comparison, the clinical PA isolates were also shown to be affected by induced prophages and similar results were obtained. The CFU count showed that no significant reduction in bacterial burden in the biofilms was achieved.

Conclusion: Here we show that upon induction of prophages in biofilm a diversification of the population occurs without reducing the bacterial burden but increasing resistance towards bacteriophages and antimicrobials. The Pf1- like prophages are widely present in CF isolates and thus might strongly contribute to the pathogenesis in CF patients. The question arises whether inhibition of prophage-escape could synergistically support current CF therapy by antimicrobials and future phage therapy.

On-site

Poster session: Biofilm applications

Pilot reactor for anoxic biocatalytically assisted electrode-based oxidation of substrates for continuous production of value-added chemicals

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In recent years, electrochemical processes with microorganisms as biocatalysts, so-called bioelectrochemical systems (BES), have increasingly become subject of research. While fuel cells are already being used experimentally in the field of wastewater treatment, synthesis or partially oxidative processes in the sense of electrofermentation are still largely restricted to laboratory research. In theory, electrofermentation in a continuously running reactor has the advantage that the product can be selectively produced with the help of specialized microorganism strains, thus, eliminating the need for expensive purification of the product. In addition, due to the anaerobic mode of operation, the product-substrate ratio is high and only a small portion of the substrate is incorporated into biomass. A major obstacle to date has been the low current densities of the working electrode the microorganisms can generate, so that the space-time yield of the entire reactor suffers. The pilot reactor presented here aims to generate a high surface-to-volume ratio using a conductive anodic sphere packing of non-porous material to compensate for this drawback. In this process, the microorganisms are to grow as a biofilm on the surface of the anode fill. Model process of the anaerobic (partial) oxidation to be investigated is the production of acetoin from glucose by a genetically modified *Shewanella oneidensis* strain. The protons produced are reduced to hydrogen at the cathode elements located within the anode packing. The packing is completely fluidizable, so that potential clogging of the interstitial spaces by excessive growth of biomass can be counteracted.

On-site

Poster session: Biofilm applications

Targeting the biofilm matrix of oral biofilms using multiple-enzyme treatment

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The oral microbiota is important both in sickness and in health. While dental biofilms are involved in disease-causing processes such as development of caries and periodontitis, the bacteria in oral biofilms also contributes positively to human physiology through nitrate metabolism and blood pressure control. This project aims to elucidate enzyme combinations that are able to target the diversity of extracellular polymeric substances (EPS) in multi-species biofilms by applying non-biocidal matrix-degrading enzymes to selectively remove the disease-causing biofilm without harming the healthy oral microbiome. The composition of the oral microbiome varies from person to person, and hence so does the composition of the extracellular matrix. The specificity of EPS-degrading enzymes makes targeting the different matrix components of a multi-species biofilm, such as dental biofilms, especially challenging, and likely requires a combination of several EPS-degrading enzymes.

In a large-scale enzyme screening, we tested 44 different matrix-degrading enzymes in 149 combinations with up to six enzymes in each formulation on multi-species in vitro biofilms grown from saliva inoculum, and quantified them by crystal violet staining. Ten promising formulations were selected and applied to in situ-grown biofilms. In situ biofilms were grown for 48 h inside the oral cavity of healthy volunteers fitted with an additively manufactured intraoral device for biofilm collection. The biofilms were subsequently enzyme treated and quantified by confocal microscopy. The bacterial diversity of in vitro and in situ biofilms was assessed by 16SrRNA amplicon sequencing.

The best performing enzyme formulations consisted of 4-6 matrix-degrading enzymes, and removed up to 70 % in vitro biofilm and 50 % in situ biofilm. Formulations that performed well in vitro were not necessarily the ones that performed best in situ. Amplicon sequencing showed that in vitro biofilms were dominated by Streptococci, while in situ-grown biofilms were more diverse. This difference may explain why a greater variety of EPS-degrading enzymes are required to remove in situ biofilm.

