proceedings
of the

biofilms 9

online conference

29 September 2020 – 01 October 2020
Karlsruhe, Germany

Table of Contents

	page
Preface	3
Schedule	4
Exhibitors	5
Sponsors	6
Topic 1 – Biofilm Control	**7**
Oral Presentations	*8*
Poster Presentations	*21*
Topic 2 – Biofilm Heterogeneity	**84**
Oral Presentations	*85*
Poster Presentations	*93*
Topic 3 – Biofilm Matrix	**106**
Oral Presentations	*107*
Poster Presentations	*114*
Topic 4 – Productive Biofilms (syngas, bioelectrochemical)	**135**
Oral Presentations	*136*
Poster Presentations	*144*
Topic 5 – Biofilm Lifecycle and its Regulation	**167**
Oral Presentations	*168*
Poster Presentations	*173*
Topic 6 – Synthetic, Artificial Biofilm Development and its Optimisation	**194**
Oral Presentations	*195*
Poster Presentations	*199*

Preface

Biofilms is a well-established conference series for biofilm research in Europe. The biofilms conference series was launched in 2004, based on the idea of Rainer Abraham, Thomas Neu and Hans-Curt Flemming. The first conference took place in Osnabrück, Germany in a meeting during 2004 organised by Wolfgang Streit's lab and Dirk Wenderoth. The conference was received very positively and thus repeated every 2 years. At the biofilms 3 conference in Munich, Thomas Neu indicated in his closing remarks that the biofilms series should become European. Since then, the biofilms conference series has established itself in Europe for non-medical biofilm research and is attracting more and more participants.

Due to the current COVID-19 situation we decided that **biofilms 9** will take place as online conference only starting on 29 September 2020 at 11:00 am (CEST) and ending on 1 October 2020 6:00 pm (CEST). Especially regarding the current situation, we are happy to chair a lively conference with more than 150 abstracts submitted.

We are looking forward to virtually meet you at the first online biofilms conference!

Prof. Dr. Harald Horn, Karlsruhe Institute of Technology, Engler-Bunte-Institut, Chair of Water Chemistry and Water Technology

Prof. Dr. Johannes Gescher, Karlsruhe Institute of Technology, Institute for Applied Biosciences

Previous *biofilms* conferences

2004 – **1st European conference on biofilms** - prevention of microbial adhesion
Deutsche Bundesstiftung Umwelt, Osnabrück, Germany

2006 – **biofilms 2** Attachment and Detachment in Pure and Mixed Cultures
Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

2008 – **biofilms 3** international conference
Technical University, Munich, Germany

2010 – **biofilms 4** international conference
Winchester, UK

2012 – **biofilms 5** international conference
Paris, France

2014 – **biofilms 6** international conference
Vienna, Austria

2016 – **biofilms 7** international conference
Porto, Portugal

2018 – **biofilms 8** international conference
Aarhus, Denmark

Schedule

	Topic 1	Topic 2	Topic 3	Topic 4	Topic 5	Topic 6
Chair	Michael Wagner	Katrin Sturm-Richter	Per Halkjær Nielsen	Johannes Gescher	Regine Hengge	Ioannis Ieropoulos
Co-chair	Fernando Morgan-Sagastume	Knut Drescher	Harald Horn	Katja Bühler	Hans-Curt Flemming	Kersten Rabe

Day 1, Tuesday, 29.09.2020

Session	Time	Duration	Session
	11:00 – 11:10	10 min	**Welcome**
Session 1 Biofilm (Structure) Control	11:10 – 11:40	30 min	**Invited talk by Fernando Morgan-Sagastume** AnoxKaldnes Malmö, Schweden
Session 1 Biofilm (Structure) Control	11:40 – 12:00	20 min	**ID 140** **Recupido, Karapantios, Greece/Italy, UTC +2.5**
Session 1 Biofilm (Structure) Control	12:00 – 12:20	20 min	**ID 14** **Vigue, Ploux, France, UTC +2**
Session 1 Biofilm (Structure) Control	12:20 – 12:40	20 min	**ID 16** **Fish, Boxall, UK, UTC +1**
Session 1 Biofilm (Structure) Control	12:40 – 13:00	20 min	**ID 35** **Magalhaes, Wright, UK, UTC +1**
	13:00 – 13:40	40 min	Break Exhibitor: JPK BioAFM, Bruker Nano
Session 1 Biofilm (Structure) Control	13:40 – 14:00	20 min	**ID 53** **Gudzuhn, Streit, Germany, UTC +2**
Session 1 Biofilm (Structure) Control	14:00 – 14:20	20 min	**ID 146** **Bajrami, Mizaikoff, Germany, UTC +2**
Session 1 Biofilm (Structure) Control	14:20 – 14:40	20 min	**ID 117** **Papadatou, Salter, UK, UTC +1**
Session 1 Biofilm (Structure) Control	14:40 – 15:00	20 min	**ID 106** **Li, Nerenberg, USA, UTC -4**
Session 1 Biofilm (Structure) Control	15:00 – 15:20	20 min	**ID 121** **Guo, Davies, Canada, UTC -4**
	15:20 – 15:30	10 min	Break
Session 2 Biofilm Heterogeneity	15:30 – 16:00	30 min	**Invited talk by Knut Drescher** Max Planck Institute for Terrestrial Microbiology Marburg, Germany
Session 2 Biofilm Heterogeneity	16:00 – 16:20	20 min	**ID 31** **Chodorski, Ulber, Germany, UTC +2**
Session 2 Biofilm Heterogeneity	16:20 – 16:40	20 min	**ID 26** **Jeckel, Drescher, Germany, UTC +2**
	16:40 – 16:50	10 min	Break
Poster Session	16:50 – 18:20	90 min	**1 Biofilms Control** 2x 23 Posters

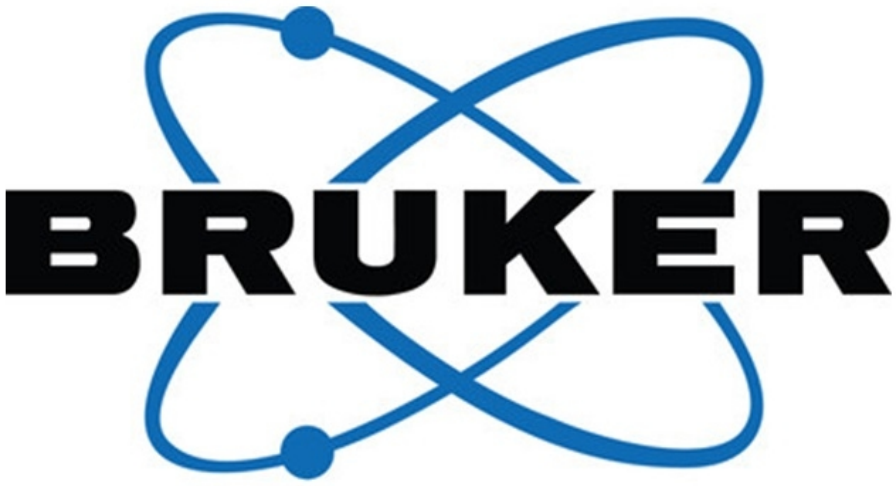
Day 2, Wednesday, 30.09.2020

Session	Time	Duration	Session
	11:00 – 11:10	10 min	**Welcome**
Session 2 Biofilm Heterogeneity	11:10 – 11:30	20 min	**ID 64** **Youssef, Henry, France, UTC +2**
Session 2 Biofilm Heterogeneity	11:30 – 11:50	20 min	**ID 66** **Longyear, Stoodley, UK, UTC +1**
Session 2 Biofilm Heterogeneity	11:50 – 12:10	20 min	**ID 104** **Lyng-Røder, Burmølle, Denmark, UTC +2**
Session 3 Biofilm Matrix	12:10 – 12:40	30 min	**Invited talk by Per Halkær Nielsen** Aalborg University Aalborg, Sweden
Session 3 Biofilm Matrix	12:40 – 13:00	20 min	**ID 10** **Secchi, Stocker, Switzerland, UTC +2**
	13:00 – 13:40	40 min	Break Exhibitor: Thorlabs GmbH
Session 3 Biofilm Matrix	13:40 – 14:00	20 min	**ID 11** **Devlin, Casey, Ireland, UTC +1**
Session 3 Biofilm Matrix	14:00 – 14:20	20 min	**ID 85** **van den Berg, de Kreuk, Netherlands, UTC +2**
Session 3 Biofilm Matrix	14:20 – 14:40	20 min	**ID 152** **Tarsitano, Zorreguieta, (BA), Argentina, UTC -3**
Session 3 Biofilm Matrix	14:40 – 15:00	20 min	**ID 112** **Steinbach, Yunker, Atlanta, US, UTC -4**
	15:00 – 15:30	30 min	**Invited talk Thomas Seviour** **"Phase transitions in the extracellular matrix mediate aggregation and biofilm formation"**
	15:30 – 15:40	10 min	Break
Session 4 Productive Biofilms	15:40 – 16:10	30 min	**Invited talk by Alfred M. Spormann** Stanford University Stanford, USA
Session 4 Productive Biofilms	16:10 – 16:30	20 min	**ID 54** **Pillot, Kerzenmacher, Germany, UTC +2**
	16:30 – 16:40	10 min	Break
Poster Session	16:40 – 18:10	90 min	**2 Biofilms Heterogeneity** **3 Biofilms Matrix** 2x 19 Posters

Day 3, Thursday, 01.10.2020

Session	Time	Duration	Session
	11:00 – 11:10	10 min	**Welcome**
Session 4 Productive Biofilms	11:10 – 11:30	20 min	**ID 55** **Hackbarth, Horn, Germany, UTC +2**
Session 4 Productive Biofilms	11:30 – 11:50	20 min	**ID 65** **Gomes, Mergulhao, Portugal, UTC +1**
Session 4 Productive Biofilms	11:50 – 12:10	20 min	**ID 80** **Bühler, Karande, Germany, UTC +2**
Session 4 Productive Biofilms	12:10 – 12:30	20 min	**ID 158** **Edel, Gescher, Germany, UTC +2**
	12:30 – 13:10	40 min	Break
Session 5 Biofilm Lifecycle and its Regulation	13:10 – 13:40	30 min	**Invited talk by Regine Hengge** Humboldt University Berlin, Germany
Session 5 Biofilm Lifecycle and its Regulation	13:40 – 14:00	20 min	**ID 71** **Morales, Lasa, Spain, UTC +2**
Session 5 Biofilm Lifecycle and its Regulation	14:00 – 14:20	20 min	**ID 32** **Kasparova, Martatkova, Czechia, UTC +2**
Session 5 Biofilm Lifecycle and its Regulation	14:20 – 14:40	20 min	**ID 20** **Boon, Boon, NY, US, UTC -4**
	14:40 – 14:50	10 min	Break
Session 6 Synthetic, artificial biofilm development and its optimisation	14:50 – 15:20	30 min	**Invited talk by Ioannis Ieropoulos** UWE Bristol Bristol, UK
Session 6 Synthetic, artificial biofilm development and its optimisation	15:20 – 15:40	20 min	**ID 128** **Volke, Nikel, Denmark, UTC +2**
Session 6 Synthetic, artificial biofilm development and its optimisation	15:40 – 16:00	20 min	**ID 56** **Hayta, Lieleg, Germany, UTC +2**
Session 6 Synthetic, artificial biofilm development and its optimisation	16:00 – 16:20	20 min	**ID 72** **Ma, Johnson, Switzerland, UTC +2**
	16:20 – 16:30	10 min	Break
Poster Session	16:30 – 18:00	90 min	**4 Productive Biofilms** **5 Biofilm Lifecycle** **6 Synthetic Biofilm Development** 22 + 21 Posters
	18:00 – 18:10	10 min	**Closing Remarks**

Exhibitors



Sponsors

National Biofilms Innovation Centre

C3 PROZESS- UND ANALYSENTECHNIK GmbH

npj | biofilms and microbiomes

pyroscience
sensor technology

SYMCEL

Funding

DFG Deutsche Forschungsgemeinschaft
German Research Foundation

Topic 1
Biofilm Control

The optimal performance of industrial processes often depends on the control over biofilm growth and distribution. Hence, we welcome contributions describing the newest methods for both biofilm monitoring and control at the biofilms 9 conference. Applications can range from drinking water and membrane processes to processes in traditional industries with water reuse (pulp and paper, food, etc.). Especially, advanced examples or cutting-edge research projects combining, for example, on-line monitoring and disinfection/cleaning strategies based on monitoring data are highly appreciated.

Oral Presentations

biofilms9-140
https://doi.org/10.5194/biofilms9-140
biofilms 9 conference

Wetting properties of biofilm-coated surfaces produced at controlled shear flow conditions

Federica Recupido[1], Maria Petala[1], Margaritis Kostoglou[1], Sergio Caserta[2], Stefano Guido[2], and Thodoris D. Karapantsios[1]

[1]Aristotle University of Thessaloniki, Chemistry, Greece (federecu@chem.auth.gr)

[2]Department of Chemicals, Materials and Production Engineering, University of Naples, Federico II, Italy

Biofilms prevention and removal are crucial in many industrial and medical applications. Their complex and cohesive structure provides resistance to cleaning even to strong disinfectants. A key factor for their behavior is the wetting properties of their surfaces.

The main goal of this work is to study the wetting properties of biofilms produced by bacteria *Pseudomonas fluorescens*. Biofilms are obtained on glass coupons under well controlled flow conditions, using custom-made flow cell devices. Different nutrient concentration and shear flow conditions are investigated.

Biofilm wetting properties are examined under imposed external body forces (forced wetting) through a specialized device, named Kerberos®. Kerberos® is capable of subjecting sessile droplets to varying tilting angles and centrifugal forces while monitoring the variation of the droplet shape in X, Y and Z-directions through three Wi-Fi cameras. Wetting experiments are carried out using water-based solution (dye solution) droplets on biofilm-coated glass coupons. In this work, spreading/sliding behaviour of droplets are investigated only on horizontal substrates (no tilting) under the action of centrifugal forces. Apart from wetting properties, biofilm growth kinetics and surface morphology at different nutrient and shear flow conditions are also assessed.

Results show that, according to the different growth conditions, biofilms present different wetting properties. At lower nutrient concentration and shear flow conditions, spreading and sliding behaviour are similar to that observed in glass coupons in the absence of biofilm. At higher nutrient and shear flow conditions, spontaneous wicking of the biofilm occurs the moment of droplet deposition on the biofilm leading to irregular and jagged shapes of droplets, while on the contrary water droplets look like smooth spherical sections on pure glass. The spontaneous wicking affects the droplet initial shape and so the wetting behaviour during the subsequent rotation tests. In each examined condition, biofilms show hydrophilic properties.

How to cite: Recupido, F., Petala, M., Kostoglou, M., Caserta, S., Guido, S., and Karapantsios, T. D.: Wetting properties of biofilm-coated surfaces produced at controlled shear flow conditions, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-140, https://doi.org/10.5194/biofilms9-140, 2020

biofilms9-14
https://doi.org/10.5194/biofilms9-14
biofilms 9 conference

Soft coatings, a new antimicrobial strategy for biomaterials?

Annabelle Vigué[1], Dominique Vautier[1], Julie Hardouin[2], Youri Arntz[1], Vincent Ball[1], Bernard Senger[1], Thierry Jouenne[2], and Lydie Ploux[1]

[1]CNRS/INSERM/University of Strasbourg, U1121 Biomaterials and Bioengineering, France (ploux@unistra.fr)

[2]CNRS/University of Rouen/INSA Rouen, UMR 6270 Polymers-Biopolymers-Surfaces, France

Fighting microbial biofilms on biomaterials is usually addressed by incorporating antimicrobial agents. Nevertheless, as usual in the natural life, intrinsic properties of the material surface can also be a complementary approach. They may drastically reduce the quantity of adhered microorganisms and the remaining microorganisms can be treated with classical antimicrobial agents. Mechanical properties of material surfaces recently emerged as a possible way to impact biofilm formation, but many questions have still to be elucidated so far.

We have especially investigated whether hydrogel and non-hydrogel soft and stiff films may differently impact, microbial behavior and biofilm formation. The films have been thoroughly characterized in terms of viscoelasticity, hydration and chemistry. Microbial mobility, adhered quantity and production of organelles such as pili have been specifically investigated. Surface properties, especially mechanical ones, have been thoroughly characterized. The study has been conducted with yeast (Candida albicans) and bacteria species (Escherichia coli) as models. Our results reveal that the stiffness differently impacts the amount and mobility of the adhered cells according to the nature of the film. These softness- and hydration-dependent microbial phenomena also vary with bacteria and yeast species.

Finally, this confirms the relevance of using some soft coatings to prevent biofilm formation on a material but also clarifies the risks to get opposite effects as desired if other crucial surface properties have not been associated.

How to cite: Vigué, A., Vautier, D., Hardouin, J., Arntz, Y., Ball, V., Senger, B., Jouenne, T., and Ploux, L.: Soft coatings, a new antimicrobial strategy for biomaterials?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-14, https://doi.org/10.5194/biofilms9-14, 2020

biofilms9-16
https://doi.org/10.5194/biofilms9-16
biofilms 9 conference

Residual-chlorine concentration impacts the ecology of biofilms in drinking water pipes and their water quality response

Katherine Fish[1], Paul Gaskin[2], and Joby Boxall[1]
[1]University of Sheffield, University of Sheffield, Civil and Structural Engineering, United Kingdom of Great Britain and Northern Ireland (k.fish@sheffield.ac.uk)
[2]Dwr Cymru Welsh Water, Newport, Wales, UK

Drinking water distribution systems (DWDS) are an engineered system designed to protect water quality during delivery from treatment works to consumers' taps. Biofilms form on the vast internal surfaces of DWDS, impacting water quality by their activity and/or mobilisation into the bulk-water. Disinfection-residuals are often maintained in drinking water to mitigate planktonic microbial contamination (and associated water quality/health risks). However, the impact of residual-disinfection upon biofilms, and the subsequent unintended risk they may present to water quality, is unclear.

To address this, an internationally-unique, temperature-controlled, full-scale DWDS test facility, fed with water from the local DWDS, was used to grow biofilms (for 28 days). The facility enables three simultaneous conditions to be run in replicate pipe loops (each ~200m long, 79mm internal diameter, PE100 pipe). Conditions studied were Low-, Medium- and High-chlorine regimes. Various water quality parameters were monitored throughout, biofilms were sampled every two weeks (n=5). Physical, chemical and molecular analyses were applied to characterise the matrix (structure and composition) and microbial communities (via analysis of bacterial 16S rRNA and fungal ITS genes) of biofilms developed under the different chlorine regimes. After growth, a "mobilisation" test was conducted simulating hydraulic changes that occur in DWDS. Biofilms from each chlorine regime were exposed to increasing shear stresses to determine any water quality degradation as a consequence of biofilm mobilisation.

High-chlorine residual concentration during development reduced biofilm bacterial concentrations but increased inorganics and selected for unique bacterial and fungal communities. Ultimately the biofilms developed under a High-chlorine residual resulted in the greatest decrease in water quality, in response to mobilisation, and the Low-chlorine regime resulted in biofilms which had the lowest impact on water quality. These unanticipated findings suggest chlorine-boosting should be considered carefully and may actually exacerbate water quality issues. The derived understanding could impact the long-term management of DWDS water quality and biofilm, whilst challenging the current mind-set of continuous residual-disinfection control strategies.

How to cite: Fish, K., Gaskin, P., and Boxall, J.: Residual-chlorine concentration impacts the ecology of biofilms in drinking water pipes and their water quality response, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-16, https://doi.org/10.5194/biofilms9-16, 2020

biofilms9-35
https://doi.org/10.5194/biofilms9-35
biofilms 9 conference

Real-Time Monitoring of Industrial Biofilms using Confocal Raman Microscopy and Multivariate Analysis

Ana Magalhaes[1,2,3], Pola Goldberg Oppenheimer[1], Tim Overton[1,2], and Kevin Wright[3]
[1]School of Chemical Chemical Engineering, University of Birmingham, Birmingham, United Kingdom
[2]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom
[3]Procter & Gamble, Greater London Innovation Centre, Egham, United Kingdom

Biofilm development in industrial settings can prove costly to manufacturing and consumer health. The presence of contaminants in raw materials, finished products and on process contact surfaces negatively impacts on product quality and safety. Rapid and accurate identification of spoilage and pathogenic microorganisms is crucial to implement effective biofilm control strategies that enhance product safety. The application of confocal Raman microscopy (CRM) for non-invasive and rapid characterisation of clinical and food isolates has been reported. The question remains whether the technique can be used as an online monitoring tool for real-time measurement of biofilm build-up in dynamic manufacturing conditions.

In this study we investigated if CRM could be used in the manufacturing environment as an alternative microbiological quality control method. We assessed if this technology is able to differentiate between bacterial species and their growth phenotype, as well as detect contaminants from process samples.

Laboratory and industrial isolates grown under different culture conditions (planktonic, agar plates, and CDC grown biofilms), and formulated products were analysed using a confocal Raman microscope (Thermofisher DXR2xi) under optimised settings. Reference and experimental Raman spectra were collected and analysed for all test conditions [1]. Spectral similarities were evaluated by developing a microbial multivariate predictive model using a two-way orthogonal partial least squares (O2PLS) regression for cluster analysis [2].

Optimal spectra for microbes were obtained in the fingerprint region at approximately 600 - 1800 cm^{-1} where characteristic peaks could be assigned to different biological macromolecules (nucleic acids, proteins, lipids and carbohydrates). Cluster analysis showed good group separation with low variation within but high variation between bacterial strains, enabling bacterial differentiation. It also highlighted the variations observed in the spectral fingerprint for planktonic, agar and biofilm growth modes. Comparative studies suggest that the peak associated with nucleotide ring stretching (~ 700 cm^{-1}) could be used as a microbial marker for contamination in formulation.

Our findings indicate that confocal Raman microscopy can be used for at-line monitoring of contamination in product streams. Raman spectra provide biochemical data for microbial characterisation but variations in the spectra are often difficult to observe and interpret. Multivariate statistical methods permit rapid interrogation of spectral data, with the potential to improve microbial identification. In combination with multivariate analysis, CRM can be used as an analytical tool for rapid identification of industrial isolates and differentiation of their growth phenotype.

References

[1] Beier, B.D., R.G. Quivey, and A.J. Berger,Raman microspectroscopy for species identification and mapping within bacterial biofilms.AMB Express, 2012. **2**(1): p. 35-35.

[2] Zou, X., et al., Automatic Spectroscopic Data Categorization by Clustering Analysis (ASCLAN): A Data-Driven Approach for Distinguishing Discriminatory Metabolites for Phenotypic Subclasses.Analytical Chemistry, 2016. **88**(11): p. 5670-5679.

How to cite: Magalhaes, A., Goldberg Oppenheimer, P., Overton, T., and Wright, K.: Real-Time Monitoring of Industrial Biofilms using Confocal Raman Microscopy and Multivariate Analysis, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-35, https://doi.org/10.5194/biofilms9-35, 2020

biofilms9-53, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-53
biofilms 9 conference

Substances based on natural fungal compounds inhibit the biofilm of various Stenotrophomonas maltophilia isolates

Mirja Gudzuhn[1], Ifey Alio[1], Jörg Steinmann[2], Nina Schützenmeister[3], and Wolfgang R. Streit[1]
[1]Department of Microbiology and Biotechnology, IPM Klein Flottbek, Universität Hamburg, Hamburg, Germany
[2]Medical Microbiology and Clinical Infectiology, Institute for Clinical Hygiene, Universitätsinstitut der Paracelsus Medizinischen Privatuniversität Klinikum Nürnberg, Nürnberg, Germany
[3]Institute for pharmacy, Universität Hamburg, Hamburg, Germany

Stenotrophomonas maltophilia is a multidrug resistant human nosocomial opportunistic pathogen. It contributes to disease progression in cystic fibrosis patients and is found in wounds, other infected tissues and on catheter surfaces. *S. maltophilia* is globally distributed and forms 23 distinct phylogenetic clusters (1, 2). Due to its multidrug resistance, it is extremely difficult to heal *S. maltophilia* caused infections. Colistin is a last-resort antibiotic against multidrug resistant pathogens. However, this study reveals that the minimal inhibitory concentration (MIC) of colistin varies strongly between 22 tested clinical isolates by ranging from 6.25 - >100 µg/ml. The minimal biofilm inhibitory concentration (MBIC) was detected to be much higher. On 41% of the isolates, colistin proved to be very effective on planktonic cells (MIC-value ≤6.25 µg/ml), but less effective on biofilm cells represented by only 18% of the isolates (MBIC-value <100 µg/ml). Thus, we screened for substances, which prevented specifically the biofilm formation or were involved in the removal of established biofilms. We identified several natural fungal compounds and synthetically produced analogues that affect the biofilm of *S. maltophilia*. In microtiter plate assays, the three substances HH-R6, HH-R8 and HH-R9, which belong to the rubrolides, had with 63 - 83 % the strongest biofilm reduction effect on the biofilm of *S. maltophilia* K279a. However, microscopy of the biofilms still revealed some living adhered cells although the biofilm structure was strongly impaired. Furthermore, the antibiofilm effect and the impact on the biofilm structure varied strongly among different clinical *S. maltophilia* isolates. Ongoing transcriptome analyses are expected to shed light on the biofilm inhibiting mechanism of these substances and to get further evidences how they can be used in a clinical setting in the future.

1 Steinmann J., Mamat U., Abda E.M., *et al*. Analysis of Phylogenetic Variation of *Stenotrophomonas maltophilia* Reveals Human-Specific Branches. Front Microbiol. 2018, 9:806 (2018). doi:10.3389/fmicb.2018.00806

2 Gröschel, M.I., Meehan, C.J., Barilar, I. *et al*. The phylogenetic landscape and nosocomial spread of the multidrug-resistant opportunist *Stenotrophomonas maltophilia*. Nat Commun 11, 2044 (2020). https://doi.org/10.1038/s41467-020-15123-0

How to cite: Gudzuhn, M., Alio, I., Steinmann, J., Schützenmeister, N., and Streit, W. R.: Substances based on natural fungal compounds inhibit the biofilm of various Stenotrophomonas maltophilia isolates, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-53, https://doi.org/10.5194/biofilms9-53, 2020

biofilms9-146
https://doi.org/10.5194/biofilms9-146
biofilms 9 conference

IR-ATR spectroscopy for *in situ* long-term monitoring of *Lactobacillus parabuchneri* biofilms

Diellza Bajrami[1], Christine Kranz[1], Stephan Fischer[2], Holger Barth[2], Maria C. Sportelli[3], Nicola Cioffi[3], and Boris Mizaikoff[1]

[1]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Ulm, Germany (diellza.bajrami@uni-ulm.de)

[2]Institute of Pharmacology and Toxicology, Ulm University Medical Center, Ulm, Germany

[3]Chemistry Department, University of Bari "Aldo Moro", Bari, Italy

Microbial biofilms are a crucial problem in many areas including the food processing industry, biotechnology, water quality and medical scenarios. The complexity of biofilm formation and subsequent prevention strategies - requires a fundamental understanding of the involved molecular mechanisms and the possibility of long-term monitoring biofilm formation. Infrared attenuated total reflection (IR-ATR) spectroscopy is a versatile analytical technique for monitoring biofilm formation of bacteria isolates in situ, non-destructively, and close to real time as an innovative approach providing molecular insight into biofilm formation [1]. The utility of IR-ATR to investigate microorganism behavior within biofilms derives from the evanescent field penetrating few micrometers into the biofilm formed directly at the interface of a multi-reflection ATR waveguide and the sample. In the present study, isolates from food biogenic amine (BA)-producing bacteria, *Lactobacillus parabuchneri* DSM 5987 strains formed in cheese are analyzed for developing a deeper understanding on the formation of biofilms, which are significant contributors to the presence of histamine in dairy food products [2]. Infrared spectra were recorded using a custom flow-through ATR assembly for revealing the metabolism of microorganisms within such biofilms along with the effects of the substrate functionality and culture conditions on the extracellular biopolymeric matrices [3,4]. The appearance of key IR bands in the region of 1600-1200 cm^{-1} indicates the production of lactic acid or lactate and the presence of amide groups, while most pronounced intensities in 1140-950 cm^{-1} correspond to phospholipids, polysaccharides and nucleic acids. In this study, the spectral region between 1700 and 600 cm^{-1} was determined to be the representative region for the identification of *Lactobacillus parabuchneri* biofilms enabling to study bioadhesion mechanisms and physico-chemical property changes during extended periods of biofilm growth. Real time monitoring has led to concrete steps for inhibition and disintegration via suitable antimicrobials by deposition on the IR inactive region of ATR waveguide. Multivariate data evaluation and classification strategies were applied to enable efficient multiparametric analysis for providing molecular information facilitating a better understanding of biofilm formation, maturation and changes in biofilm architecture via IR spectroscopic data.

Keywords: IR-ATR spectroscopy, *in situ* monitoring, *Lactobacillus parabuchneri*, biofilm, ATR waveguide, flow-through ATR, lactic acid, multivariate data analysis.

References: [1] Stenclova P, Freisinger S, et al. *Appl. Spectro.*, **2019**; Vol.73 (4) 424-432 [2] Yunda E, Quilès F, et al. *Biofouling*, **2019**; Vol.35 (5) 494-507 [3] Diaz M, del Rio B, et al. *Food Microbiol.,* **2016**; Vol.7 (591) 85-91 [4] Lorite G, de Souza A, et al. *Colloids Surfaces B. Biointerfaces*, **2013**; Vol. 102 519-525

How to cite: Bajrami, D., Kranz, C., Fischer, S., Barth, H., Sportelli, M. C., Cioffi, N., and

Mizaikoff, B.: IR-ATR spectroscopy for in situ long-term monitoring of Lactobacillus parabuchneri biofilms , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-146, https://doi.org/10.5194/biofilms9-146, 2020

biofilms9-117
https://doi.org/10.5194/biofilms9-117
biofilms 9 conference

Functionality and composition of marine biofilms on antifouling coatings.

Maria Papadatou[1], Samuel Robson[2], Joy Watts[1,2], Sergey Dobretsov[3], and Maria Salta[1]
[1]School of Biological Sciences, University of Portsmouth, King Henry I St PO1 2DY, Portsmouth, UK
[2]Centre for Enzyme Innovation, University of Portsmouth, White Swan Road PO1 2DT, Portsmouth, UK
[3]Center of Excellence in Marine Biotechnology, Sultan Qaboos University, Al Khoud 123, PO Box 50, Muscat, Oman

Marine biofilms are assemblages of microbial cells irreversibly attached to living or non-living surfaces, embedded in a self-produced matrix of hydrated extracellular polymeric substances (EPS). The phenomenon of biofouling occurs upon the adhesion and accumulation of biofilms, composing the primary colonizers that are capable of EPS production, followed by the sequential growth of secondary colonizers on submerged structures. Biofouling constitutes a significant issue in marine industries (e.g. maritime transportation) and problems related to biofilm fouling include an increase in drag force, modification of surface properties (e.g. metal corrosion) and production of chemical compounds with inhibition effects to other foulers. The use of powerful biocides exhibits a good performance against biofouling, however, often their efficacy is evident to a lesser degree against biofilms. These chemically active compounds have been found to have toxicity effects for marine life and there is a need to discover high-performance environmentally acceptable products.

The aim of the present study was to investigate the biofilm community composition and gene-expression on commercial antifouling (AF) coatings employing next-generation sequencing approaches. Natural mixed-species biofilms were examined after a four-month immersion of two commercial AF coatings, including a biocidal (BAF) and a fouling release (FR), and a control non-treated surface in Langstone Harbour UK. Replicated biofilm samples were used for nucleic acid extraction and sequenced targeting the 16S rRNA gene and metatranscriptome.

We uncovered distinct biofilm community profiles between the two coatings; the BAF samples were dominated by Bacillariophyceae (diatoms), contrary to the FR and control samples where Oscillatoriophycideae (phylum Cyanobacteria) were prevailing. Alphaproteobacteria and Gammaproteobacteria contributed to a high abundance in all samples. Biofilms on BAF samples exhibited a lower species diversity compared to the FR. Here, we introduce a set of functional genes present across all biofilm-associated communities and highlight the differing gene transcriptional profiles in biocidal treatments. The gene transcriptional analysis uncovered highly enriched genes coding for proteins involved in biofilm regulation and formation. We demonstrated that biocidal-associated biofilms harbor genes that regulate defense mechanisms. Overall, the findings highlight links between differentially expressed protein functions and effects of AF coating type during biofilm development. We anticipate these results to contribute towards further development of antibiofilm strategies and fill gaps related to marine biofilm functions.

How to cite: Papadatou, M., Robson, S., Watts, J., Dobretsov, S., and Salta, M.: Functionality and composition of marine biofilms on antifouling coatings., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-117, https://doi.org/10.5194/biofilms9-117, 2020

biofilms9-106
https://doi.org/10.5194/biofilms9-106
biofilms 9 conference

Prediction of biofilm deformation and detachment using shear rheometry, phase-field modeling, and OCT

Mengfei Li[1], Karel Matouš[2], and Robert Nerenberg[1]
[1]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, Indiana, United States (mli14@nd.edu; nerenberg.1@nd.edu)
[2]Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, Indiana, United States (kmatous@nd.edu)

In many environmental systems, such as membrane filtration systems, biofilm control is essential, but costly and requiring harsh chemicals. More effective biofilm control may be obtained using a "materials science" approach. Biofilms can be characterized as viscoelastic materials, and biofilm "disruptors" can be characterized for their weakening effect on biofilm mechanical strength. By using a novel mathematical model that incorporates biofilm mechanical properties, fluid flow, and diffusion and reaction of disruptors, better cleaning strategies can be devised.

Phase-field models, where the biofilm is treated like a viscoelastic fluid, are one of the few types of models that can predicting deformation and detachment based on mechanical properties. While several related studies have proposed phase-field models for predicting biofilm deformation, there has not been any validation of these models with experimental data. As a first step towards developing a material science strategy for biofilm control, this study validated the ability of a phase-field model to capture biofilm viscoelastic behavior.

In this study, a two-dimensional continuum biofilm model was implemented with finite element method (FEM) using COMSOL Multiphysics (COMSOL v5.4, Comsol Inc, Burlington, MA). We applied the phase-field model with the Cahn-Hilliard equation to simulate biofilm mechanical behavior under fluid flow. The Oldroyd-B model, the simplest viscoelastic constitutive model, was applied to capture biofilm viscoelasticity. The biofilm was modeled as an incompressible viscoelastic fluid, with EPS and a water solvent. The phase-field physics were adapted from previous studies and applied to biofilm-fluid interactions. Two types of incompressible, immiscible fluids (EPS and water solvent) were studied as two components of a single fluid, with a fluid-fluid interface between the two.

Homogeneous alginate was used as a synthetic biofilm for the experimental validation. The viscoelastic parameters of alginate were obtained by shear rheometry using stress relaxation tests. In experimental tests, the deformation behavior was observed in real time using optical coherence tomography (OCT). By importing the 2-D geometry from OCT and viscoelastic parameters from rheometry, the model was simulated and compared with real deformation in the flow cell.

With the applied constant flow (Re=6), biofilm demonstrated viscoelastic behavior.The same behavior was observed in modeling as well. By tracking the movements of several locations of the

biofilm geometry, it was concluded that the deformation of alginate biofilms was consistent with the computational results of phase-field models. The relative error between experiment and model for this certain location were 12.8%. Heterotrophic counter-diffusional biofilms cultured in membrane-aerated biofilm reactors were also tested in this study, with a relative error of 22.2%.

In conclusion, the phase-field model, coupled with Oldroyd-B equation, could properly capture biofilm viscoelastic behavior. In a complex system, the phase-field model could be used as a tool to characterize the viscoelastic parameters from the observed deformation. With this information, the model can be used to predict the required disruptor dose to achieve high amounts of biofilm removal with a minimal amount of chemical addition. This can reduce operating costs and minimize the use of harsh chemicals.

How to cite: Li, M., Matouš, K., and Nerenberg, R.: Prediction of biofilm deformation and detachment using shear rheometry, phase-field modeling, and OCT, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-106, https://doi.org/10.5194/biofilms9-106, 2020

biofilms9-121
https://doi.org/10.5194/biofilms9-121
biofilms 9 conference

Blocking Vibrio cholerae-mediated hemagglutination with short peptide antagonists

Shuaiqi Guo[1], Cameron Lloyd[2], Brett Kinrade[1], Mustafa Sherik[1], Ilja Voets[3], Karl Klose[2], and Peter Davies[1]

[1]Queen's University, Biochemistry, Biomedical and Molecular Sciences, Canada (9sg52@queensu.ca)

[2]South Texas Center for Emerging Infectious Diseases, University of Texas at San Antonio, San Antonio, Texas

[3]Eindhoven University of Technology, Eindhoven, The Netherlands.

Many bacteria use repeats-in-toxin (RTX) adhesins to mediate binding to host cells and facilitate subsequent colonisation and infection by forming biofilms. Vibrio cholerae, the causative agent of cholera, uses a 230-kDa RTX adhesin, FrhA, to facilitate intestinal colonization. FrhA also mediates hemagglutination of red-blood cells (erythrocytes). Here we have demonstrated that the hemagglutination capability of FrhA is localized to a ~ 20-kDa domain near its C terminus. Bioinformatic analyses indicated this erythrocyte-binding domain (VcEBD) is 65% identical to a peptide-binding module found in the 1.5-MDa ice-binding RTX adhesin that helps its Antarctic bacterium, Marinomonas primoryensis, form symbiotic biofilms with diatoms on the underside of sea ice. This suggested that the FrhA binds V. cholerae to proteins present on the cell surface of erythrocytes. X-ray crystallography revealed that VcEBD has an oblong β-sandwich fold with a shallow, Ca^{2+}-dependent ligand-binding cavity that can anchor a peptidyl ligand with a free terminal carboxyl group. Using a structure-guided approach, we screened a small library of ~ 60 short peptides and optimized the affinity of VcEBD's peptidyl ligands by roughly 1,000-fold. Importantly, the high-affinity ligands are effective at blocking V. cholerae from binding to erythrocytes at nano-molar concentrations. Structures of VcEBD in complex with three different peptides further elucidated the molecular basis for their interactions, which sets the stage for the development of ligand-based antagonists that may help disrupt V. cholerae interaction with intestinal cells to prevent or treat cholera. With the spread of antibiotic-resistant pathogenic bacteria, this work sheds light on an anti-adhesion approach for combating bacterial infections without the excessive use of antibiotics.

How to cite: Guo, S., Lloyd, C., Kinrade, B., Sherik, M., Voets, I., Klose, K., and Davies, P.: Blocking Vibrio cholerae-mediated hemagglutination with short peptide antagonists, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-121, https://doi.org/10.5194/biofilms9-121, 2020

Poster Presentations

biofilms9-69
https://doi.org/10.5194/biofilms9-69
biofilms 9 conference

"Adhere today, here tomorrow" – how is exopolysaccharide production by Pseudomonas aeruginosa affected by high-flow shear conditions?

Wendy Allan[1,2], Mark Webber[3], Kevin Wright[4], and Timothy Overton[1,2]
[1]School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom (WCA299@student.bham.ac.uk)
[2]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom
[3]Quadram Institute Bioscience, Norwich, UK
[4]Procter and Gamble London Innovation Centre, Egham, UK

Biofilms provide physical, mechanical and chemical protection for microbes from their external environment, necessitating the use of harsh chemicals (such as sanitisers and antimicrobials) and abrasive cleaning (brushing or pigging) for their control. Biofilms have a broad impact upon the manufacturing of a wide range of fast-moving consumer goods, and biofilm contamination during their manufacture can lead to production interruption and significant economic costs to industry for cleaning and sanitisation. Biofilms formed by *Pseudomonas aeruginosa* (*Ps. a.*), a major contaminant of industrial processes, have yet to be studied in-depth with respect to the changes that occur in response to high-flow shear conditions from a combined physical and biological perspective.

The central aims of this work were to understand and elucidate the biological response of *Ps. a.* biofilms when grown under different, industrially-relevant fluid flow conditions; to characterise the mechanisms through which *Ps. a.* produces phenotypic responses, and how these in turn affect biofilm architecture. Two strains of *Ps. a.*, PA01 and PA14, were used, known to differentially produce two exopolysaccharides (Psl and Pel respectively), integral components in the biofilm's protective extracellular matrix (Colvin et al. 2012).

To investigate the effect of shear stress on biofilm formation, the CDC Bioreactor (CBR) was used to grow biofilms on polyethylene coupons under high or low shear conditions for 96 hours. At 24, 48, 72 and 96-hour timepoints, coupons containing biofilms were removed from the CBR and analysed by confocal laser scanning microscopy (CSLM) and biochemical assays.

Exopolysaccharide (EPS) production was quantified by CSLM image analysis in Fiji. In order to determine how EPS organisation effects wider biofilm architecture and individual structures within a maturing biofilm, Psl and Pel localisation and distribution throughout biofilms was assessed. Under low and high shear conditions, the architecture of PA01 and PA14 biofilms was compared to further identify similarities and differences in their phenotypic responses to shear stress.

We will present data that shows that Psl and Pel have distinct localisation patterns throughout PA01 and PA14 biofilms over our selected time course. PA01 and PA14 were shown to produce varying amounts of the exopolysaccharides Psl and Pel in mature biofilm structures (i.e. mushroom colonies) and throughout the entire biofilm population. Early adhesion, colony morphology and overall biofilm architecture was shown to be considerably affected by shear. Hydrodynamic conditions impose shear stress on *Ps. a.* biofilms, in turn affecting the structural components that make up their mature architecture.

Reference: Colvin, K.M., Irie, Y., and Tart, C.S. et al. (2012). The Pel and Psl polysaccharides

provide Pseudomonas aeruginosa structural redundancy within the biofilm matrix. *Environmental Microbiology*, 14(8):1913-28.

How to cite: Allan, W., Webber, M., Wright, K., and Overton, T.: "Adhere today, here tomorrow" – how is exopolysaccharide production by Pseudomonas aeruginosa affected by high-flow shear conditions?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-69, https://doi.org/10.5194/biofilms9-69, 2020

biofilms9-114
https://doi.org/10.5194/biofilms9-114
biofilms 9 conference

Adaptation of biofilm communities in a feast-famine regime: implications for degradation of organic micropollutants

Chuanzhou Liang[1], Nadieh de Jonge[2], Pedro N. Carvalho[1], Jeppe Lund Nielsen[2], and **Kai Bester**[1]
[1]Department of Environmental Science, Aarhus University, Frederiksborgvej 399, Roskilde 4000, Denmark
[2]Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, DK-9220, Aalborg, Denmark

Feast-famine moving bed biofilm reactors (MBBRs) were found to be removing a number of organic micropollutants effectively from wastewater in previous studies. It was hypothesized that micropollutant-degrading organisms in the biofilm communities were possibly enriched by feast-famine selective pressure. We established a MBBR operated in feast-famine regimes (alternating influent/effluent wastewater) to test the hypothesis. The development of degradation kinetics of 36 micropollutants and the microbial communities in the biofilm were assessed simultaneously for 19 time points during the 70-day adaptation.

During this adaptation, 16S rRNA gene amplicon sequencing showed that the microbial communities shifted greatly from the initial biofilm composition in the first 8 days toward a more steady development afterwards. Ammonia oxidizing bacteria (Nitrosomonas) and nitrite oxidizing bacteria (Nitrospira) were strongly enriched (both > 18 % relative abundance at day 43), which led to high nitrification capability. Notably, the biofilm absorbed and nitrified ammonia during the feast regime, while releasing stored nitrate during the famine regime. Twenty-four out of studied 36 micropollutants showed enhanced reaction rate constants k (especially for propranolol up to 6600 %) during the adaptation. Maximum k values were observed between day 22 and 67 during the adaptation. DNA concentration in the biofilm was used as a proxy for biomass, and normalized reaction rate constants relative to the DNA concentration as k_{DNA} were used for understanding the degradation reaction rates of MPs per DNA concentration unit. During the adaptation, the DNA concentration continuously increased suggesting growth and accumulation of microorganisms. However, k_{DNA} of 21 micropollutants showed a decreased removal after day 11, which suggests the relative abundance of the respective degraders decreased while their absolute abundance increased. It suggests that the colonization rates of the MP degraders were slower than the non-degraders under the selective pressure of the feast-famine regime. By mining correlations between the microbial community and k_{DNA} of micropollutants, 88 operational taxonomic units (OTUs) belonging to different taxonomic groups were found to correlate significantly with removal rates of micropollutants (Pearson correlation coefficients, $r > 0.5$, $p < 0.05$). Thus, these identified OTUs are potential candidates as the degraders of the respective micropollutants. In summary, the feast-famine strategy was successful for enhancing the degradation of some compounds, but the feast-famine regime in this study was not successful in selecting microorganisms in biofilm with high removal capability for many micropollutants. Nevertheless, this study contributed to a better understanding of what occurred during the adaptation process of biofilms with potential for micropollutant degradation.

How to cite: Liang, C., de Jonge, N., Carvalho, P. N., Lund Nielsen, J., and Bester, K.: Adaptation of biofilm communities in a feast-famine regime: implications for degradation of organic micropollutants, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-114, https://doi.org/10.5194/biofilms9-114, 2020

biofilms9-137
https://doi.org/10.5194/biofilms9-137
biofilms 9 conference

Alanine racemase as target to inhibit the Campylobacter jejuni biofilm formation by L and D-amino acids

Bassam Elgamoudi, Taha taha, and Victoria Korolik
Institute for Glycomics, Griffith University, Gold Coast campus, QLD 4222, Australia.

The ability of bacterial pathogens to form biofilm is an important virulence mechanism in relation to its pathogenesis and transmission. Biofilms play a crucial role in survival in unfavourable environmental conditions, act as reservoirs of microbial contamination and antibiotic resistance. For intestinal pathogen Campylobacter jejuni, biofilms are considered to be a contributing factor in transmission through the food chain and currently, there are no known methods for intervention. Here we present an unconventional approach to reducing biofilm formation by C. jejuni by the application of D-amino acids (DAs), and L-amino acids (LAs). We found that DAs not LAs, except L-alanine, reduced biofilm formation by up to 70%. The treatment of C. jejuni cells with DAs changed the biofilm architecture and reduced the appearance of amyloid-like fibrils. In addition, a mixture of DAs enhanced antimicrobial efficacy of D-Cycloserine (DCS) up to 32% as compared with DCS treatment alone. Unexpectedly, D-alanine was able to reverse the inhibitory effect of other DAs as well as DCS. Furthermore, L-alanine and D-tryptophan decreased transcript levels of alanine racemase (alr) and D-alanine-D-alanine ligase (ddlA). Our findings suggest that a combination of DAs could reduce biofilm formation, viability and persistence of C. jejuni.

How to cite: Elgamoudi, B., taha, T., and Korolik, V.: Alanine racemase as target to inhibit the Campylobacter jejuni biofilm formation by L and D-amino acids, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-137, https://doi.org/10.5194/biofilms9-137, 2020

biofilms9-155
https://doi.org/10.5194/biofilms9-155
biofilms 9 conference

A new surface wiping test to study surface disinfection by a novel chemical combination

Joana F. Malheiro[1,2,3], Fernanda Borges[3], Jean-Yves Maillard[2], and Manuel Simões[1]
[1]LEPABE, Department of Chemical Engineering, Faculty of Engineering of University of Porto, Porto, Portugal
[2]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, United Kingdom
[3]CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences University of Porto, Porto, Portugal

Effective biofilm disinfection is difficult to be implemented in healthcare settings and industry. In particular, surface disinfection is crucial to prevent microbial contaminations. However, disinfectants misuse has led to an increased concern on the existence of resistance and cross-resistance phenomena due to inadequate disinfection practices. The purpose of this study was the development of a formulation to be used for surface disinfection with wipes. The idea was to produce a formulation based on the combination between the quaternary ammonium compound - cetyltrimethylammonium bromide (CTAB) and a natural product - cinnamaldehyde. In addition, a new disc methodology to assess wiping efficiency was developed based on the Wiperator test (E2967-15) and on the quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area, 4- field test (EN 16615:2015). The combination of CTAB and cinnamaldehyde was synergic in terms of antimicrobial action against Escherichia coli and Staphylococcus aureus. After stablishing the final formulation, wiping efficacy was assessed with the new methodology. In this case, a contaminated surface (6.20 ± 0.21 $\log_{10}$ CFU of E. coli and 7.10 ± 0.06 $\log_{10}$ CFU of S. aureus) was wiped using two different wipes in terms of composition, thickness and porosity (A and B). After wiping the contaminated surface with wipe A, without the formulation, 3.42 ± 0.46 $\log_{10}$ CFU (E. coli) and 5.38 ± 0.20 $\log_{10}$ CFU (S. aureus) remained on the surface while in the presence of the formulation the bacteria present were under the limit of detection for E. coli and 2.76 ± 0.22 $\log_{10}$ CFU for S. aureus. The formulation was also able to prevent the transfer of bacteria to clean surfaces after wiping the contaminated surface. In the case of wipe A, after wiping the contaminated surface and the subsequent 2 clean surfaces, a total reduction of 4.35 ± 0.22 $\log_{10}$ CFU and 4.27 ± 0.22 $\log_{10}$ CFU was achieved when the wipe was impregnated with the formulation in comparison with 2.45 ± 0.41 $\log_{10}$ CFU and 1.50 ± 0.35 $\log_{10}$ CFU of removal just by mechanical action for E. coli and S. aureus, respectively. For wipe B a general lower reduction was observed but the same behaviour was detected with the use of the formulation when comparison to just mechanical action. This work highlights the enormous potential of combinatorial approach to increase the efficacy of already used biocides diminishing their in-use concentration and consequently their environmental and public health burden.

Acknowledgements

This work was financed by: UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); POCI-01-0145-FEDER-030219, POCI-01-0247-FEDER-035234; POCI-01-0145-FEDER-028397; POCI-01-0247-FEDER-033298; POCI-01-0145-FEDER-006939, funded by FEDER through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. Grant attributed by

Portuguese Foundation for Science and Technology (FCT) to Joana Malheiro (SFRH/BD/103843/2014) and Manuel Simões (SFRH/BSAB/150379/2019).

How to cite: Malheiro, J. F., Borges, F., Maillard, J.-Y., and Simões, M.: A new surface wiping test to study surface disinfection by a novel chemical combination, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-155, https://doi.org/10.5194/biofilms9-155, 2020

biofilms9-148
https://doi.org/10.5194/biofilms9-148
biofilms 9 conference

Antibiofilm effect of Temporin-L on Pseudomonas fluorescens, in static and dynamic conditions.

Arianna Cirillo[1,2], **Angela Di Somma**[2,4], Alessia Romano[1], Federica Recupido[2], Sergio Caserta[1,2], Stefano Guido[1,2], Alessandra Romanelli[3], and Angela Duilio[2]
[1]Ceinge- Biotecnologie Avanzate, Naples, Italy (ariannacirillo23@gmail.com)
[2]University of Naples Federico II, Naples, Italy
[3]University of Milan, Milan, Italy
[4]National Institute of Biostructures and Biosystems (INBB), Rome, Italy

Introduction

Biofilm consists of a complex self-produced matrix of polysaccharides, DNA and proteins that protects bacteria from the environment including the host immune system and constitutes the main cause of bacterial resistance against antibiotics. Research is then focused on finding alternative antimicrobial substances able to either hamper biofilm formation or to prevent bacterial growth. Recently, we showed that the antimicrobial peptide Temporin-L impairs E.coli growth by inhibiting cell division (Di Somma et al.; 2020; BBA). Here we investigate the effect of Temporin-L (TL) on biofilm formation in Pseudomonas fluorescens (P. fluorescens) both in static and dynamic conditions,

showing that TL displays antibiofilm properties.

Materials and methods

Biofilm formation in static conditions was performed on coverslips and analyzed by the Crystal Violet

assay. Biofilm morphology was assessed using imaging techniques. Investigation of biofilms in dynamic conditions was performed in a flow chamber using a microfluidic system and images were recorded by confocal microscopy.

Results

The P. fluorescens cells were either grown in the presence of TL or incubated with the antimicrobial peptide after biofilm formation both in static and dynamic conditions using different concentrations of the peptide. When TL was added during cell growth, the peptide affected biofilm formation at 25 μM. Confocal microscopy demonstrated that at this concentration P. fluorescens cells were still alive but a clear disruption of the biofilm architecture was observed. These results had to be ascribed to

a

specific antibiofilm effect of TL. At 100 μM TL antibiofilm activity biofilm thickness was nearly negligible.

When P. fluorescens cells were treated with TL following biofilm formation, confocal images demonstrated that the peptide exerted a strong antibiofilm effect leading to cell detachment and disruption the biofilm architecture.

Discussion and Conclusions

Investigation of TL effect on P. fluorescens showed that when added during bacterial growth this peptide exerted antibiofilm activity at low concentration impairing biofilm formation both in static and dynamic conditions, leaving most of bacterial cells still alive. However, confocal microscopy measurements could not detect the long necklace-like structures observed in E.coli indicating a different mechanism of action of TL on P. fluorescens. Furthermore, when TL was added to a preformed P. fluorescens biofilm, the peptide showed a strong antibiofilm activity both in static and dynamic conditions, suggesting that TL might penetrate biofilm architecture with a still unknown mechanism leading to disruption of P. fluorescens biofilm.

How to cite: Cirillo, A., Di Somma, A., Romano, A., Recupido, F., Caserta, S., Guido, S., Romanelli, A., and Duilio, A.: Antibiofilm effect of Temporin-L on Pseudomonas fluorescens, in static and dynamic conditions. , biofilms 9 conference, 29 September–1 Oct 2020, biofilms9-148, https://doi.org/10.5194/biofilms9-148, 2020

biofilms9-151
https://doi.org/10.5194/biofilms9-151
biofilms 9 conference

Antimicriobial Activity of Silver-Nanoparticles studied by Scanning Probe Microscopy

Giada Caniglia[1], Anna Heinzmann[1], Maria Chiara Sportelli[2], Antonio Valentini[3], Nicola Cioffi[2], and Christine Kranz[1]

[1]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Albert-Einstein-Allee, 11 – 49081 Ulm, Germany

[2]Chemistry Department, University of Bari "Aldo Moro", V. Orabona, 4, 70126, Bari, Italy

[3]Physics Department, University of Bari "Aldo Moro", V. Orabona 4, 70126, Bari, Italy

Biofilms are well-organized sessile communities which exhibit an increased tolerance against antimicrobial and antibiotic treatments in comparison with their planktonic counterparts. Biofilms are ubiquitous and due to their high resilience, the problem with contamination of medical implants leads to serious health problems [1]. Within the last decades, novel therapies to prevent the formation of biofilms have been developed and, among others, antimicrobials based on metal nanoparticles (NPs) have been intensively studied [2], due to their ability to reduce biofilm formation. Silver nanoparticles (AgNPs) are known to be effective antimicrobial agents, as silver(I) has the ability to penetrate the cell and produce oxidative stress via the generation of reactive oxygen species (ROS) [3]. To understand the release mechanism of silver(I) ions, scanning electrochemical probe microscopy such as scanning electrochemical microscopy (SECM) is highly suitable.

In this contribution, biocompatible AgNPs-fluoropolymer (Ag-CF_x) composite films, prepared by ion beam sputtering (IBS) deposition [4], are investigated in respect to silver(I) release associated to the swelling of the antimicrobial film. The mechanism of the silver(I) release is studied real-time by scanning electrochemical microscopy (SECM) in combination with square-wave stripping voltammetry and the relation between controlled silver(I) release and the swelling of Ag-CF_x films will be presented, combining electrochemical techniques and atomic force microscopy (AFM).

References

[1] G.C. Anderson, et al. Innate and Induced Resistance Mechanisms of Bacterial Biofilms. In Bacterial Biofilms; Romeo, T., Ed.; Springer Berlin Heidelberg: Berlin, Heidelberg, **2008**; pp 85–105.

[2] M.C. Sportelli, et al. Nano-Antimicrobials Based on Metals. In Novel Antimicrobial Agents and Strategies; John Wiley & Sons, Ltd, **2014**; pp 181–218.

[3] N. Durán, et al. Nanomedicine, **2016**, 12(3), 789-799.

[4] M.C. Sportelli, et al. **Sci. Rep**. 2017, 7 (1), 11870.

Acknowledgements

Financial support is acknowledged from European Union's 2020 research and innovation program under the Marie Sklodowska-Curie Grant Agreement No. 813439.

How to cite: Caniglia, G., Heinzmann, A., Sportelli, M. C., Valentini, A., Cioffi, N., and Kranz, C.: Antimicriobial Activity of Silver-Nanoparticles studied by Scanning Probe Microscopy, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-151, https://doi.org/10.5194/biofilms9-151, 2020

biofilms9-131, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-131
biofilms 9 conference

Bacteria Adhesion on Polydimethylsiloxane Surfaces Impacted by Material Viscoelasticity or Surface Chemistry?