This study investigated the effect of treatment with multiple matrix degrading enzymes on both in vitro and in situ-grown biofilms and showed that treatment with enzyme formulations containing multiple matrix-degrading enzymes were able to significantly remove biofilm, and thus has considerate potential for dental biofilm control

On-site

Poster session: Biofilm applications

Fermentative granules for production of caproic acid

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A new type of fermentative granular biofilm for the production of bio-based chemicals has been recently observed in an open culture expanded granular sludge reactor fed with a real waste stream (i.e. thin stillage) or with synthetic medium containing glucose. The granules were obtained after a sufficient decrease in hydraulic retention time without using any carrier material. However, the physical integrity of granules was weak as unpredictable degranulation events happened throughout different operations, preventing a stable process.

The main product of the system was caproic acid, which is a platform chemical that can be used directly as an antimicrobial substance, as an animal feed or as an anti-corrosive agent, or indirectly for the production of fragrances and flavours, fuels, plasticisers, solvents, and other commodities. Although the granular biofilm has already proven its application potential, we lack mechanistic knowledge regarding its physico-chemical characteristics, the impact of operational parameters on biofilm properties and the role of different microorganisms in the granulation process.

In this project, we aim at improving the stability of granules, and with it the stability of bioprocess, by disassembling the system and generating fundamental knowledge about its individual parts, namely the microorganisms, the extracellular polymeric substances (EPS) and the operational conditions.

First, we have identified the four key genera (Lactobacillus, Caproiciproducens, Oscillibacter and Olsenella) comprising over 90 % of the community. In next steps, the species of these genera will be isolated and characterized for their ability of biofilm formation and caproic acid production. A synthetic community will be constructed and grown axenically under the flow conditions using the modified Robbins device. Knowledge about the formation and maturation of biofilm will be gained by following the community dynamic using molecular methods (i.e. qPCR) and identifying the role of EPS using confocal microscopy in the combination with fluorescent stains. Afterwards, we will assess the impact of various parameters (e.g. pH, up-flow velocity, carbon source, nitrogen source, presence & type of cations) on physico-chemical properties of biofilm (e.g. EPS structure, product profile, mechanical strength).

The obtained information will enable future knowledge-driven process design and mediate the development of this technology towards the industrial scale.

On-site

Poster session: Biofilm applications

Model-based evaluation of novel thermophilic partial nitritation/anammox process in a granular sludge reactor

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The discovery of the anaerobic ammonium oxidation (anammox) process has revolutionized nitrogen removal from wastewater. It is often realized via the partial nitritation/anammox (PN/A) process. However, the application of PN/A process has been limited to mesophilic conditions (<40℃). Recent developments in thermophilic anammox and nitrification open up the possibility of thermophilic PN/A, which may enable more sustainable treatment of ammonium-rich hot wastewaters (>45°C), e.g. centrate of thermophilic sludge digestion. For the first time, this contribution evaluated the feasibility of thermophilic PN/A and compared it with its mesophilic counterpart, using a 1-D biofilm model. The results revealed potential impact of key operational factors and insights in the microbial ecology of thermophilic PN/A, such as a narrower optimal dissolved oxygen range and potential rate limitation due to the lower growth rate of ammonia oxidizers (archaea instead of bacteria) at thermophilic conditions.

On-site

Poster session: Biofilm applications

The microbiome of anaerobic granules during volatile fatty acid production is shaped by fluctuations in pH