Fei Pan
Laboratory for Biointerfaces, Empa, Switzerland (fei.pan@empa.ch)

Among nosocomial infections, materials associated infections are the most frequent and severe due to biofilm formation. To prevent bacterial colonization, understanding the underlying interaction between bacteria and surface is fundamental. Herein we focused on studying how material viscoelasticity and physicochemistry can influence bacterial adhesion, using polydimethylsiloxane (PDMS) as a model material. To delineate the impact caused by bulk material from interfacial physicochemical properties, a 2 nm PDMS-like polymer layer was coated onto PDMS surfaces of different stiffness to confer comparable surface chemical properties, while retaining similar viscoelasticity for coated and uncoated PDMS species. Although the uncoated samples displayed increasing interfacial adhesion force with the decreasing Young's modulus, the nanolayer coating ensured comparable forces independent of material stiffness. The Gram negative strains Escherichia coli and Pseudomonas aeruginosa and the Gram positive strain Staphylococcus epidermidis were found to adhere respectively in similar numbers on the coated surfaces of different PDMS species, whereas the amount on the uncoated surfaces increased several fold with the decreasing modulus. The similar adhesion behaviour was noticed for abiotic polystyrene beads of similar size to bacteria, demonstrating that the interfacial chemistry of the PDMS rather than the material viscoelasticity plays a crucial role in bacterial adhesion.

How to cite: Pan, F.: Bacteria Adhesion on Polydimethylsiloxane Surfaces Impacted by Material Viscoelasticity or Surface Chemistry?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-131, https://doi.org/10.5194/biofilms9-131, 2020

biofilms9-9
https://doi.org/10.5194/biofilms9-9
biofilms 9 conference

Biocorrosion research: Are we barking up the right trees?

Hans-Curt Flemming[1,2], Brenda Little[3], Dan Blackwood[4], Jamie Hinks[2], Federico Lauro[2], Enrico Marsili[5], Akihiro Okamoto[6], Scott Rice[2], and Scott Wade[7]

[1]Duisburg-Essen, Biofilm Centre, Chemistry, Germany (hc.flemming@uni-due.de)
[2]Singapore Center for Environmental Life Science Engineering
[3]B.J. Little Corrosion Consulting
[4]National Technical Univ. Singapore, Dep. Mat. Sci.
[5]Nazarbayan Univ. Kazakhstan
[6]Nat. Inst. Mat. Sci. Ibaraki, Japan
[7]Swineburne Univ. Technol. Hawthorn, Australia

Microbially influenced corrosion (MIC), is acknowledged to be the direct cause of catastrophic corrosion failures, with damages ranging to many billions of US$. In spite of extensive research and numerous publications, fundamental questions still remain unanswered. In 1993, J.F.D. Stott published a review paper in Corrosion Science, entitled "What progress in the understanding of microbially influenced corrosion has been made in the last 25 years?" He concluded, "The most commonly asked question about MIC is: what will be the expected corrosion rate of material x in an environment where aggressive microorganisms proliferate?... For many materials we can no more answer this question now than we could 25 years ago." Now, over 50 years later, that question is still open. Current MIC research does not provide data related to detection and verification in the field, diagnosing, modelling or prediction. Laboratory experiments seldom attempt to recreate relevant natural or industrial electrolytes. A sober, solution-oriented contemplation of the state-of-art and acknowledgement of the substantial deficiencies in our understanding may help shift MIC research into a direction which could actually produce useful answers.

How to cite: Flemming, H.-C., Little, B., Blackwood, D., Hinks, J., Lauro, F., Marsili, E., Okamoto, A., Rice, S., and Wade, S.: Biocorrosion research: Are we barking up the right trees?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-9, https://doi.org/10.5194/biofilms9-9, 2020

biofilms9-7
https://doi.org/10.5194/biofilms9-7
biofilms 9 conference

Bioinspired surfaces to delay biofilm formation: different surfaces with different mechanisms

Jinju Chen

Biofilm associated infections are the fourth leading cause of death worldwide, within the U.S. about 2 million annual cases lead to more than $5 billion USD in added medical costs per annum. Therefore, it is important to control biofilm growth and reduce the instances of infections. Physical strategies, in particular the use of rationally designed surface topographies or surface energies, have present us with an interesting approach to prevent bacterial adherence and biofilm growth without the requirement for antimicrobials.

A variety of natural surfaces exhibit antibacterial properties. Examples of such surfaces include rose petals with hierarchical structures and Nepenthes pitcher plants with slippery liquid-infused porous surfaces.

In this study, we fabricated different biomimetic surfaces (rose-petal surfaces and slippery liquid-infused porous surfaces). We have demonstrated that rose-petal surface can delay early stage P. aeruginosa and S. epidermidis biofilms formation (2 days) by about 70% and control biofilm formation according to surface structures. The mechanisms of hierarchical structures of rose-petal influence biofilm formation are two folds: 1) Papillae microstructure block the bacterial clusters in between the valleys, limiting the potential for cell-cell communication via fibrous networks, thereby resulting in impaired biofilm growth. 2) The secondary structure (nano-folds) on microstructures can align bacterial cells within the constrained grooves, thereby delaying cell clusters formation during short term growth of biofilm.

While, the slippery liquid-infused porous surface(s) can prevent over 90% P. aeruginosa and S. epidermidis biofilms formation for a duration of 6 days. These are mainly attributed to their high contact angle and extreme low contact angle hysteresis.

How to cite: Chen, J.: Bioinspired surfaces to delay biofilm formation: different surfaces with different mechanisms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-7, https://doi.org/10.5194/biofilms9-7, 2020

biofilms9-94
https://doi.org/10.5194/biofilms9-94
biofilms 9 conference

Can rhamnose-based glycolipids nanoparticles be an alternative to fight biofilms on medical devices?

Carolina Tomé[1], Inês Anjos[1], Victor Martin[2,3], Catarina Santos[4,5], Lidia Gonçalves[1], Maria H Fernades[2,3], Ana F. Bettencourt[1], Pedro Gomes[2,3], and **Isabel A.C. Ribeiro**[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal (iribeiro@ff.ul.pt)
[2]Faculty of Dental Medicine, Universidade do Porto
[3]LAQV/REQUIMTE, Universidade do Porto
[4]EST Setúbal, CDP2T, Instituto Politécnico de Setúbal
[5]C2TN DECN Instituto Superior Técnico, Universidade de Lisboa

Biofilm development on medical devices is of particular concern and finding new strategies for preventing surface colonization and infection development are urgent. Antimicrobial biosurfactants such as rhamnolipids (RLs), emerge as one possible solution due their lack of resistance development. Using nanoparticles as delivery systems for these compounds may be a promising alternative in the context of biofilm-infections control. As such, the aim of this study was to encapsulate RLs into chitosan nanoparticles (RLs-NPs), test their antimicrobial activity and their biocompatibility profile.

Blank nanoparticles (b-NPs) and RLs-NPs were prepared by ionic gelation. For particles characterization, zeta potential, size distribution and encapsulation efficiency were performed. Minimal inhibitory concentration and biofilm inhibition ability were evaluated towards Staphylococcus aureus (ATCC 25923). To access NPs cytocompatibility the in vitro tetrazolium dye assay (MTT) and morphology observation were performed with a mouse fibroblastic cell line (L929).

RLs-NPs presented an encapsulation efficiency of 74.2±1.3%, a size ranging from 300 to 400 nm and a zeta potential of 37±1 mV. The minimum inhibitory concentration of RLs-NPs was 130 mg/mL and a 99% biofilm inhibition was achieved with these NPs meaning that their antimicrobial activity is also effective towards sessile bacteria. When compared to control, cell cultures grown in the presence of RLs-NPs presented no significant differences regarding the MTT reduction values and morphology analysis, suggesting that NPs up to 500 mg/mL did not significantly interfere with viability and proliferation.

The results revealed that the RLs-NPs were able to inhibit bacterial growth showing adequate cytocompatibility and might become, after additional studies, a possible approach to fight S. aureus biofilm associated infections.

Acknowledgments: Support for this work was provided by FCT through Portuguese government, PTDC/BTM-SAL/29335/2017 and Pest-UID/DTP/04138/2019

How to cite: Tomé, C., Anjos, I., Martin, V., Santos, C., Gonçalves, L., Fernades, M. H., Bettencourt, A. F., Gomes, P., and Ribeiro, I. A. C.: Can rhamnose-based glycolipids nanoparticles be an alternative to fight biofilms on medical devices?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-94, https://doi.org/10.5194/biofilms9-94, 2020

biofilms9-41, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-41
biofilms 9 conference

Characterisation of biofilm hotspots at a can filling line for beer

Eva M. Wagner[1], Sarah Thalguter[1], Kathrin Rychli[2], and Martin Wagner[1,2]
[1]FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, 3430 Tulln, Austria
[2]Institute for Food Safety, Food Technology and Veterinary Public Health - Unit of Food Microbiology, University of Veterinary Medicine Vienna, Vienna, Austria

Biofilms are thought to play a serious role in the food processing environment. Direct contact with or detachment of microorganisms of biofilms could lead to product contamination resulting in diminished shelf life of the product. Packaging and filling are essential key steps for product safety, as any contamination within this step will directly impact the shelf life and safety of the product. For bottled and canned beverages filling lines are used for filling huge amounts of product in a standardised manner. Most parts of these lines are cleaned and disinfected automatically during cleaning in place (CIP) procedures. The design of these filling lines is of great importance, as accessibility and materials are crucial regarding the success of automatic cleaning and disinfection programs. Some companies implemented additional manual cleaning strategies for the complete removal of potential problem-causing sites. In the brewery setting the term biofilm is manifested since the 90s. However, until now all studies focus only on the presence of microorganisms, neglecting the presence of matrix components, which constitutes an essential component of a biofilm.

Within this study a filling line for cans, capable of filling 60000 cans per hour (volume 0.5 l), was investigated regarding critical sites for biofilm formation. The filling line is used primarily for beer, but mixed beer drinks are also filled. We sampled 23 sites using a scraper-flocked-swab method at two time points. The first sampling was done during operation and the second sampling was conducted one month later after the automated cleaning and disinfection procedure. The samples were characterised regarding their microbial load (qPCR for 16S rRNA and 18S rRNA genes) and the presence of biofilm matrix (phenol-sulfuric assay for carbohydrates, precipitation and SDS-PAGE with subsequent silver staining for proteins and precipitation and spectrophotometric quantitative measurements for extracellular DNA).

During operation, we could identify three sites harbouring a biofilm by applying the definition of presence of microorganisms and at least two matrix components. Furthermore, there were seven sites harbouring microorganisms and one matrix component. After cleaning and disinfection no biofilm could be detected. At one site, microorganisms and one matrix component could be detected. The drastic reduction of biofilm positive sites indicates the successful removal of biofilms by the cleaning and disinfection process.

The design of future filling plants should emphasise on the principles of hygienic design, as this can help to prevent biofilm formation and targeted removal of biofilms during cleaning and disinfection. The here identified biofilm hotspots indicate potential problem-causing sites and weak-points in the design of the filling line.

How to cite: Wagner, E. M., Thalguter, S., Rychli, K., and Wagner, M.: Characterisation of biofilm hotspots at a can filling line for beer, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-41, https://doi.org/10.5194/biofilms9-41, 2020

biofilms9-154
https://doi.org/10.5194/biofilms9-154
biofilms 9 conference

Combinatorial effects of juglone and fusidic acid in controlling Staphylococcus aureus biofilms

Diana Oliveira[1,2,3], Francisco Couto[1], Anabela Borges[1,2], Fernanda Borges[3], and Manuel Simões[1,2]
[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[2]DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[3]CIQUP, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre s/n, 4169-007, Porto, Portugal

Chronic skin wounds are disruptions of the skin that remain in the inflammatory state for more than one month. They affect approximately 3% of the people with more than 60 years and are mostly associated with diabetic foot, pressure ulcers, blood vessels or trauma[1]. Besides that, it is expected that 578 million people worldwide will suffer from the disease by 2030, that will subsequently increase the risk of developing infection and potentially result in amputation or osteomyelitis[2]. One of the main difficulties in the wound healing process is the fact that bacteria persist due to biofilm formation. Biofilms delay not only healing, but they also make bacteria more resistant to the antimicrobial therapy. Among the bacteria present at the wound site, *Staphylococcus aureus* was found to be one of the most prevalent biofilm producers[3]. In this sense, other alternatives to fight biofilm infections should be considered due to the resistance to the current antibiotics. One possible strategy consists of using antibiotic adjuvants to enhance the activity of current drugs and to minimize or even block resistance. For that, plants are used as a resource of such adjuvant compounds. The use of natural compounds from plants have been applied in skin wound care for millennials, generating a lot of interest from the scientific community. Indeed, not only are phytochemicals great antibiotic potentiators but they also possess numerous therapeutic properties[4]. Therefore, in this study, the phenolic compound juglone (5-hydroxy-1,4-naphtoquinone) was investigated, alone and combined with fusidic acid antibiotic, for its potential to eradicate pre-formed *S. aureus* biofilms. Although no biomass removal was observed, there was a total loss of cell culturability (about 6-log CFU/cm^2 reduction) and a considerable metabolic activity reduction. Juglone reduced metabolic activity by 83% both alone and in combination with fusidic acid, which is an improvement over the 70% reduction obtained by the antibiotic. Therefore, an additive interaction of juglone when combined with fusidic acid was attained in the control of *S. aureus* biofilms. In addition, according with Lipinsksi's rule of five, it was assessed that juglone possesses important molecular properties with respect to pharmacokinetics in the human body. Overall, this study reveals the great potential for the topical application of juglone as an adjuvant to the widely used fusidic acid to combat multi-drug resistant wound infections.

References:

(1) Manu, C., et al. J. Wound Care 27.3 (2018): 186–192.

(2) International Diabetes Federation. https://www.diabetesatlas.org (accessed May 27, 2020).

(3) Kadam, S., et al. Biomedicines 7.2 (2019): 35.

(4) Borges, A.; et al. Molecules 21.7 (2016): 877.

Acknowledgements: This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); - PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219; POCI-01-0247-FEDER-035234; POCI-01-145-FEDER-006939, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through CEECIND/01261/2017 (Anabela Borges), SFRH/BD/138217/2018 (Diana Oliveira) and SFRH/BSAB/150379/2019 (Manuel Simões).

How to cite: Oliveira, D., Couto, F., Borges, A., Borges, F., and Simões, M.: Combinatorial effects of juglone and fusidic acid in controlling Staphylococcus aureus biofilms , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-154, https://doi.org/10.5194/biofilms9-154, 2020

biofilms9-136
https://doi.org/10.5194/biofilms9-136
biofilms 9 conference

Comparative assessment of biofilm sampling methods on stainless steel surfaces in a CDC biofilm reactor

Nissa Niboucha, Coralie Goetz, and Julie Jean
Faculty of Agriculture and Food Sciences, Laval University, Quebec, Canada (nissa.niboucha.1@ulaval.ca)

The presence of biofilms on stainless steel surfaces in the dairy industry greatly limits the efficiency of the cleaning procedures. The matrix of extracellular polymeric substances produced by the embedded bacteria is largely responsible for this irreversible binding. Therefore, to detach the biofilm in its entirety from the surface for microbiological identification and physico-chemical characterization is limited with the classical methods commonly used for surface sampling such as swabbing. The objective of this study is to optimize an extraction technique of biofilm formed using a dynamic CDC bioreactor system by a strain of *Pseudomonas fluorescens* isolated from the dairy industry during a biofilm issue. Three methods: swabbing, scraping and sonic brushing were tested in order to determine which one of these techniques allows a better recovery of the biofilm. They were also compared to sonication which is the standard method established by ASTM International. The results demonstrated that the total viable counts obtained by scraping (8.65 ± 0.07 CFU/cm2) were not significantly different from those achieved by sonication (8.74 ± 0.06 CFU/cm2) in contrast to the other two approaches, while scanning electron microscopy showed an effective removal of biofilms from surfaces by sonic brushing. In conclusion, other combinations including brushing, sonication and/or scraping must be investigated for representative sampling of biofilm on the surfaces of dairy plants.

How to cite: Niboucha, N., Goetz, C., and Jean, J.: Comparative assessment of biofilm sampling methods on stainless steel surfaces in a CDC biofilm reactor, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-136, https://doi.org/10.5194/biofilms9-136, 2020

biofilms9-19
https://doi.org/10.5194/biofilms9-19
biofilms 9 conference

Drinking Water Biofilm Management and Monitoring

Frances Pick, Katherine Fish, Stewart Husband, and Joby Boxall
Civil & Structural Engineering, University of Sheffield, United Kingdom of Great Britain and Northern Ireland

Biofilms within drinking water distribution systems can pose risks to consumers, especially when mobilised, as high concentrations of microorganisms and associated material can be released leading to degradation of water quality. Access and sampling of biofilms within drinking water pipelines can be difficult without disrupting supply in these extensive and buried systems. A novel biofilm monitoring device was developed to determine if biofilm formation rates can be used to assess microbiological water quality, track fouling rates and ultimately indicate distribution system performance. The device comprises a sample-line pipe with multiple, independent removable sections (allowing for biofilm sampling) that can be easily connected to sampling points in the distribution system. Biofilm is removed from the device and flow cytometry used to determine total and intact cell concentrations. The biomonitoring device was tested in a series of laboratory trials, to establish the impact of different flow rates and orientations on biofilm formation and to determine the optimum configuration that achieves accurate and repeatable results. Subsequently, these devices were installed in two operational systems, with different water qualities, and biofilms were sampled for two months to obtain biofilm growth rates. The results provide the first direct evidence of different biofilm formation rates in distribution systems with different water qualities. This evidence is now being used to investigate fouling rates via risk analysis and modelling. The use of the device has potential to improve understanding of biofilm behaviour and help inform biofilm and asset management to safeguard the quality of delivered drinking water.

How to cite: Pick, F., Fish, K., Husband, S., and Boxall, J.: Drinking Water Biofilm Management and Monitoring, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-19, https://doi.org/10.5194/biofilms9-19, 2020

biofilms9-124, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-124
biofilms 9 conference

Effect of selected terpenoids on antibiotic potentiation and eradication of Staphylococcus aureus biofilms – a structure activity relationship study

Anabela Borges[1,2], Ana Catarina Alves[1], and Manuel Simões[1,2]
[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal
[2]DEQ - Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

Research on the discovery of new drugs to treat bacterial biofilm infections has been a priority for biomedical and scientific communities. Actually, the sessile mode of growth accounts for more than 80% of human bacterial infections and displays increased resistance to antibiotics. To date, there are no drugs with total efficacy against biofilms and the treatment failure is a recurrent clinical situation. This lack of alternatives boosted up again the exploration of the pharmacological properties of health-promoting agents from natural origin. In this connection, the potential of phytotherapeutic agents for the treatment/prevention of complex infectious diseases, such as those that involve biofilm formation has been intensified[1]. In view of that, the main aim of the present study was to evaluate the activity of four phytochemicals bellowing to terpenoids class [cis-6-nonen-1-ol (CIS), citronellic acid (CITACID), citronellol (CITRO) and 3-7-dimethyl-1-octanol (3,7DOC)] against *Staphylococcus aureus*, both in planktonic and sessile state. Firstly, the minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined by the broth microdilution method and culturability on plate count agar, respectively. Then, the potential of each terpenoid as resistance modifying agent was assessed by the disc diffusion method, using antibiotics from different classes. Besides, its potential to eradicate pre-formed S. aureus biofilms (24-h old) was performed using a microtiter plate assay and characterized in terms of biofilm mass removal (crystal violet staining), metabolic activity reduction (alamar blue staining) and culturability (colony forming units - CFU - counts). Considering that the selected terpenoids are chemical structurally related, i.e. present a similar backbone and differ only on the functional groups location, a structure activity relationship (SAR) analysis was also established. Both CITO and 3,7DOC presented the lowest MIC value (200 µg/mL) followed by CIS (400 µg/mL) and CITACID (1000 µg/mL). The MBC was found to be 1000 µg/mL with CIS, 2000 µg/mL with CITACID and > 2000 µg/mL (the maximum concentration tested) with CITO/3,7DOC. Apparently, the hydrophobicity of the molecules appear to affect positively its inhibitory proprieties – molecules with higher hydrophobicity presented lower MIC values. Moreover, it seems that the hydroxyl and methyl functional groups play the major influence on the antimicrobial properties. Indeed, CITO and 3,7DOC presented the higher hydrophobicity values and both had hydroxyl and methyl functional groups, possessing the lowest MIC value. Independently of the terpenoid tested, all combinations (terpenoid-antibiotic) resulted in a potentiation effect. Regarding biofilm eradication, although no biomass removal was observed, metabolic activity reductions from 25% (CIS at 5×MIC) up to 44% (CITACID at 10×MIC) and total loss of culturability (CITACID at 10×MIC; 6-log CFU/cm^2 reduction) was found. These effects were found to be dose dependent. Overall, the results obtained suggest that all the tested terpenoids might be interesting antibiotic adjuvants and emphasize the use of CITACID for biofilm cells inactivation. The results obtained are promising since the terpenoids studied are natural occurring flavoring ingredients, generally recognized as safe by the FDA, which are usually applied as food additives for human consumption.

References: [1] A.Borges, A.Abreu, C.Dias, M.Saavedra, F.Borges, M.Simões, Molecules 21(2016)877.

How to cite: Borges, A., Alves, A. C., and Simões, M.: Effect of selected terpenoids on antibiotic potentiation and eradication of Staphylococcus aureus biofilms – a structure activity relationship study, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-124, https://doi.org/10.5194/biofilms9-124, 2020

biofilms9-149, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-149
biofilms 9 conference

Electrosynthetized copper based nanoantimicrobials for the inhibition of biofilms

Syed Imdadul Hossain[1,3], Maria Chiara Sportelli[1,3,2], Rosaria Anna Picca[1,3], Nicoletta Ditaranto[1,3], and Nicola Cioffi[1,3]

[1]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Bari, Italy (syedimdadul.hossain@uniba.it)

[2]CNR, Istituto di Fotonica e Nanotecnologie UOS, Bari, Italy

[3]CSGI (Center for Colloid and Surface Science) c/o Dept. Chemistry, via Orabona 4, 70125 Bari, Italy.

[1]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Bari, Italy; [2]CNR, Istituto di Fotonica e Nanotecnologie UOS, Bari, Italy; [3]CSGI (Center for Colloid and Surface Science) c/o Dept. Chemistry, via Orabona 4, 70125 Bari, Italy.

Copper nanoparticles (CuNPs) are considered as potential antimicrobial agents due to their improved stability and safety, and longer active period than that of organic nanomaterials, with multi-targeted mechanism of action [1]. Nevertheless, metal NPs can suffer from agglomeration, reducing their antibacterial activity [2]. Cu incorporation in inorganic substrates such as metal oxides or montmorillonite (MMT) plays an important role due to the possibilities of creating an antibacterial nanomaterial with slow release of Cu species in order to obtain a prolonged antibacterial activity. Therefore, CuNPs were synthesized via a rapid electrochemical method using the inorganic micro-powders as carrier. Characterization studies on the nanocomposite were done by Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy (XPS). The as-prepared Cu-based nanocomposites could be employed for inhibiting the growth of biofilms.

References

Acknowledgements

"Financial support is acknowledged from European Union's 2020 research and innovation program under the Marie Sklodowska-Curie Grant Agreement No. 813439."

How to cite: Hossain, S. I., Sportelli, M. C., Picca, R. A., Ditaranto, N., and Cioffi, N.: Electrosynthetized copper based nanoantimicrobials for the inhibition of biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-149, https://doi.org/10.5194/biofilms9-149, 2020

biofilms9-15
https://doi.org/10.5194/biofilms9-15
biofilms 9 conference

Evaluation of biological risks related to the use of different kind of road surfaces

Beata Świeczko-Żurek[1], Jerzy Ejsmont[1], and Anna Pałubicka[2]
[1]Gdańsk University of Technology, -, Mechanical Department, Poland (jejsmont@pg.edu.pl)
[2]Hospital in Kościerzyna, Microbiological Department, Poland (apalubicka@op.pl)

The paper presents the biological degradation of different road surfaces. Microorganisms are able to damage the structure and function of synthetic polymers. Some of road pavements, that are still in developing stage are porous and contain high amount of polyurethane (up to 10% by weight) or polymer modified asphalts. The same fungal enzymes can break down the polyurethanes or metabolize the plasticizers in various polymers, resulting in embrittlement and loss of strength. Many sources, indicate that enzymes produced by fungi (so also produced by lichens) have adverse effect on polyurethane resins. According to literature, bacteria and lichens may deteriorate polyurethane resin resulting in loss of bonds between particles (rubber and stone chippings). It may be possible to decrease sensitivity of the road pavements to attack of bacteria and lichens by adding certain substances (inhibitors) to the mix. The paper presents pilot study related to biological interactions with poroelastic road pavements containing rubber and polyurethane resin.

How to cite: Świeczko-Żurek, B., Ejsmont, J., and Pałubicka, A.: Evaluation of biological risks related to the use of different kind of road surfaces, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-15, https://doi.org/10.5194/biofilms9-15, 2020

biofilms9-144
https://doi.org/10.5194/biofilms9-144
biofilms 9 conference

Evidence of cannibalism during long-term biofilm-antimicrobials interaction

Maria Chiara Sportelli[1], Giada Caniglia[2], Ruggiero Quarto[1], Rosaria Anna Picca[1], Antonio Valentini[3], Holger Bart[4], Boris Mizaikoff[2], Christine Kranz[2], and Nicola Cioffi[1]

[1]University of Bari, Chemistry Department, Italy (maria.sportelli@uniba.it)

[2]Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany

[3]University of Bari, Physics Department, Italy

[4]Institute of Pharmacology and Toxicology, Ulm University Medical Center, Germany

Biofilms are considered a major cause of serious health issues in human medicine and food industry, due to their resistance against harsh conditions and pharmacological treatment [1]. Biofilms are defined as three-dimensional structures encasing bacterial communities rooted in extracellular polymeric substances (EPS). These complex systems are strongly influenced by a variety of parameters including biofilm age, external conditions, nutrient deficiency, attack of exogenous agents [2]. Moreover, bacterial colonies may activate survival strategies when subjected to stress such as the presence of antimicrobial agents. Even cannibalistic behavior may occur [3], which involves the secretion of cannibalism toxins inducing the generation of lysed cells providing nutrients.

Several methodologies were developed for or adapted to biofilm formation studies enabling a more comprehensive understanding of biofilm physiology, structure, and composition. This information should facilitate the development of more effective eradication strategies. Infrared spectroscopy in attenuated total reflectance (IR-ATR) mode provides in-situ and close to real time monitoring of biofilm lifecycles providing molecular information on the various stages of biofilm formation. Given the antibiotic resistance of biofilms [4], it is of increasing importance to develop innovative methodologies for the treatment of biofilm-related infections. While our research team has shown the generic utility of antimicrobial nanoparticles (NPs) such as ZnONPs, AgNPs, CuNPs, etc. in the past [5], the current study focuses on AgNPs embedded within fluoropolymer matrices with tunable loading of the NPs. Next to morphological studies by TEM and AFM, detailed XPS investigations revealed the surface chemical composition. In addition, the kinetics of antimicrobial ion release enabled correlating the behavior of the nanocomposite to its swelling properties and 3D modification after immersion in liquids. Biofilm growth and inhibition was studied via AFM, optical microscopy and IR-ATR. The IR analysis of the biofilm allowed collecting molecular information on the biofilm behavior during long-term contact with antimicrobial surfaces. It was demonstrated that bacterial cells may re-colonize on top of dead biomass once the latter is thick enough to prevent direct interaction with the antimicrobial surface. In summary, this study represents an excellent foundation for developing an in depth understanding on the behavior of bacterial colonies and nascent biofilms in contact with surfaces decorated with nanoantimicrobials over extended periods of time. It is anticipated that an improved understanding on the stages of biofilm formation provides insight into the processes governing antimicrobial resistance phenomena. Finally, present antimicrobial material may be a useful strategy against Corona viruses. An outlook to this urging topic will be also presented.

[1] N. Billings et al., Rep. Prog. Phys., 2015, 78, 036601. [2] D.O. Serra et al., MBio., 2013, 4, e00103. [3] C. Höfler et al., Microbiology, 2016, 162, 164. [4] M.C. Sportelli et al., Sci. Rep., 2017,

7, 11870. [5] M.C. Sportelli et al., TrAC, 2016, 84, 131.

How to cite: Sportelli, M. C., Caniglia, G., Quarto, R., Picca, R. A., Valentini, A., Bart, H., Mizaikoff, B., Kranz, C., and Cioffi, N.: Evidence of cannibalism during long-term biofilm-antimicrobials interaction, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-144, https://doi.org/10.5194/biofilms9-144, 2020

biofilms9-44, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-44
biofilms 9 conference

Identification of multi-species biofilms in the meat processing environment and characterisation of involved bacteria in a mono- and multi-species biofilm model

Eva M. Wagner[1], Nadja Pracser[2], Sarah Thalguter[1], Katharina Fischel[2], Nicole Rammer[2], Clara Beer[2], Anna Lena Palmetzhofer[2], Beate Pinior[3], Franz-Ferdinand Roch[3], Lucie Pospíšilová[4], Merima Alispahic[5], Kathrin Rychli[2], and Martin Wagner[1,2]

[1]FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, 3430 Tulln, Austria
[2]Institute for Food Safety, Food Technology and Veterinary Public Health - Unit of Food Microbiology, University of Veterinary Medicine Vienna, Vienna, Austria
[3]Institute for Food Safety, Food Technology and Veterinary Public Health - Unit of Veterinary Public Health and Epidemiology, University of Veterinary Medicine Vienna, Vienna, Austria
[4]Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic
[5]University Clinic for Poultry and Fish Medicine - Clinical Unit of Poultry Medicine, University of Veterinary Medicine Vienna, Vienna, Austria

Biofilms are suggested to be a source of contamination in the food producing environment leading to food spoilage or the transmission of food-borne pathogens. However, to date, research has mainly focused on the presence of (biofilm-forming) bacteria within food processing environments, without analysing the associated biofilm matrix components.

The aim of this study was to identify biofilm hotspots in a meat processing environment by analysing the presence of microorganisms (by cultivation and targeted quantitative real-time PCR based on 16S rRNA) and the major matrix components carbohydrates, extracellular DNA and proteins. Sampling included 47 distinct food contact surfaces and 61 distinct non-food contact surfaces from eleven rooms within an Austrian meat processing plant, either during operation or after cleaning and disinfection. Additionally, we isolated and characterized bacteria found in biofilms. The biofilm forming capacity of eleven isolates, was tested, using a static biofilm model. Additionally, two different multi-species settings were tested combining three strains, each. Biofilms were grown on stainless-steel slides for seven days at 10 °C, to mimic conditions found in the food producing environment.

Overall, we identified ten biofilm positive sites, among them seven of which were sampled during operation and three after cleaning and disinfection. Five biofilms were detected on food contact surfaces (cutters and associated equipment and a screw conveyor) and five on non-food contact surfaces (drains and water hoses) resulting in 9.3 % of the sites being classified as biofilm positive. From these sites we cultivated bacteria of 29 different genera. The most prevalent bacteria belonged to the genera *Brochothrix*, *Pseudomonas* and *Psychrobacter*. From each biofilm we isolated bacteria from four to 12 different genera, indicating the presence of multi-species biofilms.

Culturing of eleven isolates of different species (all detected in the mentioned biofilms, representing typical residential and spoilage bacteria in the meat processing environment) showed that there are differences of individual strains to produce matrix components and biomass on stainless steel slides. *Brochothrix*, *Carnobacterium* and *Kocuria* produced only detectable amounts of carbohydrates but neither eDNA nor proteins. The *Acinetobacter* and the *Flavobacterium* isolates were able to produce two of the measured components and six strains were capable of producing all types of analysed matrix components, among them a *Pseudomonas fragi* isolate. The minimal mean

bacterial load detected was 5.4 log CFU/cm^2 formed by the *Psychrobacter* strain.

Different isolates showed differences in matrix formation ability, possible contributing in different amounts to the matrix production in multi-species biofilms, indicating that multi-species biofilms are a key survival mechanism for microorganisms within the food processing environment.

Currently, we are testing two different multi-species biofilms in our model. Hereby we cultivate three species detected in the cutter-associated biofilms and other three species detected in the water hose-associated biofilms together to mimic these biofilms. This work ultimately showed the presence of multi-species biofilms within the meat processing environment, thereby identifying various sources of potential contamination. Data on the presence, formation and composition of biofilms (i.e. chemical and microbiological) will help to prevent and reduce biofilm formation within food processing environments.

How to cite: Wagner, E. M., Pracser, N., Thalguter, S., Fischel, K., Rammer, N., Beer, C., Palmetzhofer, A. L., Pinior, B., Roch, F.-F., Pospíšilová, L., Alispahic, M., Rychli, K., and Wagner, M.: Identification of multi-species biofilms in the meat processing environment and characterisation of involved bacteria in a mono- and multi-species biofilm model, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-44, https://doi.org/10.5194/biofilms9-44, 2020

biofilms9-123
https://doi.org/10.5194/biofilms9-123
biofilms 9 conference

Influence of different copper materials on biofilm control using chlorine and mechanical stress

Inês B. Gomes[1], Lúcia Simões[2], and Manuel Simões[1]
[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[2]CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057 Braga, Portugal.

The selection of materials for plumbing application has potential implications on the chemical and microbiological quality of the delivered water. This work aims to evaluate the action of materials with different copper content (0, 57, 96 and 100%) on biofilm formation and control by chlorination and mechanical stress. A strain of *Stenotrophomonas maltophilia* isolated from drinking water was used as model microorganism and biofilms were developed in a rotating cylinder reactor (RCR) using realism-based shear stress conditions. Biofilms were characterized phenotypically and exposed to three control strategies: 10 mg/l of free chlorine for 10 min; an increased shear stress (equivalent to 1.5 m/s of fluid velocity); and the combination of both treatments. Biofilms formed on the copper materials had lower wet mass and produced significantly lower amounts of extracellular proteins than those formed on stainless steel (0% of copper content). Although, the effects of copper materials on biofilm cell density was not significant, these materials had important impact on the efficacy of chemical and/or mechanical treatments. Biofilms formed on 96 or 100% copper materials had lower content of culturable bacteria than that observed on stainless steel after exposure to chlorine or shear stress. The mechanical treatment used had no relevant effects in biofilm control. The combination of chemical and mechanical treatments only caused higher culturability reduction than chlorine in biofilms formed on 57% copper alloy. The number of viable cells present in bulk water after biofilm treatment with chlorine was lower when biofilms were formed on any of the copper surface. The overall results are of potential importance on the selection of materials for drinking water distribution systems, particularly for house and hospital plumbing systems to overcome the effects from chlorine decay. Copper alloys may have a positive public health impact by reducing the number of viable cells in the delivered water after chlorine exposure and improving the disinfection of DW systems. Moreover, the results demonstrate that residual chlorine and mechanical stress, two strategies conventionally used for disinfection of drinking water distribution systems, failed in *S. maltophilia* biofilm control.

Acknowledgements:

This work was the result of the projects: UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219; POCI-01-0145-FEDER-006939, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

How to cite: Gomes, I. B., Simões, L., and Simões, M.: Influence of different copper materials on biofilm control using chlorine and mechanical stress , biofilms 9 conference, Karlsruhe, Germany, 29

September–1 Oct 2020, biofilms9-123, https://doi.org/10.5194/biofilms9-123, 2020

biofilms9-50
https://doi.org/10.5194/biofilms9-50
biofilms 9 conference

Influence of essential oils on the biofilm formation and cell agglomeration of Burkholderia cepacia from industrial environment

Katrin Huth-Herms, Alexander Kintzel, Annika Brehmer, Christoph Hein, and Prof. Dr. h. c. Dr.-Ing. Eckart Uhlmann
Fraunhofer Institute for Production System and Design Technology IPK, Microproduction technology, Germany (katrin.huth-herms@ipk.fraunhofer.de)

Burkholderia cepacia (*B. cepacia*) is one of nine species the *Burkholderia cepacia* complex, a group of gram-negative, motile, non-spore-forming and rod-shaped bacteria. Contamination by *B. cepacia* is found in different industrial issues. *B. cepacia* affect manufacturing process chains by contaminating the working fluids with planktonic cells and biofilms. Because of the opportunistic pathogenicity to plants, animals, humans and and the multi-drug resistance, *B. cepacia* is difficult to treat. An alternative treatment method could be the use of herbal raw materials, such as essential oils and their active ingredients. This study aims: (i) to identify the antimicrobial potential of essential oils on the growth of four *B. cepacia* isolates, (ii) to analyse the influence of active ingredients, on planktonic growth and biofilm formation, (iii) to better understand the impact of commercial and naturally biocides to cell agglomeration as a precursor to mature biofilms. Starting with agar dilution method to evaluate the antimicrobial potential of twenty-three essential oils against *B. cepacia* (*Burk_09*, *Burk_23*, *Burk_52* and *Burk_309*) isolated from cathodic dip coating systems and the wild type (*DSM_7288*), it was all ready possible to identify eight essential oils that inhibit the growth of *B. cepacia*. Serial microdilution was used to determine the minimal inhibitory concentration (MIC) of the essential oils for growth and biofilm formation inhibition of *B. cepacia*. The MIC of *Melaleuca alternifolia* and *Citrus aurantium dulcis* essential oils were tested equally for all strains. Essential oils contain active ingredients against the growth of multi-drug resistant and pathogenic bacteria. From twelve active substances among others, Terpinen-4-ol and Geraniol were identified that inhibited growth and biofilm formation. It is concluded that essential oils and active ingredients have a good antimicrobial potential, demonstrating a possible more environmental-friendly alternative to commercial biocides applying in industrial fluids.

How to cite: Huth-Herms, K., Kintzel, A., Brehmer, A., Hein, C., and Uhlmann, P. Dr. h. c. Dr.-Ing. E.: Influence of essential oils on the biofilm formation and cell agglomeration of Burkholderia cepacia from industrial environment, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-50, https://doi.org/10.5194/biofilms9-50, 2020

biofilms9-57
https://doi.org/10.5194/biofilms9-57
biofilms 9 conference

Inhibitory effect of microstructured PVDF surfaces on the microbial attachment of respiratory and oral biofilm forming microorganisms

Prof. Dr. h. c. Dr.-Ing. Eckart Uhlmann[1,2], Christoph Hein[1], **Annika Brehmer**[1], and Katrin Huth-Herms[1]

[1]Fraunhofer Institute for Production Systems and Design Technology IPK, Berlin, Germany
[2]Institute for Machine Tools and Factory Management IWF, Technische Universität Berlin, Germany

Bacterial resistance to conventional antibiotics combined with the increasing awareness of the essential role of biofilms in nosocomial infections caused by medical devices has led to a growing interest in new antimicrobial strategies. Since the formation of bacterial resistances represents a permanent risk in the drug treatment of biofilms, the optimisation of surface properties to avoid microbial attachment is gaining further attention. Besides the haematological field, especially in the respiratory and oral sectors, biofilm-forming microorganisms cause major problems. Due to microbial attachment being mainly determined by the surface properties of the respective substrate material, the medically established polymer PVDF was provided with different microstructures in the size s of 1 µm ≤ s ≤ 200 µm in order to influence the wettability. These structures were applied to injection moulding tools by high and ultra precision milling, electrical discharge machining as well as laser machining. The injection moulded, microstructured PVDF samples showed pyramidal, cup-shaped, channel-shaped and random structures in the micrometer range and led to contact angles □ in the range of 50° ≤ □ ≤ 110°. These samples were then tested for their influence on bacterial attachment by typical representatives of haematologic as well as respiratory and oral biofilm formers *Pseudomonas stutzeri* and *Streptococcus salivarius*. The microbial growth and the formed biofilms were analysed after 24 h and 72 h via crystal violet staining and fluorescence microscopy. In comparison to unstructured surfaces, a significant reduction of bacterial attachment was found, which correlated with the respective contact angle and surface roughness, the microgeometry of the structures and the cell morphology of the tested microorganisms. Especially the laser structured, channel-shaped surface showed a highly reduced biofilm formation for both strains. The results offer great potential for the reduction of biofilm formation on medical devices. This technology can also be used in the water treatment sector, such as pipe linings, filter surfaces and sensor housings. The economic large-scale implementation of these microstructures requires further research.

How to cite: Uhlmann, P. Dr. h. c. Dr.-Ing. E., Hein, C., Brehmer, A., and Huth-Herms, K.: Inhibitory effect of microstructured PVDF surfaces on the microbial attachment of respiratory and oral biofilm forming microorganisms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-57, https://doi.org/10.5194/biofilms9-57, 2020

biofilms9-88
https://doi.org/10.5194/biofilms9-88
biofilms 9 conference

Interaction of flow field and biofilm formation in a dripper supplied by reclaimed wastewater

Nassim Ait Mouheb[1], Jueying Qian[2], Michael Wagner[2], and Harald Horn[2]
[1]INRAE, UMR GEAU, France (nassim.ait-mouheb@inrae.fr)
[2]Water Chemistry and Water Technology, Engler-Bunte-Institut, Karlsruhe institute of Technology, Karlsruhe, Germany

In the reclaimed wastewater reuse using drip irrigation, one of the main issues is the bio-clogging of drippers and decrease of water distribution efficiency in field level. However, the relation between the complex flow created along the dripper (in general formed by a milli-channel with labyrinth geometry) and the biofouling development are rarely studied.

In order to improve the knowledge of these mechanisms, the objective was to combine the numerical flow simulations to three-dimensional measurements of biofilm along a milli-fluidic system (nominal flow rate 1L/h) fed by treated wastewater. At first, using the Optical Coherence Tomography (OCT) method and based to Qian et al, 2018 studies, the bio-clogging structure was measured at different levels of fouling (up to 77% of channel volume). Secondly, the new fouled dripper geometries were integrated to 3D CFD models (using comsol multiphysics software) to analyse the effect of biofilm on flow topology and the dripper hydraulic parameters (pressure drop, shear stress, turbulence kinetic energy in particular).

The results show that the main areas of biofilm growth correspond to vortices zones where fluid velocity, turbulent kinetic energy values and shear stress are lowest. When the level of clogging increases, the numerical plot of stream lines show local perturbation and reduction of vortices areas caused by their interactions with the biofilm structure. There is also a gradual increase in pressure drop along the milli-channel comparing to initial clean dripper. Finally, by characterising the flowrate in function of inlet pressure and according to Karmeli, 1977, the increase of biofilm formation induces also a modification of the global flow regime in the dripper, i.e. the transfer from a turbulent to a laminar regime.

Qian, J., Horn, H., Tarchitzky, J., Chen, Y., Katz, S., Wagner, M., (2017). Water quality and daily temperature cycle affect biofilm formation in drip irrigation devices revealed by optical coherence tomography. Biofouling 33, 211–221.
Karmeli, D. (1977). Classification and flow regime analysis of drippers. J Agric Eng Res 22:165–173

How to cite: Ait Mouheb, N., Qian, J., Wagner, M., and Horn, H.: Interaction of flow field and biofilm formation in a dripper supplied by reclaimed wastewater, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-88, https://doi.org/10.5194/biofilms9-88, 2020

biofilms9-75
https://doi.org/10.5194/biofilms9-75
biofilms 9 conference

Iron as a biofilm control agent: manipulation of biofilm development and differentiation

Luisa Gierl, Harald Horn, and Michael Wagner
Engler-Bunte-Institut, Water Chemistry and Water Technology, Karlsruhe Institute of Technology, Karlsruhe, Germany

In order to optimize operative parameters in wastewater treatment plants, drip irrigation systems as well as in biofilm reactors, it is necessary to understand biofilm development and proliferation under certain conditions. Additionally, the physical structure of biofilms is of great interest since it determines the interaction with its microenvironment, while knowledge about the mechanical behavior of biofilms is important for applying e.g., cleaning procedures.

In the past two years we refined a fully automated monitoring and cultivation setup that enabled replicate biofilm cultivations and investigation by means of optical coherence tomography (OCT). OCT as an imaging modality is ideal for biofilms since it allows for the monitoring of structure and deformation in real-time and noninvasively.

With this setup it was possible to analyze the effect of iron on biofilm growth and behavior with a minimum of N = 10 biofilm replicates including a statistical treatment. At least eight structural parameters of biofilms grown in flow cells could be analyzed and statistically quantified, providing insights into the structural integrity of biofilms and to their interface. Thereby, the results clearly show the positive effect of iron on Bacillus subtilis biofilms regarding biomass production and differentiation to mature biofilms. Further gravimetric and optical analyses prove the incorporation of iron as iron oxide-hydroxides and explain the positive effect on the biofilm's matrix. Initial experiments under mechanical stress confirm the withstanding of high flow rates as well as a high compressibility of the biofilms.

How to cite: Gierl, L., Horn, H., and Wagner, M.: Iron as a biofilm control agent: manipulation of biofilm development and differentiation, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-75, https://doi.org/10.5194/biofilms9-75, 2020

biofilms9-101
https://doi.org/10.5194/biofilms9-101
biofilms 9 conference

Magnetic resonance imaging (MRI) as non-invasive approach for quantifying the transport of particulate organic matter within a bed of settled aerobic granules

Florian Ranzinger[1], Maximilian Matern[1], Manuel Layer[2], Gisela Guthausen[1,3], Michael Wagner[1,4], Nicolas Derlon[2], and Harald Horn[1,5]

[1]KIT, Engler-Bunte-Institut, Wasserchemie und Wassertechnologie, Germany (florian.ranzinger@kit.edu)
[2]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Process Engineering, CH-8600, Dübendorf, Switzerland
[3]Institute for Mechanical Process Engineering and Mechanics, Karlsruhe Institute of Technology, Adenauerring 20b, 76131, Karlsruhe, Germany
[4]Karlsruhe Institute of Technology, Institute of Biological Interfaces (IBG-1), Hermann-von-Helmholtz-Platz 1, 76344, Eggenstein-Leopoldshafen, Germany
[5]DVGW Research Laboratories, Water Chemistry and Water Technology, Engler-Bunte-Ring 9, 76131 Karlsruhe, Germany

Aerobic granular sludge is one of the most promising technologies in wastewater treatment of the past decades. By now, around 50 full scale plant have been set up at full scale under the trademark Nereda©.

With approx. 50 % of the chemical oxygen demand (COD) particulate organic matter is a crucial fraction of municipal wastewater. Theories about its degradation in granular sludge reactors typically start with the assumption that particulate organic matter is adsorbed at the granule surface after the feeding phase (De Kreuk et al. 2010, Pronk et al. 2015). Despite of the ideal case, unattached particles after the feeding phase would be available for degradation under aerobic conditions or could be washed out with the effluent. To extent the knowledge about the degradation process, the present study aimed at visualizing the transport and fate of particulate organic matter into and through a bed of granular sludge. The main perspectives are to directly show their distribution and retention mechanism inside a granular sludge bed.

Magnetic Resonance Imaging (MRI) was successfully applied to visualize the different fractions of a granular sludge bed resolved in time and space (x, y, z). According to particle size, three particle consortia have been chosen to represent municipal wastewater:

Dextran coated super paramagnetic iron oxide nanoparticles (SPIONs, mean diameter d_{mean} = 20 nm) served as model particles for colloids. As a reference for toilet paper, paramagnetically tagged microcrystalline cellulose with a size fraction between 1 and 20 µm was used. The results are supplemented by the use of real wastewater particles with a size fraction between 28 and 100 µm. No paramagnetic tagging was applied in the latter case.

The retention mechanism is found to be size dependent. Colloidal particles are able to attach and penetrate the granules. Therefore, they will constantly release substrate during their degradation inside the granule. In contrast, larger particles accumulate within the void space between the granules. Moreover, the formation of particle layers indicates that most of the particles are not attached to the biomass and remain mobile after an initial feeding phase. Thus, they remain available under aerobic conditions and might be partially washed out with the effluent if no attachment is taking place in the aerobic mixing phase (Ranzinger et al. 2020).

References:

De Kreuk, M., Kishida, N., Tsuneda, S. and Van Loosdrecht, M. (2010) Behavior of polymeric substrates in an aerobic granular sludge system. Water research 44(20), 5929-5938.

Pronk, M., Abbas, B., Al-Zuhairy, S., Kraan, R., Kleerebezem, R. and Van Loosdrecht, M. (2015) Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. Applied microbiology and biotechnology 99(12), 5257-5268.

Ranzinger, F., Matern, M., Layer, M., Guthausen, G., Wagner, M., Derlon, N. and Horn, H. (2020) Transport and retention of artificial and real wastewater particles inside a bed of settled aerobic granular sludge assessed applying magnetic resonance imaging. Water Research X, 100050.

How to cite: Ranzinger, F., Matern, M., Layer, M., Guthausen, G., Wagner, M., Derlon, N., and Horn, H.: Magnetic resonance imaging (MRI) as non-invasive approach for quantifying the transport of particulate organic matter within a bed of settled aerobic granules, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-101, https://doi.org/10.5194/biofilms9-101, 2020

biofilms9-153
https://doi.org/10.5194/biofilms9-153
biofilms 9 conference

Millimetric particles functionalized with biocide to improve biofouling control in RO system

Ana C. Barros, Ana Pereira, and Luís F. Melo
Faculty of Engineering of the University of Porto, Laboratory for Process Engineering, Environment , Biotechnology and Energy, Department of Chemical Engineering, Portugal (acbarros@fe.up.pt)

Biofouling is responsible for more than 45% of all membrane fouling associated problems and is highly relevant for the performance of Reverse Osmosis systems (RO). Biofouling has a tremendous negative impact on the quality and quantity of permeate water and is responsible for high operational and maintenance costs associated with such systems. Current strategies targeting biofilm control on membrane systems often include the overuse of disinfectants which most of the time fail to effectively prevent biofouling build-up, can lead to the formation of dangerous disinfection by-products and represents high amounts of discharged biocides.

The present work aims to study how millimetric (1-3 mm length) alumina particles, functionalized with a well-known quaternary ammonium compound biocide (benzalkonium chloride) and immobilized into a Particle Biocide Bed Reactor can effectively contribute to mitigate biofilm formation in membrane systems. For that, the functionalized particles were chemically characterized, and their antimicrobial activity was assessed in batch and recirculation assays and quantified in terms of Culturability and Propidium Iodide (PI) uptake. Special attention has been given to biocides's (free and immobilized) mechanism of action and potential biocide release was evaluated by High Performance Liquid Chromatography (HPLC) measurements.

The preliminary experiments indicate that the immobilized biocide (equivalent biocide concentration of 3 g/L) has an antimicrobial activity against Pseudomonas fluorescens (initial concentration 10^8 CFU/mL) by reducing 4 logs after 30 min and 8 logs after 1 h. On the other hand, the control assays (functionalized particles in water with no bacteria), also shows a biocide release between 0.8 and 1% to the bulk water after 30 min, both in batch and in the Particle Bed Reactor with recirculation experiments. No significant biocide increase is observed in the bulk liquid studies for two weeks. Nonetheless, some changes in the functionalization approach are being made to improve the biocidal anchoring to the particle.

How to cite: Barros, A. C., Pereira, A., and Melo, L. F.: Millimetric particles functionalized with biocide to improve biofouling control in RO system , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-153, https://doi.org/10.5194/biofilms9-153, 2020

biofilms9-48
https://doi.org/10.5194/biofilms9-48
biofilms 9 conference

Multi-stage assessment of biofilm growth by drinking water bacteria on polymeric pipe materials

Olga Sójka[1,2], Patrick van Rijn[2], Henny van der Mei[2], and Maria Cristina Gagliano[1]
[1]Wetsus, Leeuwarden, the Netherlands
[2]University of Groningen and University Medical Center Groningen, Department of Biomedical Engineering, Groningen, the Netherlands

Introduction

The presence of biofilms in drinking water distribution systems (DWDS) leads to a number of issues, i.e. secondary (biological) drinking water contamination, pipe damage and increased flow resistance. Among other operational factors, the selection of pipe material plays an important role in biofilm development. Up to now, the studies that have investigated this correlation provide contradictory results in terms of which material might be the most advantageous in the DWDS biofilm control strategy. Hence, to understand the influence of pipe material on biofilm formation, we focused on developing a standardized methodology that allows a multi-stage assessment of biofilm development on real pipe materials.

Results

Development of the methodology consisted of three steps: 1) material coupon sterilization, 2) biofilm cultivation and 3) biofilm analysis, using transparent polyvinyl chloride (PVC) as a study material. For the coupon sterilization, methods utilizing immersion in different disinfectant solutions with and without pre-cleaning by rubbing the coupons in a surfactant solution. The results showed that mechanical cleaning before washing is crucial and without it, reproducible sterilization was difficult to achieve. Biofilm formation on the PVC coupons was performed in a 6-well plate assay (24, 48 and 72 h; under agitation) using DWDS biofilm strains (*Sphingomonas spp*. and *Pseudomonas extremorientalis*) and *Pseudomonas aeruginosa* as a positive control. Bacterial fitness and ability to secrete EPS and form biofilms on the PVC surfaces were tested by monitoring optical density (OD600 nm), chemical oxygen demand (COD) and protein concentration. The formed biofilm and the morphology of attached bacteria were visualized using crystal violet staining (that allow qualitative (bright field microscopy) and quantitative (OD at 570 nm) evaluation), by scanning electron microscopy (SEM) and DNA staining (4’,6-diamidino-2-phenylindole; DAPI) with fluorescence microscopy. Combination of those techniques gave a complete overview of patterns involved in biofilm development by selected drinking water bacterial strains in presence of a PVC surface. The developed methodology was also applied for the analysis of bacterial growth on real-grade pipe materials, such as PVC and polyethylene (PE), to understand their role in biofilm formation.

Conclusions

Implementation of various analytical and microscopic techniques is important in understanding mechanisms behind biofilm development in DWDS and the influence of pipe material in the process. The proposed approach allows the observation of biofilm formation in time, but also of the typical bacterial morphology of attached cells. In this study it was shown that to obtain reproducible results, it is crucial to select an appropriate sterilization technique and the influence of mechanical cleaning

cannot be ignored in preparation of polymeric surfaces.

How to cite: Sójka, O., van Rijn, P., van der Mei, H., and Gagliano, M. C.: Multi-stage assessment of biofilm growth by drinking water bacteria on polymeric pipe materials , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-48, https://doi.org/10.5194/biofilms9-48, 2020

biofilms9-27
https://doi.org/10.5194/biofilms9-27
biofilms 9 conference

Nitrogen and phosphorus removal using fluidized-carriers in a full-scale A2O biofilm system

Keke Xiao
Huazhong University of Science and Technology, School of Environmental Science and Technology, China (563861304@qq.com)

A full-scale biofilm system using fluidized-carriers integrated with anaerobic-anoxic–aerobic process (treatment capacity of 3.75×10^5 m^3 d^{-1}) was used for municipal wastewater treatment. The results indicated relatively higher removal efficiencies of 86% total nitrogen (TN), 97% ammonium (NH_4-N) and 97% total phosphorus (TP) were achieved, with 0.32 mg L^{-1} TP, 0.81 mg L^{-1} NH_4-N and 8.07 mg L^{-1} TN in the effluent, which meet the Class A of Discharge standard of pollutants for municipal wastewater treatment plant (GB18918-2002) of China. The results of microbial analysis indicated that the dominant microorganisms in the suspended sludge were Proteobacteria and Bacteroidetes at phylum level and β-Proteobacter at class level. The dominant microorganism in the biofilm was Proteobacteria at phylum level, with γ-Proteobacter (17.5%), β-Proteobacter (14%) and δ-Proteobacter (13.08%) distributed at class levels. The presence of Proteobacteria and Bacteroidetes in this system may be related with the phosphorus removal. A reddish color biofilm was formed on the surface of fluidized-carriers in the anaerobic tank and showed specific anammox ability, this may be related with the dominance of 0.0278% Planctomycetaceae at family level and 0.0278% Planctomycetales at order level. Besides the denitrification effects, the possible anammox bacteria present in the anaerobic tank might have also contributed to high nitrogen removal efficiency.

How to cite: Xiao, K.: Nitrogen and phosphorus removal using fluidized-carriers in a full-scale A2O biofilm system, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-27, https://doi.org/10.5194/biofilms9-27, 2020

biofilms9-150, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-150
biofilms 9 conference

Preventing biofilms by chitosan-based nanoantimicrobials (NAMs)

Ekaterina A. Kukushkina[1], Maria Chiara Sportelli[1,2], Nicoletta Ditaranto[1], Rosaria Anna Picca[1], and Nicola Cioffi[1]
[1]Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", and CSGI, Bari-unit, Bari, Italy (ekaterina.kukushkina@uniba.it)
[2]CNR, IFN UOS, Bari, Italy (maria.sportelli@uniba.it)

Chitosan (CS), a natural non-toxic polysaccharide, shows intrinsic antimicrobial activity against a wide range of pathogens. CS and CS-based biomaterials can be effective additives in food and medicine-related industries to inhibit growth of pathogens. The application of inorganic nanophases, such as metal and metal oxide nanoparticles, has received attention due to their broad and pronounced antimicrobial activity. Upon combination with CS, which can act as stabilizer, with active inorganic nanophases, robust synergistic nanoantimicrobial (NAM) systems can be produced. These hybrid NAMs offer an alternative strategy to fight antimicrobial resistance and overcome limitations of conventional antibiotics. Bioactive ZnO, Cu and Ag nanophases produced by green electrochemical approach [Nanomaterials, 10(3) (2020), 473] and laser ablation in solution [(Coll. Surf. A, 559 (2018), 148-158), (Food packaging shelf, 22 (2019), 1000422)] can be combined with antimicrobial CS to develop synergistic antimicrobial nanohybrids with amplified biological action. CS-based NAMs were preliminary characterized by electron microscopies and spectroscopic techniques. Hybrid NAMs may find application in the control and inhibition of biofilm growth.