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Volatile fatty acids (VFA) are used on several applications, ranging from the pharmaceutical and textile to food and chemical industries. The bio-based production of VFA from waste-streams is attracting attention as it represents a more sustainable and environmentally friendly approach. Microbial communities are key for the VFA fermentation performance and yield and are affected by parameters such as pH, temperature, organic loading rate, retention time. Knowledge on the microbiota is needed to further advance the technology. The microbiome structure of anaerobic granules from an up flow anaerobic sludge blanket (UASB) reactor fed with hydrolyzed Brewer's spent grain (BSG) was evaluated over 311 days during which fluctuations on the pH occurred. At pH 4.5, the predominant genera within the biomass were *Olsenella* (11%), *Prevotella* (15%), *Clostridium* (13%) and *Caproiciproducens* (30%). These taxa belong to families that are known to be involved in the production of acetate and butyrate, which coincided with the main products obtained during this phase. When the pH was reduced to 3.9, the fermentation products profile changed, resulting in lactate predominance in the VFA mixture. The microbiome structure at genus level also changed and *Lactobacillus* became the dominant bacterial genera (ca. 88%). The restoration of the pH to 4.5 led to the restoration of VFA profile favoring the production of acetate and butyrate. Also, the genera *Olsenella* (14%), *Prevotella* (17%) and *Caproiciproducens* (21%) became again dominant within the microbiome. Knowledge on the dominant microbiota under specific process conditions can help devising robust approaches for efficient and stable VFAs production.

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On-site

Poster session: Tools and modelling

Mapping the antimicrobial properties of polydopamine films and the role of surface charge via atomic force spectroscopy and infrared spectroscopy

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Biofilms are ubiquitous microbial communities that can attach to both biotic and abiotic surfaces. In comparison with planktonic cell cultures, biofilms exhibit an increased tolerance against antimicrobial and antibiotic treatments due to the presence of extracellular polymeric substances (EPS), which provide bacteria physical protection against external stressors.

The first step of the formation of a bacterial biofilm takes place when bacteria come into contact with a surface, followed by the adhesion and proliferation steps [1]. Hence, one of the strategies to prevent the formation of biofilms is using antimicrobial coatings to inhibit or weaken the attachment step.

In the last decades, novel coatings frequently in combination with nanoparticles have been developed to prevent the formation of biofilms. Among antimicrobial polymers (AMP), poly-lysine and polydopamine (PDA) have been investigated, due to their versatility in terms of chemical modification and antimicrobial properties [2]. PDA, a unique nature-inspired polymer, is derived from mussel foot proteins and exhibit exceptional adhesion properties due to the presence of functional groups, such as amines and catechols. Furthermore, it has been shown that the mechanical, chemical, and electrical properties of PDA are strongly dependent on the deposition method as well on the experimental conditions, in which the polymer is studied. For instance, the adhesion properties and the antimicrobial properties of PDA [3] are strongly influenced by pH and the oxidation state of the polymer [4, 5].

In this contribution, atomic force microscopy-based (AFM) force spectroscopy and infrared attenuated total reflection spectroscopy (IR-ATR) are presented to investigate the relation of the surface charge density of PDA with the early stages of bacterial attachment. The effect of the pH on the surface charge density and the point of zero charge of PDA will be also described.

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On-site

Poster session: Tools and modelling

Visualization of glycoconjugates in dental biofilms by fluorescence lectin-binding analysis (FLBA)

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Glycoconjugates are matrix components that play a major role in the adhesion, cohesion, and stability of biofilms. Dental biofilm is a classic example of a complex, disease-related biofilm, but current knowledge on its extracellular glycoconjugate structure derives almost exclusively from studies on monospecies biofilm models of Streptococcus mutans. Fluorescently labelled lectins are carbohydrate-binding proteins with high affinities to different sugar moieties, and they represent a promising tool to map the glycoconjugate architecture in the biofilm matrix. This study investigated the binding properties of 10 selected lectins in in situ-grown dental biofilms. Biofilms (n=192) from three healthy volunteers were grown for 48 h on intraoral splints equipped with glass slabs exposed to sucrose (4%, 8x2 min/day) or physiological saline solution. After growth, biofilms were fixed and stained with the following FITC-labelled lectins: Aleuria aurantia lectin (AAL), Agaricus bisporus agglutinin (ABA), Allium sativum agglutinin (ASA), Helix pomatia agglutinin (HPA), Lycopersicon esculentum agglutinin (LEA), Morniga agglutinin G (MNA-G), Maclura pomifera agglutinin (MPA), Pisum sativum agglutinin (PSA), Vicia graminea agglutinin (VGA) or wheat germ agglutinin (WGA). Microbial cells were counterstained with SYTO 60. The fluorescence signal strength, spatial distribution, and relative matrix biovolumes for each lectin were analyzed by confocal scanning microscopy. Of all investigated lectins, MNA-G, AAL and ASA showed the strongest fluorescence signals and stained the largest biovolumes (170.1%±117.2SD; 133.6%±127.9SD and 114.2%±64.9SD of the microbial biovolume, respectively). These results may point to a hitherto overlooked role for galactose (MNA-G), fucose (AAL) and mannose (ASA) moieties in dental biofilms. MNA-G predominantly visualized cell-free areas in the biofilms, while AAL and ASA bound in both areas of low cell density and inside dense bacterial clusters. AAL and ASA, but not MNA-G showed clearly increased binding in biofilms grown with sucrose exposure, which suggests that they bind with high affinity to sucrose-induced matrix components. This pioneering work shows that FLBA may help to elucidate the complex architecture and metabolism of multispecies biofilms.

On-site

Poster session: Tools and modelling

Sulfated Glycosaminoglycan-like Polymers are Present in an Acidophilic Biofilm from a Sulfidic Cave

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Sulfated glycosaminoglycans (sGAG) are negatively charged polymers that occur in biofilms from various environments. Yet, it is unclear if these if these polymers are produced internally by the biofilm inhabitants, or are acquired externally. Therefore we analyzed the presence of sGAG-like polymers in biofilm samples acquired from Sulfur Cave in Puturosu Mountain (Romania), an environment that is largely inaccesible to contamination. SGAG-like polymers were recovered from the biofilm. Enzymatic treatment with chondroitinase ABC resulted in a decrease of the mass of the polymers, suggesting the structure of the recovered sGAG is similar to chondroitin. Subsequent FT-IR analysis of these polymers revealed a possible presence of polysaccharides and sulfate. Analysis of genomic sequences closely related to those predominant in the acidophilic biofilm, contained genes coding for sulfotransferase (an enzyme needed for the production of sGAG), which supports the hypothesis of microbial synthesis of sGAGs within the biofilm.

On-site

Poster session: Tools and modelling

MiniaTour® : Development of a high-throughput biofilm screening platform.

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Due to their stickiness, biofilms are extremely difficult to remove, thereby often causing technical and economic problems in diverse industry sectors. Therefore, there is a constant need for the development of new (bio)chemical products to remove and/or control biofilms in industrial processes. Proper validation of the products efficacy to remove or reduce biofilm formation requires a testing platform with settings that are controllable, reproducible, and well adapted to product end-application. To this end, we designed and conceptualized a compact bioreactor (MiniaTour®) that allows us to quantify biofilm formation under continuous flow (low shear stress), systematically and with high throughput (16 bioreactors in parallel), under controllable environmental conditions (closed circuit, temperature regulation…). The main objective of MiniaTour® is to monitor the inhibition or formation of biofilm in the system in response to chemical or biological agents that influence the biofilm dynamics. Each tower features a closed one-pot reactor in which growth medium is distributed and recirculated via a peristaltic pump after passage through a biocarrier packing. As a proof of concept, the biofilm reducing potential of a range of new probiotic bacterial strains (PbBs) were investigated using activated sludge as source of the biofilm-forming community (BFC). All tests were carried out in triplicate using BFC as a negative control. Diluted TSB was used as a starter growth media in all reactors. To assess the efficiency of the PbBs, the increase in wet mass of the biofilm was measured every 24h. In addition, the dry mass of the newly formed biofilm was evaluated at the end of the assay. We observed that 3 of the selected PbBs induced a significant wet biofilm reduction of up to 79% in comparison with the BFC control while 3 other PbBs strains caused a significant dry biomass reduction of up to 33%.