Acknowledgements
Financial support is acknowledged from European Union's 2020 research and innovation program under the Marie Sklodowska-Curie Grant Agreement No. 813439

How to cite: Kukushkina, E. A., Sportelli, M. C., Ditaranto, N., Picca, R. A., and Cioffi, N.: Preventing biofilms by chitosan-based nanoantimicrobials (NAMs), biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-150, https://doi.org/10.5194/biofilms9-150, 2020

biofilms9-52
https://doi.org/10.5194/biofilms9-52
biofilms 9 conference

Reconstruction of an industry related biofilm into a proxy model community – Challenges around Field and lab based microbial growth analysis

Damon Brown and Ray Turner
University of Calgary, Biological Sciences, Canada (browndc@ucalgary.ca)

In the oil and gas industry, internal corrosion represents one of the major threats to asset lifetime and integrity. Of the types of internal corrosion, microbiologically influenced corrosion (MIC) is the most difficult to predict and monitor due to the unpredictable nature of microbial growth and the minimal metal loss resulting in through wall failure (pitting). MIC results from biofilm communities interacting directly and indirectly with the metal. Due to the structure and nature of these pipelines, directly monitoring sessile growth is impossible. As a result, most MIC monitoring is done through planktonic cells retrieved from fluid samples as a proxy for sessile populations.

Growth curves are one of the most fundamental methods of quantitatively assessing microbial growth. In the lab, pure cultures are measured using optical densities, biomass staining, direct microscopic counting and counting colony forming units (CFU) on specialized media while more advanced techniques involve quantitative PCR (qPCR) of key genes. While PCR technologies are more easily transferred from the field to the lab, CFU counts are impossible in the field. Alternatives to the CFU are colorimetric activity assays such as "bug bottles" or biological activity reaction test (BART) bottles but aren't sensitive and require long incubation times. More sensitive assays such as ATP measurements are also used but can be misleading as high metabolically active samples will give higher cell count equivalents than a metabolically slow community of an identical size.

To systematically evaluate a best practice, we conducted growth curves in a lab scenario using six pure cultures and techniques predominantly used in the field to determine how these techniques compare and accurately measure microbial growth. The six species used are Acetobacterium woodii, Bacillus subtilis, Desulfovibrio vulgaris, Geoalkalibacter subterraneus, Pseudomonas putida and Thauera aromatica. The techniques used are optical density at 600 nm, ATP activity measurements using a luciferase-based assay, DNA concentration and 16S rRNA copy numbers.

It was found that most lines of data follow the expected sigmoidal growth curve to varying degrees for all species. OD_{600} readings follow the expected sigmoidal curves, exhibiting a lag phase, log growth phase and a stationary phase. ATP peaks during mid log phase and quickly declines, never showing a distinct stationary phase, while DNA concentrations closely follow the OD_{600} readings but decline to death phase more rapidly. qPCR of the 16S rRNA genes revealed this data followed the same trends but was less susceptible to fluctuations.

Assessing microbial biofilms in the environment and on anthropogenic industrial infrastructure is extremely challenging given sampling, storage and transportation to the lab. This work begins to establish best practices for growth of environmental communities to be followed. Cumulatively, this work shows that each approach supports the expected growth curve. Considerations should be made if all field data is of a single type, e.g. ATP, as it measures activity and not total cell count. Collecting even two lines of evidence in the field will greatly improve the quality of assessment and strengthen any conclusions regarding assessment of microbial growth.

How to cite: Brown, D. and Turner, R.: Reconstruction of an industry related biofilm into a proxy model community – Challenges around Field and lab based microbial growth analysis, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-52, https://doi.org/10.5194/biofilms9-52, 2020

biofilms9-86
https://doi.org/10.5194/biofilms9-86
biofilms 9 conference

Screening of fast biofilm formation on stainless steel by thermophilic sporeformers originated from dairy powder and their resistance against CIP

Louis Delaunay[1], Florence Postollec[2], Ivan Leguerinel[1], and Anne-Gabrielle Mathot[1]
[1]Université de Bretagne Occidentale, Science, Food science, France (louis.delaunay@univ-brest.fr)
[2]ADRIA Développement, UMT14.01 SPORE-RISK, Z.A. de Creac'h Gwen, F-29196, Quimper Cedex, France

Introduction:

Thermophilic sporeformers are present in raw milk at very low concentration and resist to pasteurisation applicated to destruct vegetative and pathogenic cells. Those spores can adhere to stainless steel due to their hydrophobicity and can form biofilms. Early stage biofilms are important because it can increase the matrix and the adhesion of other cells. Because of those biofilms, the three main species: Geobacillus stearothermophilus, Anoxybacillus flavithermus and Bacillus licheniformis can resists to Cleaning In Place (CIP) procedure, and contaminate a new process.

Material and Methods:

Early stage adhesion was conducted on stainless steel submerged by milk inoculated with a fresh culture of bacteria (G. stearothermophilus (N=15), A. flavithermus (N=32) and B. licheniformis (N=15)) for 6h of growth at 55°C under agitation. The ability of sporeformers to form biofilms under those conditions were measured by image analysis after a fluorescent coloration (acridine orange) and random photography. A coverage percentage was calculated by ImageJ ; and a positive threshold was set up at 5% of covering.

The efficiency of CIP procedures were obtained after a caustic soda and nitric acid treatment during different duration and temperature of treatment. Tested biofilms were formed in milk during 12h at 55°C, in stainless steel microplates (96 wells) on the same species (3 strains for each) under agitation. Surviving spores were enumerated by the microcolony method.

Results:

Early stage adhesion shows that 62.5 % (N=20) of A. flavithermus strains can form biofilm within 6h, whereas only 6.7% (N=1) of G. stearothermophilus and 0% (N=0) of B. licheniformis biofilm in 6h at 55°C on submerged stainless steel. However, the maximum covering % on A. flavithermus was 35%; while on the only biofilm forming strain of G. stearothermophilus, this percentage reach 75%. Image analysis also shows biofilm structure from 2D to 3D.

The presence and the resistance of spores to chemical cleaning was highly variable within strains. Nitric acid appears to be more effective than caustic soda against biofilms formed by vegetative cells and spores from these strains.

Significance:

Those results shows that strong biofilms are mainly composed of spore and are very resistant to CIP used in dairy industries. That is why a better understanding of control methods can lead to a finer and suitableness use of cleaning products.

How to cite: Delaunay, L., Postollec, F., Leguerinel, I., and Mathot, A.-G.: Screening of fast biofilm formation on stainless steel by thermophilic sporeformers originated from dairy powder and their resistance against CIP, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-86, https://doi.org/10.5194/biofilms9-86, 2020

biofilms9-74
https://doi.org/10.5194/biofilms9-74
biofilms 9 conference

Selective antibiofilm properties of nano-ZnO and nano-ZnO/Ag coated surfaces

Merilin Rosenberg[1,2], Meeri Visnapuu[3], Heiki Vija[1], Vambola Kisand[3], Kaja Kasemets[1], Anne Kahru[1,4], and Angela Ivask[1,5]

[1]National Institute of Chemical Physics and Biophysics, Laboratory of Environmental Toxicology, Estonia (rosenbergmerilin@gmail.com)

[2]Tallinn University of Technology, Department of Chemistry and Biotechnology, Estonia

[3]University of Tartu, Institute of Physics, Estonia

[4]Estonian Academy of Sciences, Estonia

[5]University of Tartu, Institute of Molecular and Cell Biology, Estonia

Background: Spread of pathogenic microbes and antibiotic-resistant bacteria in healthcare settings and public spaces is a serious public health challenge. Materials and surface-treatments that prevent solid surface colonization and biofilm formation or impede touch-transfer of viable microbes could provide means to decrease pathogen transfer from high-touch surfaces in critical applications. Both, ZnO and Ag nanoparticles have shown a great potential in antimicrobial applications. Although antimicrobial properties of such nanoparticle suspensions are well studied, less is known about nano-enabled solid surfaces.

Results: Here we demonstrate that solid surfaces coated with nano-ZnO or nano-ZnO/Ag composites possess species-selective medium-dependent antibiofilm activity against *Escherichia coli* MG1655, *Staphylococcus aureus* ATCC25923 and *Candida albicans* CAI4. Colonization of nano-ZnO surfaces by *E. coli* and *S. aureus* was decreased in oligotrophic (nutrient-poor, no growth) conditions with *E. coli* showing higher sensitivity to Ag and *S. aureus* to Zn, respectively. Nano-ZnO inhibited bacterial biofilm formation in a dose-dependent manner in oligotrophic conditions reaching maximum of 2.12 and 3.49 log reduction on dense nano-ZnO surface compared to uncoated surface after 72 h for *E. coli* and *S. aureus*, respectively. Minor to no effect was observed for bacterial biofilms in growth medium (nutrient-rich, supporting exponential growth). Addition of Ag to the sparse nano-ZnO surfaces had transient negative effect on *E. coli* biofilm formation in oligotrophic conditions with an additional 0.5-1.6 log reduction in harvested viable cells (3-48 h post-inoculation, respectively) compared with sparse nano-ZnO without added Ag. This additional reduction decreased to a non-significant 0.34 log by 72 h. Inversely, compared to uncoated surfaces, nano-ZnO surfaces enhanced biofilm formation by *C. albicans* in oligotrophic conditions by 1.27 log increase in viable attached cells at 48 h time point and just a minor transient negative effect was seen in nutrient-rich medium. However, enhanced *C. albicans* biofilm formation on nano-ZnO surfaces in oligotrophic conditions was effectively counteracted by the addition of Ag.

Conclusion: Our results not only showed that nano-ZnO and nano-ZnO/Ag coated solid surfaces have the potential to effectively decrease surface colonization by the bacteria *E. coli* and *S. aureus* but also indicated the importance of the use of application-appropriate test conditions and exposure medium in antimicrobial surface testing. Possible selective enhancement of biofilm formation by the yeast *C. albicans* on Zn-enabled surfaces should be taken into account in antimicrobial surface development.

This work was funded by Estonian Research Council Grants EAG20, PRG749.

How to cite: Rosenberg, M., Visnapuu, M., Vija, H., Kisand, V., Kasemets, K., Kahru, A., and Ivask, A.: Selective antibiofilm properties of nano-ZnO and nano-ZnO/Ag coated surfaces, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-74, https://doi.org/10.5194/biofilms9-74, 2020

biofilms9-68
https://doi.org/10.5194/biofilms9-68
biofilms 9 conference

Study of Biofilm Growth on Slippery Liquid-Infused Porous Surfaces Made from Fluoropor

Nico Keller[1], Julia Bruchmann[2], Thomas Sollich[2], Christiane Richter[1], Richard Thelen[3], **Kai Sachsenheimer**[1], Frederik Kotz[1], Thomas Schwartz[2], Dorothea Helmer[1], and Bastian E. Rapp[1]

[1]Department of Microsystems Engineering, University of Freiburg, 79110 Freiburg, Germany

[2]Institute of Functional Interfaces, Karlsruhe Institute of Technology, 76344 Eggenstein-Leopoldshafen, Germany

[3]Institute of Microstructure Technology, Karlsruhe Institute of Technology, 76344 Eggenstein-Leopoldshafen, Germany

Avoiding undesired growth of biofilm is a fundamental challenge for all surfaces in long-term contact with aqueous media. Slippery liquid infused porous substrates (SLIPS) are a promising type of surface for preventing biofilm attachment. The effectiveness of SLIPS is based on the liquid/liquid interface between the medium and the surface, which prevents biofilm attachment. However, the long-term stability of these surfaces is problematic: under shear force, the oil layer is removed and the repellent effect is lost. Here, we study correlations between the porosity of the infused substrate and the ability to uphold the SLIPS oil-film under low shear and high shear force conditions. For this purpose, we manufacture substrates with different porosity and surface roughness in porous fluorinated polymer "Fluoropor", which we have recently introduced. The porous layers were infused with fluorinated oil and their roughness was studied by white light interferometry. We find that SLIPS samples with smaller pores more effectively reduce Pseudomonas aeruginosa biofilm growth in a seven-day microfluidic flow cell experiment. With its easy production, simple adjustment of porosity and the possibility to attach the polymer to various technical substrates during polymerization, Fluoropor is a very promising material for producing stable SLIPS. When produced with small pores, Fluoropor is also transparent and enables the real-time observation of biofilm growth by optical examination. Thus, Fluoropor SLIPS provides an easy approach to reduce bacteria adhesion and bio fouling in many technical applications.

How to cite: Keller, N., Bruchmann, J., Sollich, T., Richter, C., Thelen, R., Sachsenheimer, K., Kotz, F., Schwartz, T., Helmer, D., and Rapp, B. E.: Study of Biofilm Growth on Slippery Liquid-Infused Porous Surfaces Made from Fluoropor, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-68, https://doi.org/10.5194/biofilms9-68, 2020

biofilms9-134
https://doi.org/10.5194/biofilms9-134
biofilms 9 conference

Study of the ability to form biofilms of microorganisms isolated from the milk industry in Canada

Coralie Goetz, Nissa Niboucha, and Julie Jean
Institut sur la Nutrition et les Aliments Fonctionnels, Sciences des aliments, Université Laval, Canada (coralie.goetz.1@ulaval.ca)

The ability of microorganisms to form biofilms has become a major problem in the dairy industry in Canada, notably by affecting the quality and the safety of the by-products. Established biofilms are difficult to remove during the CIP cleaning system and may become resistant to sanitizers. Therefore, it is important to identify and characterize the microorganisms associated to biofilm in the Canadian dairy industry, allowing to develop improvement strategies of biofilm control. The purpose of this study is to evaluate the ability to form biofilms by spoilage microorganisms isolated in processing plants in Canada. For this purpose, 19 strains were isolated from problems associated with the formation of biofilms in the dairy industry and identified using a MALDI-TOF mass spectrometer. The single species biofilm production of these isolates was then measured after a crystal violet coloration using 96-well microplates. The results revealed different biofilm formation profiles depending of the isolates in culture medium. Indeed, 7/19 isolates are moderate or strong biofilm producers and 12/19 isolates are negative or weak biofilm producers. Furthermore, enzymatic treatments revealed that the composition of the biofilms was different depending of the species but also the isolates. In conclusion, the results suggest that some of the isolates collected in the dairy industry have the ability to produce moderate or strong biofilms and thus, to facilitate the persistence of other spoilage microorganisms but also potential pathogenic microorganisms such as *Listeria monocytogene*s. The characterization of those biofilms will be helpful to the development of an effective approach allowing a better control of the biofilms in the dairy industry.

How to cite: Goetz, C., Niboucha, N., and Jean, J.: Study of the ability to form biofilms of microorganisms isolated from the milk industry in Canada , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-134, https://doi.org/10.5194/biofilms9-134, 2020

biofilms9-95
https://doi.org/10.5194/biofilms9-95
biofilms 9 conference

Talk2clean: application of probiotics to control biofilm in industrial water circuits – a innovative application

Hetty KleinJan[1], Christophe Meunier[1], Stéphane Nonet[1], and Mickael Michel[2]
[1]CEBEDEAU asbl, Allée de la Découverte, 11 Quartier Polytech 1 4000 Liège, Belgique
[2]CMI-AQUAGED, 34 rue des Marizys, 08400 Vouziers, France

Biofilms are omnipresent in industrial cooling water circuits and frequently lead to technical and economic problems. Yet, methods to remove biofilms may be inefficient, due to the EPS layer, which provides a protective layer against penetration of disinfectants. In combination with stricter legislation and increased environmental awareness, this has opened the search for alternative strategies to control biofilms in cooling water systems.

In this study we implement a novel biofilm removal strategy, in which we target the intercellular language involved in the formation of biofilm structures. This language consists of signalling molecules (autoinducers) excreted by biofilm forming bacteria, a process referred to as quorum sensing (QS). We aim to alter QS via the activation of an antagonistic process called quorum quenching (QQ). Quorum quenching is a process that naturally occurs in bacterial communities; hence the final product is environment, - and user-friendly and thus a valuable alternative to the oxidising chemical products that are often used to clean cooling water circuits.

Here we present the first application of a QQ-product tested on pilot scale using parameters that resemble industrial evaporative cooling towers. This setting is particularly interesting as it an open system, fed with various types of make-up water, and comprised of biofilms adjusted to high operating temperatures.

The QQ product was tested using a closed, tube-like system, under continuous flow, fed with a propionic, - and acetic acid rich synthetic medium. Hydraulic retention time (HRT) was gradually shifted over a time span of 6 weeks. Heterotrophic plate counts were acquired once per week from the planktonic and biofilm phase. A pilot without the addition of QQ was ran in parallel as a control.

Our results show that the QQ product reduced and delayed the formation of biofilm compared to the control. Interestingly, this difference diminished when the HRT was modified. Metagenomic analysis of the biofilm phase, revealed that 16S rRNA sequences corresponding to the QQ were also strongly reduced during this shift, indicating a wash-out of the QQ product. The exact interaction of HRT and presence of QQ will be analysed in more depth using QQ specific qPCR primers.

How to cite: KleinJan, H., Meunier, C., Nonet, S., and Michel, M.: Talk2clean: application of probiotics to control biofilm in industrial water circuits – a innovative application , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-95, https://doi.org/10.5194/biofilms9-95, 2020

biofilms9-33
https://doi.org/10.5194/biofilms9-33
biofilms 9 conference

The Impact of Shear Forces and Surface Hydrophobicity on Coccoid Cyanobacterial Biofilm Development

Sara I. Faria[1], Rita Teixeira-Santos[1], Maria J. Romeu[1], João Morais[2], Vítor Vasconcelos[2,3], and Filipe J. Mergulhão[1]
[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Chemical engineering, Portugal (sisf@fe.up.pt)
[2]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research
[3]FCUP—Faculty of Sciences, University of Porto

Biofouling is a natural process in marine environments with associated economic and ecological problems. Thus, understanding the conditions that affect cyanobacterial biofilm development is crucial to develop new antibiofouling strategies and decrease the impact of biofilms in the marine environment. In this study, we investigated the relative importance of shear forces and surface hydrophobicity on biofilm development by two coccoid cyanobacteria with different biofilm formation capacities. The strong biofilm-forming *Synechocystis salina* was used along with the weaker biofilm-forming *Cyanobium* sp. Biofilms were developed in defined hydrodynamic conditions using glass (a model hydrophilic surface) and a polymeric epoxy coating (a hydrophobic surface) as substrates. Biofilms developed in both surfaces at lower shear conditions contained a higher number of cells and presented higher values for wet weight, thickness, and chlorophyll *a* content. The impact of hydrodynamics on biofilm development was generally stronger than the impact of surface hydrophobicity, but a combined effect of these two parameters strongly affected biofilm formation for the weaker biofilm-producing organism. The antibiofilm performance of the polymeric coating was confirmed at the hydrodynamic conditions prevailing in ports. Shear forces were shown to have a profound impact on biofilm development in marine settings regardless of the fouling capacity of the existing flora and the hydrophobicity of the surface.

How to cite: Faria, S. I., Teixeira-Santos, R., Romeu, M. J., Morais, J., Vasconcelos, V., and Mergulhão, F. J.: The Impact of Shear Forces and Surface Hydrophobicity on Coccoid Cyanobacterial Biofilm Development, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-33, https://doi.org/10.5194/biofilms9-33, 2020

biofilms9-125
https://doi.org/10.5194/biofilms9-125
biofilms 9 conference

The effects of different biocides against selected drinking water-isolated bacteria in planktonic and sessile states

Isabel Maria Oliveira[1], Lúcia Chaves Simões[1,2], and Manuel Simões[1]
[1]LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal (up201204019@fe.up.pt; mvs@fe.up.pt)
[2]CEB – Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

The chemical disinfectant chlorine, as chlorine gas (Cl_2) and sodium hypochlorite (NaOCl), has been commonly used for drinking water treatment.[1,2] Although the recommended residual concentration of free chlorine allows to some extent the control of microbial growth in the bulk water, the occurrence of biofilms in chlorinated drinking water distribution systems (DWDS) has been frequently reported.[3,4] Therefore, the main goal of this study was the study of alternative biocides to control biofilm development in DWDS. The effects of sodium dichloroisocyanurate (NaDCC), trichloroisocyanuric acid (TCCA), and pentapotassium bis(peroxymonosulphate) bis(sulphate) (OXONE®) were analysed against two emerging pathogens isolated from drinking water, *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia*. The determination of the minimum bactericidal concentrations (MBC) of the selected biocides were based on the European Standard EN 1276, with MBC between 1.56 to 6.25 mg/L for NaDCC, 2.5 to 3.75 mg/L for TCCA, and 172 to 688 mg/L for OXONE®. Inactivation curves were developed and fitted to microbial survival models. The effects of biocides on cytoplasmic membrane integrity were assessed by propidium iodide uptake. The action on biofilm control was analysed against 48 h old biofilms developed on polyvinyl chloride (PVC) and stainless steel (SS) coupons using a 24-wells microtiter plate assay. The bacteria culturability and removal assessment were determined by colony forming units (CFU) enumeration on R2A agar, and by 4',6-diamidino-2-phenylindole (DAPI) staining, respectively. This study reinforces biofilms as chronic contaminants of DWDS and highlights that the understanding of antimicrobial susceptibility of microorganisms to biocides is an important step in the design of effective biofilm control strategies in order to provide to consumers drinking water of adequate microbiological quality.

References

[1] K.V. Ellis (1991), Crit. Rev. Environ. Control. 20:5-6, 341-407.

[2] N. Pichel, M. Vivar, and M. Fuentes (2019), Chemosphere. 218, 1014-1030.

[3] S. Liu, C. Gunawan, N. Barraud, S.A. Rice, E.J. Harry, and R. Amal (2016), Environ. Sci. Technol. 50, 8954-8976.

[4] L.C. Simões and M. Simões (2013), RSC Adv. 3, 2520-2533.

Acknowledgements

This work was financially supported by: Base Funding - UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE - funded by national funds through the FCT/MCTES (PIDDAC); PTDC/BII-BTI/30219/2017 - POCI-01-0145-FEDER-030219; POCI-01-0145-FEDER-006939, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through

FCT/MCTES; and PhD grant awarded to Isabel Maria Oliveira (SFRH/BD/138117/2018).

How to cite: Oliveira, I. M., Simões, L. C., and Simões, M.: The effects of different biocides against selected drinking water-isolated bacteria in planktonic and sessile states, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-125, https://doi.org/10.5194/biofilms9-125, 2020

biofilms9-142
https://doi.org/10.5194/biofilms9-142
biofilms 9 conference

The glycolysis by-products glycolic acid and glyoxal cause antimicrobial and antibiofilm effects

Susana Fernandes and Manuel Simões
University of Porto, LEPABE, Chemical Engineering Department, Portugal (su.fonseca.02@gmail.com)

The development of new biocidal formulations targeting cells in biofilms is still a scientific challenge [1,2]. The current arsenal of biocides is clearly limited in controlling biofilms [3]. Therefore, novel molecules to control biofilms are needed. This study assessed the antimicrobial activity of glycolic acid (GA) and glyoxal (GO) against *Bacillus cereus* and *Pseudomonas fluorescens*, two species commonly found in industrial biofilms. GA and GO are two glycolysis by-products approved as biocides for surface disinfection, whose antimicrobial action remains to be understood. Their antimicrobial activity was determined according to the European Standard EN 1276 [4]. The mode of action was assessed according to the effects on the cell envelope (surface hydrophobicity and cell membrane damages) and cell replication. *P. fluorescens* was eradicated by both selected compounds, while *B. cereus* was only partially reduced even under high concentrations. According to the survival curves, *P. fluorescens* cells had the same susceptibility to both compounds. *B. cereus* cells were more susceptible due to cumulative damages. The dose-activity curves proposed that the selected compounds interacted chemically with cell targets - GA and GO were able to disturb cell integrity, causing changes in cell hydrophobicity and further membrane damages. In terms of cell replication, GA caused negligible changes in lag time length and in the maximum cell growth, while GO was found to act as a bacteriostatic. Thus, GA was found to be an oxidant (acid group) and membrane-active compound (alcohol group). On the other hand, GO had cell growth inhibitory (nucleophilic group) effects. These compounds were further applied against *B. cereus* and *P. fluorescens* biofilms, promoting strong inactivation and removal effects. The combination of GA and GO with traditional biocides is likely to represent a new, and much needed, generation of disinfectant formulations for industrial biofilm control.

References

1 Yuksel, F. N., Buzrul, S., Akcelik, M. & Akcelik, N. Inhibition and eradication of *Salmonella* Typhimurium biofilm using P22 bacteriophage, EDTA and nisin. Biofouling **34**, 1046-1054, doi:10.1080/08927014.2018.1538412 (2019).

2 Araújo, P. A. et al. Combination of selected enzymes with cetyltrimethylammonium bromide in biofilm inactivation, removal and regrowth. Food Res Int **95**, 101-107, doi:10.1016/j.foodres.2017.02.016 (2017).

3 Capita, R. et al. Effect of low doses of biocides on the antimicrobial resistance and the biofilms of *Cronobacter sakazakii* and *Yersinia enterocolitica*. Sci Rep **9**, 15905, doi:10.1038/s41598-019-51907-1 (2019).

4 European Standard EN-1276 Chemical disinfectants and antiseptics in: Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)

(2009).

How to cite: Fernandes, S. and Simões, M.: The glycolysis by-products glycolic acid and glyoxal cause antimicrobial and antibiofilm effects, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-142, https://doi.org/10.5194/biofilms9-142, 2020

biofilms9-143
https://doi.org/10.5194/biofilms9-143
biofilms 9 conference

The influence of the potential on single cell yield coefficients of early stage anode biofilms of Geobacter sulfurreducens

Francesco Scarabotti[1], Benjamin Korth[1], Laura Rago[1], Katja Bühler[2], and Falk Harnisch[1]
[1]Helmholtz Centre for Environmental Research GmbH - UFZ, Department of Environmental Microbiology, - 04318 Leipzig, Germany (falk.harnisch@ufz.de)
[2]2 Helmholtz Centre for Environmental Research GmbH - UFZ, Department Solar Materials, - 04318 Leipzig, Germany

Geobacter sulfurreducens is a gram- rod shaped microorganism and the model system for direct extracellular electron transfer (EET). Although several details of the molecular mechanisms of EET are deciphered [1, 2], there is still a significant lack of knowledge, for instance on kinetics or cell growth. It is the aim of this study to provide information about the influence of the anode potential on yield coefficients on the single cell level, i.e. the moles of substrate consumed or moles of electrons transferred to the anode per cell. This information is highly relevant for physiological as well as technical considerations and for further improvement of bioelectrochemical systems (BES). Therefore, single and double chamber bioelectrochemical reactors were operated in batch mode using either the "standard" graphite rods or a microscopy slide sputter-coated with 10 nm of Cr and 25 nm of AuPd as anode material for allowing analysis of the biofilms with CLSM, as a tool for cell number determination.
We demonstrate that the anode potential and the electrode material has a strong influence on the kinetics of initial growth as well as on the yield coefficients of early stage anode biofilms of *Geobacter sulfurreducens*, but does not influence other parameters like coulombic efficiency. For instance, in single chamber reactors, the observed lag time, expressed as the time when the biofilm delivered a current density of 1 µA cm^{-2}, was of 1.76±1.02, 6.99±2.26 days and 5.32±1.82 days at -200mV, 0 mV and +200mV (vs. Ag/AgCl sat. KCl) respectively with the sputter-coated glass and of 2.44±0.48 days, 1.38±0.46 days and 1.73±1.45 at -200 mV, 0 mV and +200 mV (vs. Ag/AgCl sat. KCl) respectively using graphite rods.

References

[1] Annu Rev Microbiol. 2012; 66:391-409

[2] Microbial Electrochemical Technology Sustainable Platform for Fuels, Chemicals and Remediation Biomass, Biofuels and Biochemicals 2019, Pages 339-351

How to cite: Scarabotti, F., Korth, B., Rago, L., Bühler, K., and Harnisch, F.: The influence of the potential on single cell yield coefficients of early stage anode biofilms of Geobacter sulfurreducens, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-143, https://doi.org/10.5194/biofilms9-143, 2020

biofilms9-43, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-43
biofilms 9 conference

The microbiome of water and water-associated biofilms in meat processing facilities

Eva M. Wagner[1], Sarah Thalguter[1], Kathrin Rychli[2], and Martin Wagner[1,2]
[1]FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, 3430 Tulln, Austria
[2]Institute for Food Safety, Food Technology and Veterinary Public Health - Unit of Food Microbiology, University of Veterinary Medicine Vienna, Vienna, Austria

Drinking water represents a reservoir for microorganisms. Microorganisms from water are able to attach to the inner surface of a water hose and nourish themselves by the leaking carbon molecules. Through the interaction of different species a multi-species biofilm can develop. Especially in the food processing environment biofilms in water hoses represent a risk factor. Within the food processing sector water hoses are often used to remove disinfecting agents from freshly cleaned surfaces, after the cleaning and disinfection procedure. When biofilms are located inside these water hoses, cells or cell clusters can detach, subsequently contaminating cleaned food contact surfaces.

We checked water hoses as a biofilm hotspot in a meat processing facility by using a flocked swab for biofilm sampling inside the water hose and accessory parts (i.e. nozzle). The bacterial load (culture-based and DNA-based) and the presence of matrix components (carbohydrates, proteins and extracellular DNA) were analysed.

Herby we identified three from six tested water hoses to harbour a biofilm, by being positive for microorganisms and at least two matrix components. This clearly states the need for further understanding of biofilm formation in water hoses. Within the three other hoses, microorganisms could be detected, but no matrix components. We could isolate twelve genera of the water hose associated biofilms using one growth medium (TSA) and two different incubation temperatures (10 °C and 20 °C). There was only on genus that was present within all three water hose biofilms, which was *Rhodococcus*. Previously this genus was isolated from a shower head (Lee 2013), and is known to catabolise a wide range of organic compounds. This potentially enables the growth in a nutrient poor environment like the water hose providing secondary colonisers launch aid to contribute to the biofilm. The genera *Flavobacterium*, *Microbacterium* and Stenotrophomonas were shared among two of the water hose biofilms. Experiments to assess the biofilm forming ability of *isolates* of these genera using a mono-species static biofilm model indicate that all three species are able to produce matrix and can therefore be regarded as biofilm producers.

To date, there is limited information about biofilm development and presence in water hoses, especially in the food processing environment. This first identification of biofilms in water hoses and associated parts emphasizes the need of further research on this topic and detailed monitoring at these sites to prevent recontamination. A currently ongoing microbiome study on the water, the used water hoses, and the water-contacting food contact sites in a meat processing facility will give further details about the biofilm presence and possible transmission of microorganisms encountered there.

References:

Lee, Yoonjin. 2013. "An Evaluation of Microbial and Chemical Contamination Sources Related to the

Deterioration of Tap Water Quality in the Household Water Supply System.” International Journal of Environmental Research and Public Health 10(9):4143–60.

How to cite: Wagner, E. M., Thalguter, S., Rychli, K., and Wagner, M.: The microbiome of water and water-associated biofilms in meat processing facilities, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-43, https://doi.org/10.5194/biofilms9-43, 2020

biofilms9-1
https://doi.org/10.5194/biofilms9-1
biofilms 9 conference

The role of filter media geometry in tap water biofilms

Erifyli Tsagkari, Stephanie Connelly, Zhaowei Liu, Andrew McBride, and William Sloan
University of Glasgow, College of Science and Engineering, School of Engineering, United Kingdom

Biofilms are inextricably linked to the hydrodynamics of flow through a filter and yet engineers rarely explicitly engineer this interaction. We developed a system that links computer simulation and 3-D printing to optimize filter media geometry and biofilm function. The main objective is to prototype filter media to passively induce vortices that roll down a surface, imposing oscillating flow in the channel to enhance biofilm formation. Thus, a 2-D model was developed and linked to a 3-D printer. The model was solved to determine the wall shear stress distribution with time. The experiments showed that the thickest, densest and most extended biofilms were formed for the strongest oscillations in the channel due to the presence of a filter medium. This is a speeding-up innovation in the design and implementation of small-scale biofiltration systems for rural communities.

How to cite: Tsagkari, E., Connelly, S., Liu, Z., McBride, A., and Sloan, W.: The role of filter media geometry in tap water biofilms , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-1, https://doi.org/10.5194/biofilms9-1, 2020

biofilms9-30
https://doi.org/10.5194/biofilms9-30
biofilms 9 conference

The susceptibility of E. faecalis biofilm against selected new quaternary ammonium compounds

Michaela Hympanova[1,2], Sasa Terlep[3], Aneta Markova[1,4], Lukas Prchal[1], Iztok Dogsa[3], Jan Marek[1,2], and David Stopar[3]

[1]University of Defence in Brno, Faculty of Military Health Sciences, Department of Epidemiology, Hradec Králové, Czech Republic
[2]Biomedical Research Centre, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic
[3]University of Ljubljana, Biotechnical Faculty, Department of Microbiology, Ljubljana, Slovenia
[4]University of Defence in Brno, Faculty of Military Health Sciences, Department of Toxicology and Military Pharmacy, Hradec Kralove, Czech Republic

Introduction: An increasing microbial resistance to known antibiotics raises the demand for new antimicrobials. New antibacterial agent should have a good activity against planktonic as well as biofilm bacteria. Quaternary ammonium compounds (QACs), are widely used in medicine, have proven antimicrobial properties, and are low toxic and low irritating. In this study new QACs were evaluated for their biofilm eradication efficiency as antibacterial compounds and as irrigants in combination with Er:YAG photoacoustic streaming.

Aims: To evaluate the effectiveness of new QACs against E. faecalis biofilms and to increase the effectiveness of QACs with laser treatment.

Method: The biofilm of E. faecalis were grown on titanium surface. The fraction of the dead cells and the biofilm surface coverage was determined with LIVE/DEAD™ using confocal microscopy (CLSM) before and after QACs treatment. To enhance to effectiveness the biofilm samples were pretreated with QACs followed by short laser Er:YAG photoacoustic streaming treatment.

Results: All tested QACs were effective against the E. faecalis biofilms. The best anti-biofilm compounds were N-Alkylimidazolinium derivatives. Compared to planktonic bacteria the bacteria in the biofilm were up to 10 fold more resistant. The fraction of the dead bacteria that were treated with QACs followed by Er:YAG photoacoustic streaming increased significantly compared to the chemical treatment alone. In addition, the biofilm surface coverage decreased after laser treatment.

Conclusions: The results suggest that new QACs have a great potential as antibacterial compounds effective against biofilms of E. faecalis. The laser treatment can significantly improve the effectiveness of QACs treatment.

How to cite: Hympanova, M., Terlep, S., Markova, A., Prchal, L., Dogsa, I., Marek, J., and Stopar, D.: The susceptibility of E. faecalis biofilm against selected new quaternary ammonium compounds, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-30, https://doi.org/10.5194/biofilms9-30, 2020

biofilms9-4
https://doi.org/10.5194/biofilms9-4
biofilms 9 conference

The versatile effect of L- and D-Cateslytin on bacteria and yeast biofilms according to configuration, medium and dose

Lydie Ploux[1], Min Jin[1], Sophie Hellé[1], Cosette Betscha[1], Jean-Marc Strub[2], and Marie-Hélène Metz-Boutigue[1]

[1]CNRS/INSERM/University of Strasbourg, U1121 Biomaterials and Bioengineering, France (ploux@unistra.fr)

[2]CNRS/University of Strasbourg, UMR7178, Laboratoire de spectrométrie de masse bioorganique, France

L- and D-Cateslytin (CTL) are antimicrobial peptides (AMP) derived from chromogranin A, a protein of the stress response system. Their antimicrobial properties have been thoroughly characterized and already exploited in biomaterials. However, effects on biofilms of yeast and bacteria have never been specifically addressed. We have investigated the impact of both L and D configurations of CTL on the growth of biofilms formed by Candida albicans, Escherichia coli or Staphylococcus aureus microorganisms.

The study was conducted in different media and two strategies of treatment were tested, consisting of administrating the peptide either just at the beginning of biofilm development i.e. on just adhering pioneer microbial cells or on a biofilm already allowed to develop for 24h. We also considered whether the peptide was modified in contact with the medium or/and microbial metabolites. Planktonic and sessile populations of microbial cells were analyzed by spectrophotometry, crystal violet staining, MTT and confocal microscopy with staining by Syto9Ò and propidium iodide. Identification of the peptides and their derived fragments was investigated by HPLC and Mass-Spectroscopy.

In general, CTL-D exhibited higher antibiofilm performances than CTL-L. In addition, concentrations necessary to inhibit biofilm formation were found to vary from ten to eighty times the MICs determined in planktonic cultures. Nevertheless, the results also demonstrate that sessile microorganisms and biofilms are sensitive to CTL (L and D conformations) differently that planktonic populations. Significant (p-value < 0.01) effects were observed on both sessile and planktonic populations and with both strategies of treatments, but they highly varied with medium, species and CTL configuration. Typically, better antibiofilm effect than common antibiotics was reached in some specific conditions, while enhancement of aggregation or biofilm formation occurred in another medium and for other doses. Nevertheless,

Finally, this confirms the quality of CTL peptides as new antimicrobial agents and reveals their anti-biofilm properties. This also specifies the conditions of use necessary to benefit of the highest performances.

How to cite: Ploux, L., Jin, M., Hellé, S., Betscha, C., Strub, J.-M., and Metz-Boutigue, M.-H.: The versatile effect of L- and D-Cateslytin on bacteria and yeast biofilms according to configuration, medium and dose, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-4, https://doi.org/10.5194/biofilms9-4, 2020

biofilms9-77
https://doi.org/10.5194/biofilms9-77
biofilms 9 conference

Understanding how operating conditions affect biofouling structure in spacer filled membrane filtration channels using optical coherence tomography

Kees Theo Huisman, Bastiaan Blankert, Szilard Bucs, and Johannes S. Vrouwenvelder
KAUST, Water Reuse and Decalination Center, Saudi Arabia (kees.huisman@kaust.edu.sa)

Abstract

The growth of biofilms, causing biofouling on the membrane and feed spacer surface, is an unavoidable phenomenon in reverse osmosis. Biofouling can lead to unacceptable losses in product quality and quantity, and membrane lifetime. Process conditions such as crossflow velocity and nutrient concentration in the feed water strongly affect the development of biofilms. To improve system performance, understanding the relation between process conditions, biofilm development, and system performance is key. Optical coherence tomography (OCT), is increasingly applied to characterize biofilm structure in-situ and non-destructively. In OCT, near-infrared light is used to capture 2D and 3D images from within optical scattering media. In spacer filled channels with representative biodegradable nutrient conditions in the feed, biofilms often develop heterogeneously and dispersed. In such systems, commonly used structural parameters such as average thickness, average roughness, and average porosity may not be reflected in the system performance.

In this study, biofilm structural and spatial parameters are explored with the objective to link biofouling in spacer filled channels to system performance indicators. For this purpose, biofilms are grown in membrane fouling simulators at different nutrient concentrations and flow rates. Biofilm development on the feed spacer and on the membrane and system performance (pressure drop, transmembrane pressure, rejection) are monitored. Understanding the impact of (i) feed water quality and flow rate on biofilm growth and of (ii) biofilm structure and spatial distribution on system performance will lead to the development of more effective strategies for biofouling control.

Keywords

Biofouling; desalination; drinking water production; reverse osmosis; optical coherence tomography; feed spacer; biofilm structure

How to cite: Huisman, K. T., Blankert, B., Bucs, S., and Vrouwenvelder, J. S.: Understanding how operating conditions affect biofouling structure in spacer filled membrane filtration channels using optical coherence tomography, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-77, https://doi.org/10.5194/biofilms9-77, 2020

biofilms9-132
https://doi.org/10.5194/biofilms9-132
biofilms 9 conference

Use of enzymatic detergents to remove biofilms in food industries

Mercè Berga, Irene Ylla, and Martirià Latorre
Itram Higiene, Technical Department, Spain

In the last decade concern about the presence of biofilms in food processing plants has increased. Biofilms in the environment of food processing plants represent a threat to food quality, safety and shelf-life. These biofilms can host pathogenic bacteria such as Listeria, Salmonella and Campylobacter, as well as spoilage microorganisms. Additionally, biofilms show some degree of resistance to conventional detergents and disinfectants that hinders their removal and favors re-growth. Therefore, there is a need for sanitizing products and protocols that are highly efficient at removing biofilms and suitable for food processing plants. Enzymatic detergents have recently been introduced as an alternative to conventional products against biofilms in food processing plants. These detergents contain one or more enzymes that disrupt the EPS of the biofilms, making the microorganisms present in the biofilm more vulnerable to disinfectants. Enzymatic detergents have been proofed to be more efficient in degrading biofilms than conventional detergents reducing both, EPS content and bacterial counts. Finally, higher efficiency on biofilm removal was observed after completing the entire sanitizing procedure (cleaning + disinfection) using an enzymatic detergent than a conventional detergent. These tests confirm the great potential of enzymatic detergents to remove biofilms.

How to cite: Berga, M., Ylla, I., and Latorre, M.: Use of enzymatic detergents to remove biofilms in food industries, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-132, https://doi.org/10.5194/biofilms9-132, 2020

Topic 2
Biofilm Heterogeneity

Heterogeneity is the most reliable companion of biofilm development in all types of environments. Structural heterogeneity can be described by parameters like texture, roughness, and porosity among others. Variations in these parameters are often indicative of the performance of biofilm systems in terms of turnover, substrate consumption, and mass transfer at the biofilm-water interface. Hence, structural heterogeneity will often correlate with heterogeneity on a molecular level. Challenges appear if we want to predict the amount of substrate, which can be converted or the amount of product, which can be generated by a biofilm of a certain surface area over time. We would like to approach the term heterogeneity in biofilm systems with advanced imaging techniques both on the micrometre scale but also on the mesoscale with molecular techniques like single cell sequencing. Moreover, we would like to have contributions which can show that heterogeneity can be quantified and linked to biofilm performance measures.

Oral Presentations

biofilms9-31
https://doi.org/10.5194/biofilms9-31
biofilms 9 conference

Morphological and diffusional changes in L. lactis biofilms

Jonas Chodorski[1], Jan Hauth[2], Andreas Wirsen[2], and Roland Ulber[1]
[1]Bioprocess Engineering, TU Kaiserslautern, Kaiserslautern, Germany
[2]System Analysis, Prognosis and Control, Fraunhofer ITWM, Kaiserslautern, Germany

Through their special way of life, biofilms have several advantages over organisms in planktonic growth. By being surface-attached and producing a mass of extracellular polymeric substances (EPS), microorganisms possess inherent self-immobilization, which decreases the expenditure of downstream processing in industrial applications. Furthermore, they are more resilient against environmental stress and toxic substances, such as antibiotics. An important factor here is diffusion, of substrate into the biofilm and metabolites through and out of the biofilm; however, these mechanisms are still poorly understood. By utilizing a specially developed diffusion model and CLSM FRAP microscopy, diffusion constants in the living, fully hydrated biofilm of *L. lactis* during development can be assessed. With it, heatmaps of diffusional constants and finally a diffusion profile encompassing a true 3D space of the living biofilm in growth can be generated. With those, possible hotspots and changes of diffusion inside the biofilm structure with regard to changing cultivation conditions and the substratum can be identified, thus furthering our understanding of diffusion in biofilms and how they react to their environment.

The project is funded by the DFG (UL 170/14-1) and the collaborative research center (SFB) 926 on "microscale morphology of component surfaces" (MICOS).

How to cite: Chodorski, J., Hauth, J., Wirsen, A., and Ulber, R.: Morphological and diffusional changes in L. lactis biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-31, https://doi.org/10.5194/biofilms9-31, 2020

biofilms9-26
https://doi.org/10.5194/biofilms9-26
biofilms 9 conference

BiofilmQ, a software tool for quantiative image analysis of microbial biofilm communities

Hannah Jeckel[1,2], Raimo Hartmann[1], Eric Jelli[1,2], Knut Drescher[1,2,3], and the BiofilmQ team[*]
[1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany (jeckel@physik.uni-marburg.de)
[2]Department of Physics, Philipps University Marburg, Marburg, Germany
[3]Center for Synthetic Microbiology, SYNMIKRO, Marburg, Germany
[*]A full list of authors appears at the end of the abstract

Biofilms are now considered to be the most abundant form of microbial life on Earth, playing critical roles in biogeochemical cycles, agriculture, and health care. Phenotypic and genotypic variations in biofilms generally occur in three-dimensional space and time, and biofilms are therefore often investigated using microscopy. However, the quantitative analysis of microscopy images presents a key obstacle in phenotyping biofilm communities and single-cell heterogeneity inside biofilms. Here, we present BiofilmQ, a comprehensive image cytometry software tool for the automated high-throughput quantification and visualization of 3D and 2D community properties in space and time. Using BiofilmQ does not require prior knowledge of programming or image processing and provides a user-friendly graphical user interface, resulting in editable publication-quality figures. BiofilmQ is designed for handling fluorescence images of any spatially structured microbial community and growth geometry, including microscopic, mesoscopic, macroscopic colonies and aggregates, as well as bacterial biofilms in the context of eukaryotic hosts.

BiofilmQ team:
Raimo Hartmann, Hannah Jeckel, Eric Jelli, Praveen K. Singh, Sanika Vaidya, Miriam Bayer, Lucia Vidakovic, Francisco Díaz-Pascual, Jiunn C.N. Fong, Anna Dragoš, Olga Besharova, Janne G. Thöming, Susanne Häussler, Carey D. Nadell, Victor Sourjik, Ákos T. Kovács, Fitnat H. Yildiz, Knut Drescher

How to cite: Jeckel, H., Hartmann, R., Jelli, E., and Drescher, K. and the BiofilmQ team: BiofilmQ, a software tool for quantiative image analysis of microbial biofilm communities, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-26, https://doi.org/10.5194/biofilms9-26, 2020

BiofilmQ team: Raimo Hartmann, Hannah Jeckel, Eric Jelli, Praveen K. Singh, Sanika Vaidya, Miriam Bayer, Lucia Vidakovic, Francisco Díaz-Pascual, Jiunn C.N. Fong, Anna Dragoš, Olga Besharova, Janne G. Thöming, Susanne Häussler, Carey D. Nadell, Victor Sourjik, Ákos T. Kovács, Fitnat H. Yildiz, Knut Drescher

biofilms9-64
https://doi.org/10.5194/biofilms9-64
biofilms 9 conference

Oxygen spatio-temporal distribution in a 4-species adherent community of bacteria

Wafa Ben Youssef, Amaury Monmeyran, Franck Sureau, Thomas Panier, and Nelly Henry
Laboratoire Jean Perrin, CNRS-UMR 8237, Sorbonne université, Campus Jussieu, Paris F-75005, France
(wafa.ben_youssef@upmc.fr)

More than 30 years have passed now since the pioneering work of Costerton and co-workers[e.g.,1]. We have learned that the biological functions of the cells embedded in the complex, self-produced polymeric extracellular matrix, differ radically from the ones of the planktonic cells. Emergent properties such as enhanced antimicrobial resistance appear. Biofilms are widely spread in different habitats, both in the environment and the living organisms. Mostly, the characterization of this bacterial specific phenotype has been carried out using mono-species lab models. Yet, these systems are in marked contrast to the biofilms found in the environment. Those are usually complex and contain multiple bacterial species and, in many cases, also fungi, algae, and protozoa[2]. To take this into account, researches have recently turned to multispecies communities, aiming at describing the interspecies interactions in order to decipher the mechanisms underlying the properties of these complex consortia.

We present here a simplified model community consisting of 4 species — *Bacillus thuringiensis, Kocuria salsicia, Pseudomonas fluorescens, Rhodocyclus sp.* — elaborated from a natural environment to investigate the mechanisms supporting the formation of a multispecies consortium. We have been able to grow the 4-species biofilm under flow in a millimetric channel made of PDMS, which enabled to monitor the biofilm settlement and development using video-microscopy[3]. We found a deterministic development which follows defined kinetics and spatial distribution, suggesting that the formation of this adherent community is dominated by the self-induced modulation of the environmental parameters. To clarify this hypothesis, we focused our attention on the spatio-temporal distribution of oxygen and we devised an original experiment to map *in situ* and in real-time the evolution of oxygen level within the 4-species biofilm.

We used an O_2 fluorescent probe made of a Ruthenium complex encapsulated in lipidic micelles to overcome the metal toxicity. We derived local oxygen concentration in the biofilm from fluorescent-lifetime imaging microscopy (FLIM) measurements of the probe *in situ*. The setup was equipped with a light sheet to ensure the optical sectioning for a 3D mapping. We will show here the spatial and temporal characteristics of the method and the first O_2 map obtained on a growing biofilm.

To conclude, we will discuss how the monitoring of oxygen spatio-temporal distribution in a model community can help to elucidate basic interspecies interactions and reveal general mechanisms likely to govern number of more complex natural systems.

How to cite: Ben Youssef, W., Monmeyran, A., Sureau, F., Panier, T., and Henry, N.: Oxygen spatio-temporal distribution in a 4-species adherent community of bacteria , biofilms 9 conference,

Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-64,
https://doi.org/10.5194/biofilms9-64, 2020

biofilms9-66
https://doi.org/10.5194/biofilms9-66
biofilms 9 conference

Landscape-level patterns in photosynthetic marine fouling biofilm compositional heterogeneity as revealed by hyperspectral classification

Jennifer Longyear[1] and Paul Stoodley[2,3]
[1]International Paint Ltd, AkzoNobel, Gateshead, UK
[2]National Centre for Advanced Tribology at Southampton (nCATS), University of Southampton, Southampton, UK
[3]Infectious Diseases Institute, The Ohio State University, Columbus, USA

Marine fouling biofilms typically have diverse community assemblages in which microalgae are strongly represented. The visible light absorption properties of microalgal photosynthetic pigments typically drive the overall visible light reflectance spectra of these biofilms. In some cases diagnostic spectral features can be used to infer algal taxonomy, while in mixed communities the overlapping pigment signatures of the constituent species often blur together. In this study, we apply methods common in remote sensing approaches to spectral data to extract information from subtle variations in the reflectance spectra of mixed composition marine biofilms. We demonstrate that marine biofilm community composition, as evidenced by their reflectance spectra, is both spatially heterogenous and spatially structured.

Visible-NIR hyperspectral images (3.3nm x 200 bands) of biofilms grown on 7.5cm x 7.5cm panels (n=9), immersed in a coastal marina at ~1m depth for 13 months, were captured with a benchtop line-scan imager. The hyperspectral data were smoothed and transformed to consolidate the major aspects of spectral variability. A novel active learning spectral classification method incorporating iterative spectral library building by k-means clustering and spectral angle mapping, followed by hierarchical clustering by spectral similarity, discovered more than 70 distinct spectral classes present in the biofilms. Accordingly, the hyperspectral images of the fouling biofilms were converted to spatially explicit spectral class maps, where each class was assumed representative of a distinct community compositional mix. Hyperspectral indexing calibrated to chl *a* surface area density was used to map biomass for the same images.

Cross-tabulating the spectral class and biomass data, it was apparent that for these biofilms, different biomass density levels were consistently associated with specific community compositions (spectral classes.) Only a small number of the possible classes were represented in the densest areas of biofilm, suggesting that these species composition mixes have a competitive advantage. In contrast, the full diversity of class types was present in the low biomass areas.

Our hyperspectral approach does not convey exact species composition, as would pooled metagenomic sampling or in-depth microscopy. However it does allow for the examination of spatially explicit changes in biofilm composition at relatively large scales (the landscape), and so may be a useful tool in hypothesis generation, long term monitoring, and other environmental biofilm applications.

How to cite: Longyear, J. and Stoodley, P.: Landscape-level patterns in photosynthetic marine fouling biofilm compositional heterogeneity as revealed by hyperspectral classification, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-66, https://doi.org/10.5194/biofilms9-66, 2020

biofilms9-104
https://doi.org/10.5194/biofilms9-104
biofilms 9 conference

Impact of spatial heterogeneity for selection regimes in multispecies biofilms

Henriette Lyng Røder[1], Wenzheng Liu[2], Jakob Russel[1], Jakob Herschend[1], Michala Falk Andersen[1], Jonas Stenløkke Madsen[1], Søren Johannes Sørensen[1], and Mette Burmølle[1]
[1]Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark
[2]School of Food and Pharmaceutical Engineering, Nanjing Normal University, Nanjing, China

Bacteria live in sprawling communities forming complex dynamic biofilm structures. The spatial heterogeneity found in biofilms may be a driver for the selection for optimized biofilm variants. Bacterial species that increase their matrix production can position favourably in the exterior biofilm regions in order to compete for valuable substrates. Here, we study both the spatial organisation and growth of bacterial cells in different bacterial communities over time to determine links between the structure of interspecies biofilms and selection for phenotypes adapted to growth and persistence in biofilms. This leads to the identification of driving mechanisms behind the dynamic spatial organisation combined with the performance of individual species over time in the spatial biofilm structure.

Four species, previously co-isolated from soil, were used in different combinations. We examined the formation and spatial organisation of biofilms in distinct experimental model systems, as we hypothesized that their interaction would change dependent on the specific environment. The biofilm models used included the static Calgary biofilm device assay and two flow systems: the microfluidic BioFlux model (liquid bulk flow), and the drip flow reactor (liquid-air interphase). Both chromosomal fluorescent markers and FISH were used to visualize the organisation within biofilms by confocal laser scanning microscopy.

We reveal how the changes in biofilm structure affect the overall performance of the biofilm community as well as the individual species in the biofilm. Our data indicates that a favourable localization of the individual species in a multispecies biofilm reduces selection for competitive phenotypes. Furthermore, we also observed that changes in matrix production could serve to stabilise the interspecific interaction between bacteria. This highlights the specific structural composition of a biofilm community as important for explaining biofilm dynamics.

How to cite: Røder, H. L., Liu, W., Russel, J., Herschend, J., Andersen, M. F., Madsen, J. S., Sørensen, S. J., and Burmølle, M.: Impact of spatial heterogeneity for selection regimes in multispecies biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-104, https://doi.org/10.5194/biofilms9-104, 2020

Poster Presentations

biofilms9-141
https://doi.org/10.5194/biofilms9-141
biofilms 9 conference

Biofilm formation capacity of S. aureus under diabetic environments

María Fernández Grajera[1,2,3], María Coronada Fernández Calderón[2,3,4], Miguel A. Pacha Olivenza[2,3,4], Ciro Pérez Giraldo[2,3,4], Amparo M. Gallardo Moreno[1,2,3], and María Luisa González Martín[1,2,3]

[1]University of Extremadura, Department of Applied Physics, Badajoz, Spain
[2]Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Badajoz, Spain
[3]University Institute of Extremadura Sanity Research (iNube), Badajoz, Spain
[4]University of Extremadura, Department of Biomedical Science, Badajoz, Spain

Diabetes increases the blood glucose levels above those of healthy individuals and poorly controlled diabetes is associated to ketoacidosis. Different authors have shown evidences that diabetes is linked to a higher risk of developing infections in different parts of the body. Although the reasons why diabetes enhances infection episodes are not entirely clear, different undesired physiological responses under diabetic environments are pointed out as the main causes, for example, inflammatory reactions, poor vascularization, neutrophilic chemotaxis or phagocytosis. However, it has so far not been quantified how high concentrations of glucose and ketone bodies can affect the beginning of the infectious process, i.e. the formation of biofilms.

In this sense, this research will address how the presence of glucose and ketone bodies can alter the biofilm formation capacity of Staphylococcus aureus. The research will be carried out with six different diabetic conditions, including the individual action of both components (glucose and ketone bodies) and the combined action.

The main conclusion of this work is that any studied diabetic condition is able to increase the slime index of S. aureus with respect to control (bacteria grown without diabetic supplements), so the biofilm formation capacity of this bacterium would rise in diabetic people. In addition to the change that can be as high as 400% in glucose concentrations of 1.9 mg/ml, the clustering behavior among the bacteria is also modified at all condition differently.

How to cite: Fernández Grajera, M., Fernández Calderón, M. C., Pacha Olivenza, M. A., Pérez Giraldo, C., Gallardo Moreno, A. M., and González Martín, M. L.: Biofilm formation capacity of S. aureus under diabetic environments, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-141, https://doi.org/10.5194/biofilms9-141, 2020

biofilms9-49
https://doi.org/10.5194/biofilms9-49
biofilms 9 conference

Chemical stress contribution in bacterial biofilm formation

Olga Iungin[1,2] and Andrew Spiers[3]
[1]Kyiv National University of Technologies and Design, Kyiv, Ukraine (olgaungin@gmail.com)
[2]Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, Kyiv, Ukraine (olgaungin@gmail.com)
[3]School of Applied Sciences, Abertay University, Dundee, United Kingdom (a.spiers@abertay.ac.uk)

Background: The use of different chemicals for agriculture, industry and mining has caused pollution of agrarian soils which provokes changes in rhizosphere microflora. We have studied 10 bacterial strains isolated from a winter wheat Cd-polluted field in Ukraine by their taxonomic position, biochemical properties and resistance to 3 classes of toxicants: heavy metals (Cu^{2+}, Cd^{2+}), non-metals (perchlorate-ion), organic xenobiotic (1-chloro-4-nitrobenzene, CNB).

Objectives: To study the effect of toxicants on the biofilm–formation ability of individual strains and a mixed community.

Methods: Biofilm characteristics (total microcosm growth, biofilm strength and attachment to the microcosm walls) were studied by combined biofilm assay (n = 4) with four treatments including 100 mg/L Cu^{2+}, 25 mg/L Cd^{2+}, 300 mg/L ClO_4^-, and 100 mg/L CNB, with correlations and Principal component analysis (PCA) used to investigate data.

Results: We found that microbial community had a greater resistance to the toxicants compare to individual strains. The presence of heavy metals increased the strength of biofilms, and in most cases growth and biofilm strength were positively correlated. However, in the presence of Cd^{2+} this correlation was lost. Perchlorate affected bacteria, increasing mucus production and biofilm strength though it also reduced attachment levels. Finally, we also found that CNB could be used as a source of Carbon and energy during biofilm–formation.