During this pilot study, we highlighted the wet and dry biofilm reduction potential of several probiotic bacterial strains. Future perspectives involve investigating the in-situ biofilm reduction potential of the most effective PbBs in association with their in-depth characterization. In conclusion, we demonstrated the ability of the MiniaTour® platform as a promising tool to validate the efficacy of chemical or biological/probiotic biofilm modifying products.

On-site

Poster session: Tools and modelling

Rubiginosin C inhibitory effect against Candidia albicans and Candida auris

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Abstract: In this study, Rubiginosin C was analyzed and isolated from stromatal azaphilone pigments from Hypoxoylon rubiginosum. This compound consists of an orsellinic acid (OA) core structure and a linear polyketide substituted side chain. This OA-carrying rubiginosin C exhibited diversity bioactivities in MIC, cytotoxity, and especially biofilms tests of C. auris. It is a young pathogen caused fungi, which was firstly found and identified in 2009 in a patient from japan. In the recent years, more researches are related and targeted to this strain, as the result of multidrug resistance and responsible for globally infections. No inhibition to the planktonic cells in MIC and cytotoxities to mammalian cell were observed. Furthermore, 2h old biofilm and 24h old biofilm of C. auris were treated with rubiginosin c for 24 h respectively. It performed inhibitory effects on the formation of 2h old biofilm until the concentration when it was applied at 0.13 µg/mL. In comparison with that, it led to an overgrowth of biofilm at the concentration at 250 µg/mL. In addition to this, a time-dependent biofilm assays of 2 h old biofilm with interval of 8 h, 24 h, and 48 h was carried out as well. Rubiginosin c can promise the growth of biofilms at higher concentration and reduce the formation of biofilm even at the nanogram concentration.

Poster Abstracts

Online poster session 2

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Biofilm application

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Online

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Effect of hydrophobic membrane properties on dynamic cell adhesion of microalgae

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Microalgae has a high potential for wastewater purification and the microalgal biomass can be further applied to biofuel production. One of the challenges of microalgal biomass collection is dilute suspended microalgae culture harvesting require a significant amount of energy input. The algal biofilm reactor has received attention in recent years, as the concentrated microalgae biomass can be accomplished during cultivation. The hydrophobic membrane as a substratum for microalgal biofilm cultivation (membrane carbonated biofilm reactor) exhibited high cell adhesion performance and also can promote a higher carbon dioxide utilization efficiency during the microalgal biofilm growth. In this study, we employed 5 different hydrophobic membrane materials (polypropylene, nylon, polytetrafluoroethylene, polyurethane rubber and silicone rubber) as substrata to investigate their capability on the attachment of Chlorella Vulgaris in hydrodynamic conditions. Biomass attachment on the membrane materials was monitored for 16 days of cultivation in a CDC biofilm reactor. The physicochemical surface properties of materials such as contact angle, surface energy and zeta potential were examined to clarify the role of materials properties on algal attachment. Nylon exhibited the greatest increase in biomass (up to 35.9 g/m2), while polytetrafluoroethylene came in second place with 11.7 g/m2. Based on the results, the contact angle alone can not sufficiently elucidate the selectivity of microalgae cells to get attached to a surface. The nylon membrane is suggested as the substratum for further algal biofilm reactor study due to its best adhesion performance.

Keywords: Membrane carbonated biofilm reactor; microalgal biofilm, hydrophobic membrane, surface properties.

Online

Poster session: Biofilm applications

Cu- Polysaccharide Complexes:
Physico-Chemical Characterization and Antimicrobial Activity