How to cite: Iungin, O. and Spiers, A.: Chemical stress contribution in bacterial biofilm formation, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-49, https://doi.org/10.5194/biofilms9-49, 2020

biofilms9-23
https://doi.org/10.5194/biofilms9-23
biofilms 9 conference

Driving factors for bioclogging of pores and porous media

Dorothee Kurz[1,2], Eleonora Secchi[1], Roman Stocker[1], and Joaquin Jimenez-Martinez[1,2]
[1]Department of Civil, Environmental and Geomatic Engineering, Institute for Environmental Engineering, ETH Zurich, Switzerland
[2]Department Water Resources and Drinking Water, Swiss Federal Institute of Aquatic Science and Technology, EAWAG, Dübendorf, Switzerland

Understanding the interplay between hydrodynamics and biogeochemical processes is of growing importance in environmental applications and studies, especially in the fields of bioremediation and ecology. The majority of the microbial communities living in soil have a surface-attached lifestyle, allowing them to form biofilms. The biofilm growth influences pore geometries by clogging them and thus redirecting the flow, which in return affects biofilm development and local mass transport. After initially clogging single pores, the biofilm structure expands to larger clusters before eventually clogging the porous medium entirely. We study these processes with a soil-born microorganism, *Bacillus subtilis*, in microfluidic devices mimicking porous media to get a mechanistic understanding of the driving factors of bioclogging of porous media on different scales.

Carefully designed porous geometries were used for the experiments to study biofilm growth under different flow conditions. After being seeded with bacteria, devices were exposed to a continuous nutrient flow during several days. Continuously monitoring the pressure evaluation and imaging the biofilm growth using Brightfield microscopy allowed a high temporal resolution of biofilm growth processes.

An interplay of hydraulic parameters and geometric features of the porous medium as well as the mass flow rate of nutrients drive the speed of pore clogging. Besides the pore scale clogging, the initiation of biofilm formation as well as the speed of clogging of the entire medium are influenced by the before mentioned parameters. Furthermore, the size and number of the biofilm clusters formed seem to drive the medium scale clogging. This leads to inverting trends concerning the clogging rate in one pore when compared to the porous medium scale for different pore sizes. These results shed light on the pore-scale mechanism as well as driving parameters of biofilm formation and bioclogging and their transferability to the next larger scale, e.g. a porous medium.

How to cite: Kurz, D., Secchi, E., Stocker, R., and Jimenez-Martinez, J.: Driving factors for bioclogging of pores and porous media, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-23, https://doi.org/10.5194/biofilms9-23, 2020

biofilms9-92, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-92
biofilms 9 conference

Dynamic and spatially resolved mid-infrared characterization of biofilms

Elisabeth Leiss-Holzinger[1], Robert Zimmerleiter[1], Eva Maria Wagner[2], Kathrin Rychli[3], and Markus Brandstetter[1]

[1]Research Center for non-destructive Testing, Infrared & Raman Spectroscopy, Austria (elisabeth.leiss@recendt.at)
[2]FFoQSI GmbH – Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Tulln, Austria
[3]Institute for Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

In this contribution we present results on non-destructive chemical imaging in the mid-infrared (MIR) region of well-defined biofilms formed by *Pseudomonas simiae*. Biofilms were grown on stainless steel slides using a static biofilm model (incubation lasted for seven days at 10 °C, with repetitive medium changes). The MIR spectra correlate with fundamental molecular vibrations and are therefore characteristic for chemical composition and structure [1, 2]. Besides a brief insight into the systematic of how the investigated biofilms were grown the main focus will be on MIR spectroscopic measurements including dynamic observation of drying processes of bacteria, as well as spatially resolved scans of the steel plates with an MIR microscope. The obtained hyperspectral chemical images of biofilms were analyzed by various spectroscopic data analysis techniques.

Furthermore, the dynamic spectroscopic observation of the drying process of planktonic *Pseudomonas simiae* cultures in nutrient solution gave insight in dynamic variances in certain functional chemical groups of the bacteria. These variances have also been observed in biofilm samples and may correlate with vitality. The MIR chemical images, where each pixel is composed of an entire MIR spectrum (4000-400 cm^{-1}) provide detailed information of the investigated biofilms such as their composition and spatial structure. The overlay with conventional microscope images relates spectroscopic to visual data, both laterally resolved in the µm-range, over a scan area of up to 10 x 40 mm^2.

The variation of the vibrational bands was screened, revealing high and low variance bands, to identify certain spectral regions suitable for classification of the investigated biofilm samples. Characteristic spectral bands were found and related to data from literature. Furthermore, differences in the spatial distribution of proteins and carbohydrates as part of the bacteria and extracellular polymeric substances were clearly identified.

Acknowledgment:
This work was created within a research project of the des Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence centre FFoQSI is funded by the Austrian ministries BMVIT, BMDW and the Austrian provinces Niederoesterreich, Upper Austria and Vienna within the scope of COMET -Competence Centers for Excellent Technologies. The programme COMET is handled by the Austrian Research Promotion Agency FFG.

References:
[1] Andreas Schwaighofer, Markus Brandstetter and Bernhard Lendl , "Quantum cascade lasers (QCLs) in biomedical spectroscopy", Chem. Soc. Rev. 46, 5903-5924 (2017)
[2] Jakob Kilgus, Gregor Langer, Kristina Duswald, Robert Zimmerleiter, Ivan Zorin, Thomas Berer, and Markus Brandstetter, "Diffraction limited mid-infrared reflectance microspectroscopy with a

supercontinuum laser," Opt. Express 26, 30644-30654 (2018)

How to cite: Leiss-Holzinger, E., Zimmerleiter, R., Wagner, E. M., Rychli, K., and Brandstetter, M.: Dynamic and spatially resolved mid-infrared characterization of biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-92, https://doi.org/10.5194/biofilms9-92, 2020

biofilms9-103
https://doi.org/10.5194/biofilms9-103
biofilms 9 conference

Dynamics of biofilm spatial-temporal heterogeneity in RSFs for ammonium and manganese removal from groundwaters

Thomas Etcheberry[1,2], Matthieu Peyre Lavigne[1], Rosalia Trias[2], Etienne Paul[1], and Lodovico di Gioia[2]

[1]TBI, Université de Toulouse, CNRS, INRA, INSA, 135 avenue de Rangueil, 31077 Toulouse, FRANCE

[2]Danone Research, RD 128, 91767 Palaiseau, FRANCE

Filtration through natural biofilms in Rapid Sand Filters (RSFs) is among the most used processes to remove ammonium and manganese from groundwaters[a]. However, initial biofilm seeding is relatively slow[b], and little is known about the spatial-temporal distribution of the activities. The objectives of this work were to: **(a)** understand heterogeneity of microbial populations and activities in depth and time, **(b)** discover how it impacts the process, and **(c)** develop a mathematical model to propose and experiment enhanced "start-up" strategies.

A stainless-steel column filled with sand was fed with groundwater, with the possibility to modulate inlet temperature and substrate concentrations in "enhanced configuration". Ammonium, nitrite and manganese concentrations were measured by spectrophotometry at the inlet, at several intermediate column heights and outlet of the RSF. A model was developed with Aquasim[c] software where biological reactions, and evolutions of soluble compounds, free and attached functional microbial populations are described. Sand and water at different experiment stages and in RSF depths were sampled for DNA extraction and 16S rDNA sequencing, qPCR and metagenomic analysis.

Results shed light on heterogeneity in time of the activities: nitrification systematically begins before manganese oxidation. Analysis in all depth of the RSF, "profiles", show that all activities are evenly distributed during the seeding and attachment of planktonic microorganisms. However, after the initial phase "start-up", profiles indicate logically that the biological activities migrate to the inlet of the RSF where substrates are. Most of the substrates are oxidized on the first quarter of sand media depth.

Relative abundances of microorganisms indicate that active species changed from the start-up phase to the production phase: AOB Nitrosomonas species were dominant during ammonium oxidation, while commamox Nitrospira species were mostly found in production.

The model fits pilot data in terms of elimination periods duration and distribution of the activities and allowed to estimate parameters to further simulate "start-up" configurations. By increasing temperature and substrates loading rate, effective nitrifying biofilm settlement was achieved 4.7 times faster than in conventional conditions. However, no significant improvement was observed for manganese oxidation.

At the end of the start-up phase, with both conventional and accelerated method, the filters hosted similar communities. The model, confronted to experiments in production time, validated that the spatial heterogeneity in depth of the RSF ensures robustness of the biological process to punctual over charge of ammonium and manganese.

Our study showed that **(a)** spatial-temporal heterogeneity is linked to growth of different microbial

populations in time, but also related to local conditions in time and depth, and **(b)** heterogeneity in depth is a characteristic of RSFs and is responsible for robustness and resilience of the process.

REFERENCES

[a] Tekerlekopoulou et al. J Chem Technol Biotechnol 88 (5), 751–773, 2013.

[b] Cai, et al. Bioresource Technol., 176, 149–155, 2015.

[c] Reichert. Environmental Software 10 (3), 199-210, 1995.

How to cite: Etcheberry, T., Peyre Lavigne, M., Trias, R., Paul, E., and di Gioia, L.: Dynamics of biofilm spatial-temporal heterogeneity in RSFs for ammonium and manganese removal from groundwaters, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-103, https://doi.org/10.5194/biofilms9-103, 2020

biofilms9-73
https://doi.org/10.5194/biofilms9-73
biofilms 9 conference

Global analyses imply that Stenotrophomonas maltophilia biofilms are phenotypically highly diverse despite a common transcriptome profile

Ifey Alio[1], Mirja Gudzuhn[1], Marie Schölmerich[1], Pablo Pérez García[1], Christel Vollstedt[1], Uwe Mamat[2], Anja Poehlein[3], Jorg Steinmann[4], Thomas Kohl[5], and Wolfgang Streit[1]

[1]Department of Microbiology and Biotechnology, Universität Hamburg, Hamburg, Germany (ifey.alio@uni-Hamburg.de)

[2]Molecular and Experimental Mycobacteriology, Priority Area Infections, Research Center Borstel Germany

[3]Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Georg-August University of Göttingen

[4]Institut für Klinikhygiene, Medizinische Mikrobiologie und Klinische Infektiologie, Universitätsinstitut der Paracelsus Medizinischen Privatuniversität Klinikum Nürnberg

[5]Cellular Microbiology, Priority Area Infections, Priority Area Infections, Research Center Borstel, Borstel, Germany

Stenotrophomonas maltophilia is one of the most frequently isolated multidrug resistant opportunistic pathogens. It contributes to disease progression in cystic fibrosis patients and is found in wounds, other infected tissues and on catheter surfaces. Only little is known on key processes linked to biofilm formation of S. maltophilia on a broader basis. Thus the aim of this study was the identification of key processes involved in biofilm formation of S. maltophilia on a global level. Therefore, we analyzed biofilm profiles of 300 globally collected clinical and environmental isolates of the main and recently identified lineages Sgn3, Sgn4 and Sm2 - Sm18 (Groeschel et al., 2020). These data together with the 3D structural analysis of a subset of clinical 40 clinical isolates revealed an unexpectedly high phenotypic variability on a strain specific level. Further transcriptome analysis of seven clinical isolates using biofilm grown cells identified a set of 106 shared and coexpressed genes and roughly 30-35 strain-specific genes. Based on these findings S. maltophilia employs a mostly fermentative growth modus under the biofilm conditions and uptake of iron, phosphorous and other metals appears to be of high relevance. Surprisingly, the transcriptome profiles of biofilm versus planktonic cells revealed that only 8.6% of all genes were differentially regulated when both conditions were compared. This implies that only relatively few genes contribute to the change from planktonic to biofilm life style. Thereby iron uptake appears to be a key factor involved in this metabolic shift. The transcriptome data generated in this study together with the phenotypic and metabolic analysis represent so far the largest data set on S. maltophilia biofilm versus planktonic grown cells. This study now lays the foundation for the identification of new strategies in fighting S. maltophilia infections in clinical settings.

Ref: Gröschel et al., 2020 ,The phylogenetic landscape and nosocomial spread of the multidrug-resistant opportunist Stenotrophomonas maltophilia. Nature Commun. 2020 Apr 27;11(1):2044. doi: 10.1038/s41467-020-15123-0.

How to cite: Alio, I., Gudzuhn, M., Schölmerich, M., Pérez García, P., Vollstedt, C., Mamat, U., Poehlein, A., Steinmann, J., Kohl, T., and Streit, W.: Global analyses imply that Stenotrophomonas maltophilia biofilms are phenotypically highly diverse despite a common transcriptome profile, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-73, https://doi.org/10.5194/biofilms9-73, 2020

biofilms9-113
https://doi.org/10.5194/biofilms9-113
biofilms 9 conference

Heterogeneities in biofilms from clinical isolates under flow conditions

Cindy Cardenas[1] and Roberto Rusconi[1,2]
[1]Humanitas Clinical and Research Center – IRCCS, Rozzano (MI), Italy
[2]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele (MI), Italy

Pancreatic cancer is the fourth leading cause of cancer death worldwide. The most common sign of presentation of pancreatic cancer is obstructive jaundice, which prevents the drainage of bile into the intestines and it is often associated with decreased survival in patients. Nowadays more than 70% of the patients with biliary obstructive jaundice is treated by biliary stenting; however, biliary stenting disrupts the natural anatomic barrier between the biliary and the gastrointestinal tract, strongly increasing the risk of a bacterial infection. Moreover, duodenal bacteria, by gaining access into the biliary system, can adhere to the stent surface and develop biofilms. Nevertheless, very little is known about the growth of biofilms on the stents and their role in infectious post-operative complications. In particular, the biliary system is an inherently fluid mechanical environment, where the gallbladder provides the driving pressure and the flow rate of the bile going through the ducts depends on the resistance between the gallbladder and the downstream end of the common bile duct. The average flow rate of the bile ranges between approximately 0.5 to 5 ml/min, which depends if the body is fasting or after a meal; this flow rate then corresponds – in the case for example of plastic stents, which are typically 2-4 mm in luminal diameter – to a maximum flow velocity of about 1-40 mm/s and to a shear rate at the inner surface of the stent of 1-80 s^{-1}. Therefore, the mechanical stress induced by the bile flow in the stent is likely to play a significant role in the formation of biofilms, as shown by our data. Six clinically relevant isolates from preoperative biliary stents were selected to be grown inside microfluidic channels at different flow rates, in which bacterial attachment and biofilm dynamics were recorded and quantified. We found that fluid flow largely influences biofilm morphology in all the isolates, for which the conditions of flow and shear stress that trigger heterogeneities in biofilm structure have been determined. These results will help us to improve our understanding of biofilm formation in the presence of fluid dynamic environments and eventually consider optimal parameters of flow in the design of medical devices.

How to cite: Cardenas, C. and Rusconi, R.: Heterogeneities in biofilms from clinical isolates under flow conditions, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-113, https://doi.org/10.5194/biofilms9-113, 2020

biofilms9-145
https://doi.org/10.5194/biofilms9-145
biofilms 9 conference

Pseudomonas Fluorescens biofilm in rotating annular bioreactor: formation kinetics and wetting properties

Michela Castigliano[1], Maria Petala[2], Margaritis Kostoglou[3], Stefano Guido[1,4], Sergio Caserta[1,4], and Thodoris Karapantsios[3]

[1]University of Naples Federico II, University of Naples Federico II, Chemical, Materials and Production Engineering, Italy
[2]School of Civil Engineering, University Box 10, 54 124 Thessaloniki (Greece).
[3]Division of Chemical Technology, School of Chemistry, Aristotle University of Thessaloniki, University Box 116, 541 24 Thessaloniki (Greece).
[4]CEINGE, Advanced Biotechnologies, 80145 Naples (Italy) (steguido@unina.it)

Biofilms are bacterial communities embedded in an extracellular matrix, able to adhere to surfaces. A deeper knowledge of the biofilm as a whole will aid the development of efficient methods to control deleterious biofilms (clinical biofilms, biofouling) or to enhance beneficial ones (waste-water treatment, bio-filtration). P. fluorescens has been widely studied, this strain produces bioactive secondary metabolites, and forms biofilms[1]. Several experimental set-ups have been widely used for in vitro biofilm cultivation of P. fluorescens, even if a deep characterization among different culture conditions is still lacking in the literature. This work, based on previous studies[2], is focused on the investigation of growth conditions on biofilm structure and properties. Growth kinetics of P. fluorescens biofilms was characterized in vitro under stagnant and flow-controlled conditions, using a rotating annular bioreactor. Two different supports in borosilicate glass and polycarbonate have been used. Bacterial growth kinetics has been measured through bio-turbidity analysis and TOC/DOC quantification. Biofilm morphology has been quantified through optical microscopy and image analysis by measuring the fraction of support surface covered by biofilm. The wetting properties of the biofilm layers have been investigated by using an innovative device, named Kerberos®, able to control centrifugal and gravitational forces acting on a single droplet placed on a surface[3]. The evolution of the droplet shape and position was measured as function of the imposed stress, to quantify wetting of different biofilm coated samples, following already assessed methodologies[4]. Different chemo-physical environments, investigated by changing growth medium, physical support, and imposed flow stress, induced different growth kinetics, biofilm morphology, and wetting properties. Accurate experimental measurements allowed us to estimate in a quantitative way the influence of investigated parameters on specific morphologic measurements.

[1] Brittan S. Scales and others, 'Microbiology, Genomics, and Clinical Significance of the Pseudomonas Fluorescens Species Complex, an Unappreciated Colonizer of Humans', Clinical Microbiology Reviews, 27.4 (2014), 927–48 <https://doi.org/10.1128/CMR.00044-14>.

[2] Federica Recupido and others, 'The Role of Flow in Bacterial Biofilm Morphology and Wetting Properties', Colloids and Surfaces B: Biointerfaces, 2020 <https://doi.org/10.1016/j.colsurfb.2020.111047>.

[3] Sotiris P. Evgenidis and others, 'Kerberos: A Three Camera Headed Centrifugal/Tilting Device for Studying Wetting/Dewetting under the Influence of Controlled Body Forces', Colloids and Surfaces A:

Physicochemical and Engineering Aspects, 2017 <https://doi.org/10.1016/j.colsurfa.2016.07.079>.

[4] Inmaculada Ríos-López and others, 'Effect of Initial Droplet Shape on the Tangential Force Required for Spreading and Sliding along a Solid Surface', Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2018 <https://doi.org/10.1016/j.colsurfa.2018.04.004>.

How to cite: Castigliano, M., Petala, M., Kostoglou, M., Guido, S., Caserta, S., and Karapantsios, T.: Pseudomonas Fluorescens biofilm in rotating annular bioreactor: formation kinetics and wetting properties, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-145, https://doi.org/10.5194/biofilms9-145, 2020

biofilms9-109
https://doi.org/10.5194/biofilms9-109
biofilms 9 conference

Structural differences of biofilms

Daniel Kleine[1], Paul Breuninger[2], Anna-Lena Maus[1], Sergyi Antonyuk[2], and Roland Ulber[1]
[1]TU Kaiserslautern, Bioprocess Engineering, Mechanical & Process Engineering, Germany
[2]TU Kaiserslautern, Particle Process Engineering, Mechanical & Process Engineering, Germany

Biofilms consist of bacteria immobilized in extracellular polymeric substances (EPS) with a complex three-dimensional morphology. This inevitably results in gradients (concentration, cell count, pH, etc.) directly affecting the overall behavior of biofilms [1]. Yet, comparatively little is known about the influence of surface structures beneficial for biofilms as production platforms [2,3]. This understanding is indispensable to establish stable and highly productive biofilm processes. In this study, the model organism *Lactococcus lactis* subsp. *lactis* was used, which produces the antimicrobial peptide nisin (E234). Even though its potential for clinical use has been recognized over the past two decades and the application extended to biomedical fields, its widespread use is restricted due to high production costs and relatively low yields [4]. Within this study, microstructured metallic substrata were investigated. All surface structures were characterized via optical profilometry and *L. lactis* biofilms were cultivated in custom built flow cells. Biofilm morphology was analyzed via optical coherence tomography (OCT) and qRT-PCR was used to analyze relative gene expression levels of nisin genes. Biofilm thickness as well as mushroom count varied depending on the substratum used. This morphological dependency on the surface structure rather than solely on fluid dynamics was demonstrated with a hybrid substratum which was only partly structured. Two separate and morphologically distinct sections were further investigated in order to identify structure-based variations in gene expression. Increased gene expression levels were detected for all genes investigated in the sample of the mushroom rich biofilm section. For the structural gene *nisA* and *nisP*, a gene involved in nisin processing, particularly high levels were detected. This indicates an increased activity of the entire nisin gene cluster. Even though mRNA levels cannot directly be linked to respective product titers, it is rather interesting to see different behaviors of biofilm sections on the transcriptional level. In addition to the influence of the substratum surface on biofilm morphology, this knowledge can be used to design biofilm processes based on beneficial surface structures.

The financial support by DFG - Collaborative Research Center 926 (Microscale Morphology of Component Surfaces) is gratefully acknowledged.

How to cite: Kleine, D., Breuninger, P., Maus, A.-L., Antonyuk, S., and Ulber, R.: Structural differences of biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-109, https://doi.org/10.5194/biofilms9-109, 2020

Topic 3
Biofilm Matrix

For decades, biofilm research has been trying to provide a comprehensive description of the biofilm matrix. Due to the complexity of the biofilm matrix, an easy characterisation seems somehow impossible. Recently, optical coherence tomography has been pushed and very much contributes to an advanced structural characterisation of the mesoscopic biofilm structure. However, the biofilm matrix is a highly complex matter with various physical (e.g. distribution of biomass, material properties), chemical (e.g. composition, constituents) and (micro)biological (e.g. microorganisms and interactions) properties.

We are thus looking for contributions improving the structural (physical, chemical, micro biological) understanding of the biofilm matrix. Very much appreciated are inputs correlating results of different methodologies.

Oral Presentations

biofilms9-10
https://doi.org/10.5194/biofilms9-10
biofilms 9 conference

The role of eDNA in the formation of biofilm streamers

Eleonora Secchi[1], Giovanna Savorana[1], Alessandra Vitale[2], Leo Eberl[2], Roberto Rusconi[3], and Roman Stocker[1]

[1]Institute of Environmental Engineering, ETH Zürich, Zürich, Switzerland
[2]Department of Plant and Microbial Biology, University of Zürich, Zürich, Switzerland
[3]Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy

Across many different habitats, bacteria are often found as sessile communities embedded in a self-secreted matrix of extracellular polymeric substances (EPS) [1]. The biofilm matrix enhances bacterial resistance to harsh environmental conditions and antimicrobial treatments, and thus hinders our ability to remove detrimental biofilms in medical and industrial applications. Depending on the environmental conditions, biofilms can be found as tethered filaments suspended in flow, known as streamers [2], or surface-attached communities. Despite the importance of the matrix to biofilm survival, little is known about how environmental features shape its microstructure and chemical composition.

Here, we show that a laminar flow of a diluted suspension of *Pseudomonas aeruginosa* PA14 around a pillar can trigger the formation of suspended filamentous biofilm structures known as streamers and that extracellular DNA (eDNA) plays a fundamental structural role in streamer formation [3]. We have developed a microfluidic setup that allows real time visualization of the formation of biofilm streamers and the investigation of their biochemical composition by means of lectins staining. Our experiments confirmed that this phenomenon is dominated by the interplay between the viscoelastic nature of EPS, which is extruded by local flow shear, and the secondary flow around the pillar, which promotes the growth of the filaments due to a filtration mechanism. By varying the composition of the biofilm matrix using mutant strains of PA14 and by applying targeted treatment with the enzyme DNase I, we could shed light on the structural role of the different biochemical components: eDNA is essential for streamers formation, while Pel, a positively charged exopolysaccharide which binds to eDNA [4], affects the filament morphology. In addition, since in this geometry we can study freestanding biofilm filaments [5], we could probe the shear-induced deformation of streamers to investigate their material properties and reveal that eDNA affects the elastic behaviour of the biofilm matrix, while the viscous behaviour is determine by the quantity of Pel. Finally, thanks to our mechanistic understanding of the interplay between streamers composition and microstructure, we could surprisingly promote streamers formation by adding sublethal concentration of an antibiotic commonly used to treat *P. aeruginosa* infections. In summary, using the experimental toolbox from biophysics to characterize the biofilm matrix, we could elucidate the relation between chemical composition and microstructure, use our understanding to control streamers formation and gain an insight on this biological system that could make an impact in the medical sector.

[1] H.-C. Flemming et al., Nat. Rev. Microbiol. **14**, 563 (2016).

[2] R. Rusconi, S. Lecuyer, L. Guglielmini, and H. A. Stone, J. R. Soc. Interface **7**, 1293 (2010).

[3] E. Secchi, G. Savorana, A. Vitale, L. Eberl, R. Rusconi, and R. Stocker, paper in preparation.

[4] L. K. Jennings et al., Proc. Natl. Acad. Sci. **112**, 11353 (2015).

[5] G. Savorana, R. Rusconi, A. Sartori, L. Heltai, R. Stocker, and E. Secchi, paper in preparation.

How to cite: Secchi, E., Savorana, G., Vitale, A., Eberl, L., Rusconi, R., and Stocker, R.: The role of eDNA in the formation of biofilm streamers, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-10, https://doi.org/10.5194/biofilms9-10, 2020

biofilms9-11
https://doi.org/10.5194/biofilms9-11
biofilms 9 conference

Tailoring nanoparticle-biofilm interactions to increase efficacy of antimicrobial agents against Staphylococcus aureus

Henry Devlin[1], Stephanie Fulaz[1], Stefania Vitale[1], Laura Quinn[1], James O'Gara[2], and Eoin Casey[1]
[1]UCD School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Dublin 4, Ireland
(henry.devlin@ucdconnect.ie)
[2]Department of Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland

Considering the timeline required for the development of novel antimicrobial drugs, increased attention should be given to repurposing existing drugs and improving their antimicrobial efficacy, particularly for chronic infections associated with biofilms. Methicillin-susceptible Staphylococcus aureus (MSSA) and methicillin-resistant S. aureus (MRSA) are common causes of biofilm-associated infections however each species has a distinct biofilm phenotype resulting in different biofilm matrix characteristics.. Nanoparticles (NPs) have the potential to significantly enhance the delivery of antimicrobial agents into biofilms, however the physicochemical properties which influence these interactions between NPs and the biofilm are not fully understood. The influence of NP surface chemistry on interactions with MRSA and MSSA biofilms was explored in this study. Mesoporous silica nanoparticles (MSNs) with different surface functionalizations (bare-B, amine-D, carboxyl-C, aromatic-A) were synthesised. Following interaction studies, MSNs were loaded with vancomycin (VAN) to observe biofilm eradication. The two negatively charged MSNs (MSN-B and MSN-C) showed a higher VAN loading in comparison to the positively charged MSNs (MSN-D and MSN-A). Cellular binding with MSN suspensions (0.25 mg mL^{-1}) correlated with reduced viability of both MSSA and MRSA biofilm cells. MSNs were shown to be efficient carriers of vancomycin while also displaying significantly improved efficiency compared to free VAN. This allowed the administration of low MSNs concentrations, while maintaining a high local concentration of the antibiotic surrounding the bacterial cells, indicating a promising novel therapeutic approach for S. aureus biofilm infections.

How to cite: Devlin, H., Fulaz, S., Vitale, S., Quinn, L., O'Gara, J., and Casey, E.: Tailoring nanoparticle-biofilm interactions to increase efficacy of antimicrobial agents against Staphylococcus aureus , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-11, https://doi.org/10.5194/biofilms9-11, 2020

biofilms9-85
https://doi.org/10.5194/biofilms9-85
biofilms 9 conference

How to Measure Diffusion Coefficients in Biofilms: A Critical Analysis

Lenno van den Berg[1], Mark van Loosdrecht[2], and Merle de Kreuk[1]
[1]Department of Watermanagement, Delft University of Technology, Delft, Netherlands (l.vandenberg@tudelft.nl)
[2]Department of Biotechnology, Delft University of Technology, Delft, Netherlands

Effective diffusion coefficients are often required for kinetic descriptions of biofilms. Many previous studies have measured diffusion coefficients for specific molecule-biofilm combinations. As a result, many biofilm researchers today rely on literature values of diffusion coefficients for their own biofilm system. However, the reported diffusion coefficients in literature fall within a wide range, even for the same molecule. One potential cause of this range is the accuracy of the methods used to measure diffusion coefficients. The objective of this study was to determine the precision (similarity between repeated experiments) and bias (difference between measured and true diffusion coefficient) of six common methods. The six selected methods were based on determining mass balances and on microelectrode measurements. The precision and bias were quantified based on mathematical models of the six methods, with oxygen diffusion in granular sludge as a case study. The precision was assessed by a Monte Carlo uncertainty analysis, which considers the propagation of uncertainty in the input experimental parameters. The bias was determined for six potential sources of error: solute sorption, biomass deactivation, a concentration boundary layer, granule roughness, granule shape, and granule size distribution. From the Monte Carlo analysis, it followed that the precision of the methods ranged from 4-77% relative standard deviation. The microelectrode methods were more accurate than the mass balance methods. The bias due to the combined effect of the six errors was an underestimation of the diffusion coefficient by 74%. This shows that current methods are unable to accurately determine diffusion coefficients. We do not propose improvements to the current methods, but instead discuss why inaccurate diffusion coefficients are sufficient for accurate engineering of biofilm processes.