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The anion-exchange capacity of the cell-wall sulfated polysaccharide (PS) of the red marine microalga Porphyridium sp. can be used as a platform for loading metals (e.g., Cu) to produce complexes- novel materials with bioactivities. First, we investigated PS as a platform for the incorporation of copper in the form of Cu2O and CuSO4. The complexes Cu2O–PS, and CuSO4–PS were physicochemically and structurally characterized, and their antibiofilm activity was evaluated. Significant differences were found between the two complexes as expressed in all parameters examined. A new covalent bond was detected in the Cu2O–PS complex (FT-IR) and higher antibiofilm activities were measured, especially against C. albicans. And needle-like structures (spikes) were detected in the Cu2O–PS complex protruding from the complex surface to a maximum height of 1,000 nm and 10 nm thickness and density of about 5,000/μm2. Similar spikes were not detected in the native PS nor the CuSO4–PS complex. It is suggested that the spikes on the surface of the Cu2O–PS complex are responsible for the high antimicrobial activities of the complex, i.e., for disruption of the microbial membrane, leading to cell death. To understand the mode of action of the Cu-PS complexes, we focused on the aspect of Cu valences. For this, monovalent (Cu2O, CuCl) and divalent (CuO, CuCl2) Cu-complexes were prepared. They were characterized in terms of surface morphology, physicochemical properties, and antimicrobial activities. So far, significant differences exist between the monovalent and divalent complexes in terms of their physicochemical characteristics, antimicrobial activities, and surface topographies. Again, the monovalent complexes are different than the divalent complexes that had similar effects as the CuSO4–PS complex. The surface topography of the monovalent Cu-complexes was characterized by spikes that protruded up to 1,000nm above the surface, their thickness 10nm, and density of 5,000 spikes/μm2, whereas in the divalent complexes they were 24 nm above the surface. The monovalent Cu-complexes were the most effective against C. albicans, E. coli, and B. subtilis and as compared with the divalent Cu-complexes, Cu salts alone, and the native PS. The next steps of this research will be focusing on the spike's morphology, their structural and chemical characterization, and their role in antimicrobial activity.

Online

Poster session: Biofilm applications

Biofilms of agronomic importance: interactions among plant growth-promoting rhizobacteria display potential in the development of biofertilizers

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Since the 1990s Argentina and other countries in Latin America rapidly became soybean producers. Genetically modified soybeans were engineered to be herbicide resistant, and their incorporation along with no-tillage practices led to a rapid increase in the use of agrochemicals with detrimental effects to the environment. A way to promote sustainable agriculture in developing countries is through the use of biofertilizers (based on plant growth-promoting rhizobacteria or PGPR) that help to preserve soil health and productivity. However, there are constraints affecting the effectiveness of current biofertilizers on crop yields, as survival in adverse environments or competition with native soil microbiota. In this regard, the use of PGPR biofilms is envisaged as a means to develop a new generation of biofertilizers with higher efficiency and improved field results.

The strains selected were Bradyrhizobium japonicum E109 and Azospirillum brasilense Az39. B. japonicum nodulates soybean roots carrying out N2 biological fixation, while A. brasilense is a free-living N2-fixing bacterium, that also stimulates root growth through auxin production. We have established conditions in which both strains can co-exist and also positively influence each other. Mixed Az39-E109 biofilms increased 45% from day 4 on, compared to mono-species biofilms. The presence of strain Az39 stimulated exopolysaccharide production by B. japonicum E109, an exopolymer of great importance for biofilm formation, root infection and nodulation. Moreover, A. brasilense Az39 increased morphological differentiation into cysts in the presence of strain E109. Cysts are ovoid encapsulated cells, more adhesive than vegetative cells. We observed abundance of cyst-like forms in Az39 biofilms. This phenomenon is of great interest since cyst formation and biofilm production were proposed mechanisms enabling free-living nitrogen-fixing bacteria to efficiently fix N2 in an aerobic environment.

CLSM images of mixed Az39-E109 biofilms showed the segregation of strains with a particular pattern, probably related to nutrient and/or oxygen availability. Adhesion to soybean seeds was evaluated, showing an improvement in the ability to adhere to seeds when Az39 and E109 are co-cultured. This could be explained by the augmented adhesion properties identified in the co-cultures (E109 exopolysaccharide production and Az39 cyst formation), opening the way towards the use of mixed biofilms as biofertilizers

Online

Poster session: Biofilm applications

Inadequate dosing of THPS treatment increases MIC of pipeline steel by inducing biofilm growth

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Biocides are often used to mitigate the microbially influenced corrosion (MIC) of construction materials in many fields. To study the effect of inadequate dosing of non-oxidizing biocide tetrakis (hydroxymethyl) phosphonium sulfate (THPS) on corrosion of pipeline steel caused by microorganisms, a novel marine isolate Desulfovibrio hontreensis SY-21 was selected as a test microorganism. Weight loss rate determination, morphological analyses, and corrosion product analyses combined with electrochemical measurements were performed to investigate the influence of THPS on the MIC of X70 pipeline steel. The responses of sessile and planktonic cells of D. hontreensis to THPS were also studied. Results showed that D. hontreensis cells could significantly promote steel corrosion and induce local corrosion pits. With a THPS addition within the tolerance range of D. hontreensis for the biocide, MIC of the steel was further promoted by 65%. The growth of planktonic cells was inhibited by the biocide, but the number of biofilm cells was significantly increased. This study revealed that THPS concentrations within a specific range increased the corrosive effect of the presence of D. hontreensis by promoting the growth of sessile cells and biofilm formation. Therefore, the use of the biocide in practical applications needs to be properly considered and managed.

Online

Poster session: Tools and modelling

Regulation of the Pseudomonas putida lifestyle switch by the stringent response

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Biofilm dispersal is a genetically programmed response enabling bacterial cells to exit the biofilms in response to particular physiological or environmental conditions. In Pseudomonas putida biofilms, nutrient starvation triggers c-di-GMP hydrolysis by phosphodiesterase BifA, resulting in proteolysis of the adhesin LapA and the subsequent release of biofilm cells. We have demonstrated that the stringent response, a ubiquitous bacterial stress response mediated by the alarmone (p)ppGpp, is accountable for relaying the nutrient stress signal to the biofilm dispersal machinery. Mutants lacking elements of the stringent response were defective in biofilm dispersal while ectopic (p)ppGpp synthesis restored biofilm dispersal in a (p)ppGpp null mutant. In vivo gene expression analysis showed that (p)ppGpp positively regulated transcription of bifA. Differential gene expression analysis on RNA-seq data from a (p)ppGpp null mutant revealed new stringent response targets related to biofilm development and flagellar biogenesis, including genes involved in alginate and Pea exopolysaccharides biosynthesis, as well as genes encoding adhesin lapF, phosphodiesterase yegE and motility regulators hsbAR-hptB. These results suggest that the stringent response modulates the lifestyle switch of P. putida by different pathways.

Online

Poster session: Tools and modelling

An non-instrusive electrical impedance technique for monitoring P. Fluorescens biofilm development on metallic surfaces

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Although quite a significant number of analytical techniques exist to characterize biofilms, online monitoring methods are limited, especially as regards their application in water systems. In this study, a highly sensitive, online electrical impedance spectroscopy technique was developed to monitor in real time the growth of biofilms formed on metallic surfaces inside continuous flow water systems. Single strain biofilms of Pseudomonas fluorescens were progressively developed in a laboratory-scale water flow test setup with flexible design capable of generating various hydrodynamic conditions met in water systems. Biofilm build-up over time was monitored with continuous recording of electrical signals. Increase of impedance was attributed to increase of surface area coverage with biofilm and increase of biofilm thickness. The latter two quantities were independently estimated using imaging tools, i.e., tested surfaces (coupons) on which biofilms were formed, were subjected to 3D surface metrology microscopic analysis to determine the extent of coverage and structural characteristics of biofilms. The technique developed in this study was capable of providing high resolution data to depict slight impedance changes during cells attachment and biofilm formation and growth. Proper configuration and tuning of the electronics along with proper grounding diminished electrical interferences and yielded superb sensitivity and accuracy in sensing impedance changes. Combination of electrical signals with surface coverage from optical means permitted for the first time in situ estimation of biofilm thickness on meatllic surfaces in the order of a few micrometers.