How to cite: van den Berg, L., van Loosdrecht, M., and de Kreuk, M.: How to Measure Diffusion Coefficients in Biofilms: A Critical Analysis, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-85, https://doi.org/10.5194/biofilms9-85, 2020

biofilms9-152
https://doi.org/10.5194/biofilms9-152
biofilms 9 conference

A multimeric matrix-associated lectin (RapD) affects proper exopolysaccharide processing in Rhizobium leguminosarum

Julian Tarsitano[1], Daniela Marta Russo[1], Leonardo Alonso[2], and Angeles Zorreguieta[1]
[1]Fundación Instituto Leloir, IIBBA - CONICET (juliantarsitano@gmail.com)
[2]NANOBIOTEC, CONICET-Universidad de Buenos Aires, Argentina

Rhizobium leguminosarum synthesizes an acidic polysaccharide formed by the polymerization of octasaccharide repeating units containing glucose (Glc), glucuronic acid (GlcA) and galactose (Gal) in a 5:2:1 ratio with particular substitutions; most of it is secreted to the extracellular medium (EPS) and part of it is retained on the bacterial surface as a capsular polysaccharide (CPS). Rap proteins, substrates of the PrsDE type I secretion system (TISS) share at least one Ra/CDHL (cadherin-like) domain and are involved in biofilm and matrix development either by cleaving the polysaccharide (Ply glycanases) or by altering the bacterial adhesive properties. Previous studies have shown that RapA2 is a monomeric calcium-binding lectin capable of binding specifically the *R. leguminosarum* CPS through a Ra/CDHL domain. It was shown that the absence or excess of RapA2 in the extracellular medium alters the biofilm matrix's properties.

In this work we identified a new Rap protein (RapD), which comprises an N-terminal Ra/CDHL domain and a C-terminal domain of unknown function. By Western blot analysis using specific polyclonal antibodies we showed that in planktonic cultures RapD is co-secreted with the other Rap proteins in a PrsDE-dependent manner. Furthermore, under conditions that favor EPS production, a prominent RapD secretion was observed. In addition, colony blot assays indicated that RapD is associated with the biofilm matrix. Interestingly, size exclusion chromatography of the EPS produced by the Δ*rapA2* Δ*rapD* double mutant showed differences in the EPS profiles compared with those of the single mutants and the wild type strain, thus suggesting a functional interaction between the RapA2 and RapD proteins.

Biophysical studies showed that calcium triggers proper folding and multimerization of recombinant RapD. Besides, further RapD conformation changes were observed in the presence of EPS.

ELISA and BIA (binding inhibition assay) assays showed that in the presence of calcium, RapD specifically binds the EPS and that galactose residues would be involved in this interaction.

In conclusion, RapD is a multimeric calcium-dependent EPS lectin that is co-secreted with the other Rap proteins via TISS PrsDE. Unlike RapA2, RapD is not retained on the bacterial surface but would rather interact with the released EPS. Finally, our results suggest that the interaction of RapA2 and RapD with the CPS or the EPS somehow affects the polysaccharide processing and therefore the biofilm matrix.

How to cite: Tarsitano, J., Russo, D. M., Alonso, L., and Zorreguieta, A.: A multimeric matrix-associated lectin (RapD) affects proper exopolysaccharide processing in Rhizobium leguminosarum , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-152, https://doi.org/10.5194/biofilms9-152, 2020

biofilms9-112
https://doi.org/10.5194/biofilms9-112
biofilms 9 conference

Interplay of microbial interaction and biofilm mechanics govern biofilm dynamics

Gabi Steinbach[1], Cristian Crisan[2,3,4], Siu Lung Ng[2,3,4], Brian Hammer[2,3,4], and Peter Yunker[1]
[1]School of Physics, Georgia Institute of Technology, Atlanta, GA, USA
[2]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA
[3]Center for Microbial Dynamics and Infection, Georgia Institute of Technology, Atlanta, GA, USA
[4]Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, USA

Biofilms are highly structured, densely packed bacterial consortia where many different species can coexist. During biofilm development and growth, the different species often form spatial distribution patterns that govern biofilm composition and function. In some cases, emerging structures have been explained as the result of social interactions between bacteria, e.g. cooperation and competition. Others emphasize the role of local mechanics, where spatial structuring arises from forces exerted between cells or between cells and their environment. Typically, these two lines of argumentation are treated separately. Here, we show that mechanics and social interactions can be strongly interrelated and their combination can crucially impact biofilm formation and dynamics. Using confocal microscopy and bacterial co-culture assays, we examine how bacterial antagonism impacts biofilm mechanics, and vice versa. We study competing Vibrio cholerae strains that kill on contact using the Type 6 secretion system. In case of mutual killers, i.e. two V. cholerae strains that can kill each other on contact, this social interaction leads to the formation of clonal domains of the competing strains (Mc Nally et al., Nat Commun, 2017). Intuitively, an unequal fight may enable a superior killer to invade and quickly eliminate a much weaker competitor. However, we observe that killer cells can coexist with killing-deficient target cells for very long times, and find that this results from the mechanical consequences of the deadly competition. Killing produces dead cells, which accumulates between domains of competing cells and prevents subsequent killing. Counterintuitively, our results suggest that antagonistic interactions stabilize coexistence in diverse communities. The findings demonstrate that the impact of social interactions in bacterial consortia is complex, requiring the understanding of the structural and the statistical-mechanical processes in biofilms.

How to cite: Steinbach, G., Crisan, C., Ng, S. L., Hammer, B., and Yunker, P.: Interplay of microbial interaction and biofilm mechanics govern biofilm dynamics, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-112, https://doi.org/10.5194/biofilms9-112, 2020

Poster Presentations

biofilms9-129
https://doi.org/10.5194/biofilms9-129
biofilms 9 conference

A brief exploration of EPS composition in biofilms of Staphylococcus spp ATCC reference strains

Cristina D. Cruz[1], Rebekah C. Hewitt[2], and Päivi Tammela[1]
[1]Faculty of Pharmacy, University of Helsinki, Helsinki, Finland (cristina.durantecruz@helsinki.fi; paivitammela@helsinki.fi)
[2]Faculty of Pharmacy, University of Bath, Bath, United Kingdom (rch42@bath.ac.uk)

Antibiotic resistance is expected to cause 10 million deaths per year worldwide by 2050. One of the mechanisms for the resilient nature of bacteria toward antibiotics is through the formation of biofilm. Bacterial biofilms are sessile communities of microorganisms, which exist in a matrix of proteins, carbohydrates, eDNA and other various components – collectively known as extracellular polymeric substances. Biofilms slow the penetration of drugs, and also contribute to the development of a resistant phenotype known as persisters. Thus, understanding biofilm composition might contribute to the development of anti-biofilm strategies. The aim of this study was to explore biofilm formed by five *Staphylococcus* spp ATCC strains, commonly used in research as references: *S. aureus* 25923, *S. aureus* 29213, *S. aureus* 43300 (methicillin-resistant), *S. aureus* 6538 and *S. epidermidis* 12228. Biofilm mass and its components were analysed after 24h and 72h of biofilm growth. Bacterial biofilm was prepared in 96-well microtiter plates, in Trypticase Soy Broth supplemented with 1% glucose. After incubation at 37°C, absorbance measurements and crystal violet staining were performed and the specific biofilm formation determined for each strain. Extracellular polymeric substances were extracted using a combination of physical and chemical methods; including centrifugation, vortexing and the use of 1.5M NaCl. In these assays, biofilms were grown in polystyrene tubes containing 10 ml of same media mentioned above. The concentration of protein, carbohydrate and eDNA was determined using the Bicinchoninic acid assay, phenol-sulfuric acid method and DNeasy® Blood and Tissue Kit, respectively, followed by spectroscopy. Our data demonstrated heterogeneity between the biofilm-forming capabilities and EPS components within staphylococcal strains and species. Strains 25923 and 6538 had the highest value for biofilm formation at both time points. Interestingly, strain 43300 was the only one to show a significant increase in biofilm after 72h. Contradictory to previous findings, *S. epidermidis* 12228 was found to be a good biofilm producer. At both time points studied, strains demonstrated considerably higher concentrations of protein (varying from 172 µg/mL – 345 µg/mL) and carbohydrate (56 µg/mL - 372µg/mL) in EPS compared to eDNA (2.74 µg/mL – 8.12 µg/mL). On average, strains 43300 and 12228 had the highest concentration of protein, and the latter also had the highest carbohydrate and eDNa amounts at 72h. Strains 25923 and 6538 had a significant decrease in eDNA concentration over time. Based on this brief study, the relative quantities of EPS components investigated is similar to that of other studies with protein being the most plentiful component followed by carbohydrate and then considerably lower amounts of eDNA. Differences in specific biofilm formation did not directly reflect variations observed in abundance of a particular constituent in the matrix of EPS. This study also showed that *S. epidermidis* 12228, usually classified as a weak or non-biofilm former, was able to grow a relatively substantial biofilm under the conditions tested here.

How to cite: D. Cruz, C., C. Hewitt, R., and Tammela, P.: A brief exploration of EPS composition in biofilms of Staphylococcus spp ATCC reference strains, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-129, https://doi.org/10.5194/biofilms9-129, 2020

biofilms9-76
https://doi.org/10.5194/biofilms9-76
biofilms 9 conference

A microfluidic platform for characterizing the structure and rheology of biofilm streamers

Giovanni Savorana[1], Roberto Rusconi[2], Roman Stocker[1], and Eleonora Secchi[1]
[1]ETH Zurich, Institute of Environmental Engineering, Dept. of Civil, Environmental and Geomatic Engineering, Switzerland (gsavoran@ethz.ch)
[2]Humanitas University, Dep. Biomedical Sciences, Italy

In many environmental or medical settings, biofilm formation is the most successful strategy for bacterial colonization[1,2]. In the biofilm lifestyle, bacteria embed themselves in a self-secreted matrix of extracellular polymeric substances (EPS), acting as a shield against mechanical and chemical insults[3]. When ambient flow is present, this viscoelastic EPS scaffold can take a streamlined shape, forming biofilm threads suspended in flow, called streamers[4]. In many situations, the streamer architecture can enhance the harmful effects of biofilms, bridging the spaces between obstacles in the flow path[5]. Despite their importance for biofilm survival, little is known about the material properties of the matrix. In particular, these are really hard to probe with traditional rheological techniques when the biofilm grows into the thread-like streamer shape.

In this work we present a microfluidic platform that allows to reproducibly grow biofilm streamers in controlled chemical and flow conditions and to characterize their structure and mechanical properties in situ[6].This platform overcomes the main sources of error and variability of the experiments performed with traditional flow-chambers: the randomness in the location and shape of the streamers. Our device consists of a straight channel with isolated micropillars, where a bacterial suspension is injected at a constant flow rate. The micropillars act as nucleation points for the growth of a pair of biofilm filaments, developing on the midplane of the channel under the action of secondary flows. The microfluidic technology allows to control the chemical and flow conditions and to perform live imaging of the growth process. By controlling the flow rate, we are also able to perform in situ stress tests on the streamers by inducing controlled variations of the fluid shear stress exerted on them. We developed a theoretical framework to estimate the material properties of biofilm streamers from the flow-induced deformation measured in our experiment. Thanks to this platform, we are able to investigate the role of the different EPS components[7]and the physico-chemical microenvironment in determining biofilm structure and rheology.

References

[1]Flemming and Wingender, Nat. Rev. Microbiol. 8, 623 (2010).

[2]Flemming et al., Nat. Rev. Microbiol. 14, 563 (2016).

[3]Peterson et al., FEMS Microbiol. Rev. 39, 2 (2015).

[4]Rusconi et al., J. R. Soc. Interface 7, 1293 (2010).

[5]Drescher et al., Proc. Natl. Acad. Sci. 112, 11353 (2015).

[6]Savorana et al., paper in preparation

[7]Secchi et al., paper in preparation

How to cite: Savorana, G., Rusconi, R., Stocker, R., and Secchi, E.: A microfluidic platform for characterizing the structure and rheology of biofilm streamers, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-76, https://doi.org/10.5194/biofilms9-76, 2020

biofilms9-59
https://doi.org/10.5194/biofilms9-59
biofilms 9 conference

Archaeal biofilms: Composition of extracellular polymeric substances, exopolysaccharide synthesis and secretion in Sulfolobus acidocaldarius

Laura Kuschmierz[1], Martin Meyer[2], Benjamin Meyer[1], Sonja-Verena Albers[3], Christopher Bräsen[1], Jost Wingender[4], Oliver J. Schmitz[2], and Bettina Siebers[1]

[1]Molecular Enzyme Technology and Biochemistry (MEB), Environmental Microbiology and Biotechnology, University of Duisburg-Essen, 45141 Essen, Germany

[2]Applied Analytical Chemistry (AAC), University of Duisburg-Essen, 45141 Essen, Germany

[3]Molecular Biology of Archaea, Institute for Biology II, Albert-Ludwigs-University Freiburg, 79104 Freiburg, Germany

[4]Aquatic Microbiology, Environmental Microbiology and Biotechnology, University of Duisburg-Essen, 45141 Essen, Germany

Archaea, representatives of the third domain of life, are often referred to as "extremophiles" since most of the cultivable species are adapted to extreme environments [1]. However, environmental cultivation-independent approaches (metagenomics) revealed a wide distribution of Archaea in moderate habitats suggesting a major role in geochemical processes. Similar to Bacteria, also Archaea are believed to exist predominantly in the biofilm mode, but knowledge about archaeal biofilm formation and structure, extracellular polymeric substance (EPS) composition and synthesis is scarce [2].

Sulfolobus acidocaldarius is a thermoacidophilic, aerobic Crenarchaeon (78°C and pH 2-3) that was isolated from acid hot springs [3]. The organism is easy to cultivate under laboratory conditions and a genetic system is established. In this study, we investigate *S. acidocaldarius* biofilms with a special focus on synthesis and transport of exopolysaccharides (PS). PS constitute a major EPS component beside proteins and eDNA, suggesting an important role in *Sulfolobus* biofilms, and changes in PS composition were observed in response to environmental stress [4]. A gene cluster encoding several glycosyltransferases (GTs) as well as membrane proteins (MPs), likely involved in exopolysaccharide synthesis, was identified in *S. acidocaldarius*. Several deletion mutants have been constructed lacking certain GT and MP encoding genes from the PS gene cluster. A combination of methods including the quantification of biofilm formation, isolation and quantification of EPS components, visualization of biofilm and PS structures via confocal laser scanning microscopy as well as molecular and biochemical techniques have been applied to compare biofilm characteristics of wildtype and mutant strains. First insight into the function of GTs and MPs will be presented and a model of PS synthesis and export will be proposed.

[1] Schocke et al. (2019). *Curr. Opin. in Biotechnol.* 59, 71-77.

[2] van Wolferen et al. (2018). *Nature Rev. Microbiol.* 16(11), 699-713.

[3] Brock et al. (1972). *Arch. Mikrobiol.* 84, 54-68.

[4] Jachlewski et al. (2015). *Front. Bioeng. Biotechnol.* 3, 123.

How to cite: Kuschmierz, L., Meyer, M., Meyer, B., Albers, S.-V., Bräsen, C., Wingender, J., Schmitz, O. J., and Siebers, B.: Archaeal biofilms: Composition of extracellular polymeric

substances, exopolysaccharide synthesis and secretion in Sulfolobus acidocaldarius, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-59, https://doi.org/10.5194/biofilms9-59, 2020

biofilms9-81
https://doi.org/10.5194/biofilms9-81
biofilms 9 conference

Assessing arsenic bioremediation potential of epilithic biofilms affected by acid-mine drainage

Sarah Zecchin[1,2], Nicoletta Guerrieri[3], Evelien Jongepier[2], Leonardo Scaglioni[1], Gigliola Borgonovo[1], Gerard Muyzer[2], and Lucia Cavalca[1]

[1]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano, Milan, Italy
[2]Microbial Systems Ecology, Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, Netherlands
[3]Water Research Institute, National Research Council of Italy (CNR-IRSA), Verbania Pallanza, Italy

Arsenic is a toxic but naturally abundant metalloid that globally leads to contamination in groundwater and soil, exposing millions of people to cancer and other arsenic-related diseases. In several areas in Northern Italy arsenic in soil and water exceeds law limits (20 mg kg^{-1} and 10 mg L^{-1}, respectively), due to both the mineralogy of bedrock and former mining activities. The Rio Rosso stream, located in the Anzasca Valley (Piedmont) is heavily affected by an acid mine drainage originated from an abandoned gold mine. Arsenic, together with other heavy metals, is transferred by the stream to the surrounding area. The stream is characterized by the presence of an extensive reddish epilithic biofilm at the opening of the mine and on the whole contaminated waterbed.

The aim of this study was to characterize the mechanisms allowing the biotic fraction of this biofilm to cope with extreme arsenic concentrations. The composition and functionality of the microbial communities constituting the epilithic biofilms sampled in the close proximity and downstream the mine were unraveled by 16S rRNA genes and shotgun Illumina sequencing in relation to the extreme physico-chemical parameters. In parallel, autotrophic and heterotrophic microbial populations were characterized *in vivo* by enrichment cultivation and isolated strains were tested for their ability to perform arsenic redox transformation.

Preliminary analyses indicated that the biofilm accumulated arsenic in the order of $6 \cdot 10^3$ mg kg^{-1}, in contrast to 0.14 mg L^{-1}, measured in the surrounding water. The main chemical parameter affecting the composition of the microbial community was the pH, being 2 next to the mine and 6.7 in the downstream sampling point. In both sampling sites iron- and sulfur-cycling microorganisms were retrieved by both cultivation and molecular methods. However, the diversity of the microbial community living next to the mine was significantly lower with respect to the community developed downstream. In the latter, autotrophic *Cyanobacteria* belonging to the species *Tychonema* were the dominant taxa. A complete arsenic cycle was shown to occur, with heterotrophic bacteria mainly responsible for arsenate reduction and autotrophic bacteria performing arsenite oxidation.

These observations indicate that the epilithic biofilm living in the Rio Rosso stream represents a peculiar ecosystem where microorganisms cope with metalloid toxicity likely using diverse mechanisms. Such microbial metabolic properties might be exploited in bioremediation strategies applied in arsenic-contaminated environments.

How to cite: Zecchin, S., Guerrieri, N., Jongepier, E., Scaglioni, L., Borgonovo, G., Muyzer, G., and Cavalca, L.: Assessing arsenic bioremediation potential of epilithic biofilms affected by acid-mine drainage, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-81, https://doi.org/10.5194/biofilms9-81, 2020

biofilms9-157
https://doi.org/10.5194/biofilms9-157
biofilms 9 conference

A thin line between plankton and biofilm

Iztok Dogsa, Rok Kostanjsek, and David Stopar
University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, SI-Slovenia

Planktonic bacterial cells are by definition not aggregated. However, our previous work, where we have demonstrated the invisible mechanical connections between bacterial cells in dilute planktonic suspensions, challenged this assumption. Here we provide an experimental evidence using autocorrelation analysis of micrographs that in planktonic suspensions of *B. subtilis* a size continuum of aggregated structures is formed. In the microbial aggregates viable cells were embedded in the nucleic acid network. The eDNA was released during regular cell lysis events. To determine the size distribution of planktonic bacterial aggregates a pair-wise spatial correlations of bacterial cells in microscopic images were calculated. The monotonously decreasing shape of the autocorrelation function indicated a continuous distribution of bacterial aggregate sizes from monomer to multimers. Soft bacterial aggregates in dilute suspensions provide a missing link in a continuum of organic matter in aqueous environments and can significantly improve our understanding how non-attached biofilms form during planktonic growth.

How to cite: Dogsa, I., Kostanjsek, R., and Stopar, D.: A thin line between plankton and biofilm, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-157, https://doi.org/10.5194/biofilms9-157, 2020

biofilms9-21, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-21
biofilms 9 conference

Biofilm Architectural Breakdown in Response to Antibiotics Facilitates Community Invasion

Francisco Diaz-Pascual[1] and Knut Drescher[1,2]
[1]Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
[2]Philipps-Universität Marburg, Marburg, Germany

Bacterial cells are often exposed to stress by changes in their environment. During the last decades the response of isolated cells to stress has been investigated in great detail. By contrast, little is known about the emergent multicellular level responses to stress, such as antibiotic exposure. Studying responses at the community level is key to understand the structure and function of the most common bacterial state: the multicellular communities termed biofilms. Here, by analysing *Vibrio cholerae* biofilms exposed to all different classes of antibiotics with single-cell resolution, we found that inhibition of protein synthesis cause striking changes in cell volume and biofilm architecture. The observed changes in cell volume are a single-cell level response driven by metabolic effects of the translational inhibition. The multicellular-level responses result from changes in matrix composition, matrix-cell dissociation and mechanical properties of the biofilms. We observed that these antibiotic-induced changes in biofilm architecture have strong consequences on the ecological dynamics of biofilms by making biofilms prone to invasion by bacteriophages and other bacterial cells. These mechanistic and ecological consequences of the emergent group-level architectural response to antibiotics are important to fully understand the ecological succession of biofilms and the implications of antibiotic therapy.

How to cite: Diaz-Pascual, F. and Drescher, K.: Biofilm Architectural Breakdown in Response to Antibiotics Facilitates Community Invasion, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-21, https://doi.org/10.5194/biofilms9-21, 2020

biofilms9-60
https://doi.org/10.5194/biofilms9-60
biofilms 9 conference

Cadmium selenide formation influences the production and characteristics of extracellular polymeric substances of anaerobic granular sludge

Piet Lens
(piet.lens@nuigalway.ie)

Feeding cadmium (II) and selenium (IV) simultaneously to anaerobic granular sludge with the aim to synthesize cadmium selenide (CdSe) nanoparticles induces compositional changes in the extracellular polymeric substances (EPS) matrix of this sludge. A methanogenic anaerobic granular sludge was repeatedly exposed to Cd(II) (10-50 mg L^{-1}) and selenite (79 mg L^{-1}) for 300 days at pH 7.3 and 30 °C in a fed-batch feeding regime for enrichment of Se reducing bacteria and synthesis of CdSe nanoparticles. EPS fingerprints of the granular sludge, obtained by size exclusion chromatography coupled to a fluorescence detector, showed a significant increase in the intensity of protein-like substances with >100 kDa apparent molecular weight (aMW) upon repeated exposure to Cd(II) and Se(VI). This was accompanied by a prominent decrease in protein-like substances of aMW <10 kDa. The fingerprint of the humic-like substances showed emergence of a new peak with aMW of 13 to 300 kDa in the EPS extracted from the Cd/Se fed granular sludge. Experiments on metal(loid)–EPS interactions showed that the CdSe nanoparticles interact mainly with loosely bound-EPS (LB-EPS). This study showed that the formation of Se(0) and CdSe nanoparticles occurs in the LB-EPS fraction of the granular sludge and repeated exposure to Cd and Se induces compositional changes in the EPS matrix.

How to cite: Lens, P.: Cadmium selenide formation influences the production and characteristics of extracellular polymeric substances of anaerobic granular sludge, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-60, https://doi.org/10.5194/biofilms9-60, 2020

biofilms9-58
https://doi.org/10.5194/biofilms9-58
biofilms 9 conference

Comparative study of chromatographic methods for the analysis of exopolysaccharides from archaeal biofilms

Martin Meyer[1,2], Laura Kuschmierz[3], Bettina Siebers[3], Jost Wingender[4], and Oliver J. Schmitz[1,2]
[1]Applied Analytical Chemistry (AAC), University of Duisburg-Essen, Essen, Germany
[2]Teaching and Research Center for Separation (TRC), University of Duisburg-Essen, Essen, Germany
[3]Molecular Enzyme Technology and Biochemistry (MEB), University of Duisburg-Essen, Essen, Germany
[4]Aquatic Microbiology, University of Duisburg-Essen, Essen, Germany

Microorganisms, such as archaea, favour life in a biofilm rather than the planktonic form of life. A biofilm is defined as a community of microorganisms embedded in a self-produced matrix of hydrated extracellular polymeric substances (EPS), mainly polysaccharides (PS), proteins and extracellular DNA. The polysaccharides form a three-dimensional network, which provides stability of the biofilm and mediates the adhesion to surfaces. [1] Analysis of the monomeric composition of PS requires chromatographic separation and identification by mass spectrometry (MS).

A comparative study of different chromatographic methods for the analysis of the monomeric composition of exopolysaccharides from archaeal biofilms from Sulfolobus acidocaldarius has been carried out. For this study, different chromatographic separation methods, such as supercritical fluid chromatography (SFC), hydrophilic interaction liquid chromatography (HILIC) reversed-phase liquid chromatography (RP-LC) and gas chromatography (GC), each coupled to mass spectrometry, were developed and compared by means of separation performance and sensitivity, using authentic standards.

The study revealed, that each method features distinct advantages and disadvantages over the other methods. For example, when using SFC-MS, no derivatization is necessary and soft ionization conditions can be used. [2] However, the HILIC-MS and RP-LC-MS methods show significantly greater separation performances for the analysis of the monosaccharide composition. [3] All investigated methods show similar quantification limits in the sub-mg/L range.

Finally, the developed chromatographic methods were applied to real biofilm samples of the thermoacidophilic archaeon Sulfolobus acidocaldarius. To determine the monomeric composition of the exopolysaccharides from these archaeal biofilms, the extracellular polymeric substances were extracted from the biofilm and then the PS were hydrolyzed.

Literature:

[1] H.-C. Flemming, Nat. Microbiol. Rev. 2010, 8, 623 - 633. [2] M. Lafosse, J. Chromatogr. A, 1996, 720, 61-73. [3] V. Sieber, J. Chromatogr. A, 2014, 1350, 44–50.

How to cite: Meyer, M., Kuschmierz, L., Siebers, B., Wingender, J., and Schmitz, O. J.: Comparative study of chromatographic methods for the analysis of exopolysaccharides from archaeal biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-58, https://doi.org/10.5194/biofilms9-58, 2020

biofilms9-98
https://doi.org/10.5194/biofilms9-98
biofilms 9 conference

Decrypting the matrix: how interspecies interactions influence matrix in multispecies biofilm?

Cristina Amador[1], Henriette L. Røder[1], Ute Kuhlicke[2], Thomas Neu[2], and Mette Burmølle[1]
[1]Copenhagen University, Section of Microbiology, Biology, Denmark (chierro@bio.ku.dk)
[2]Helmholtz Centre for Environmental Research – UFZ, 39114 Magdeburg, Germany

The biofilm matrix contributes to the establishment of microbial cells on very diverse surfaces, stabilizing biofilms and providing cells with protection against multiple hostile conditions. Moreover, the biofilm matrix can also retain nutrients, enzymes or quorum sensing molecules, favoring the establishment of social interactions among biofilm cells. Functional bacterial amyloids are part of the biofilm structural components of various species, and they were previously proven to bind QS molecules and strengthen the matrix. Multiple studies have been conducted to characterize matrix determinants and their regulation in single species biofilms, while these remain scarcely understood in multispecies biofilms. We have previously isolated and characterized a soil-derived consortium composed of Xanthomonas retroflexus, Stenotrophomonas rhizophila, Microbacterium oxydans and Paenibacillus amylolyticus showing enhanced biofilm biomass and differential gene/protein expression specific of the four-species biofilm.

This study aimed at exploring the effect of interspecies interactions on biofilm matrix production in the four-species biofilm. We hypothesize that interspecies interactions may result in differential expression of matrix-encoding genes responsible for biofilm emergent properties.

We searched for matrix determinant homologues in X.retroflexus and combined different techniques for characterizing the matrix identity and expression in mono-, dual- and multispecies biofilms.

The fap amyloid operon, described in Pseudomonas as a biofilm-scaffold contributing element, was deleted in X. retroflexus, replaced in the four-species model and compared to the parental community for biofilm structure and adhesion capability. The fap mutant displayed poor substrate colonization in flow cells in both mono- and multispecies biofilms with relative filamentous structure compared to the parental strain/ consortium. However, adhesion did not significantly change under static conditions. To characterize matrix composition, we tested 78 different lectins in multispecies biofilms and identified five that bound to our samples. Interestingly, some matrix glycoconjugates were only produced in the consortium.

Our data suggest that loss of matrix components, such as the Fap amyloid, and the presence of other species, influences synergistic biofilm properties in the four-species consortium. Ongoing approaches involving localized expression of matrix-encoding genes and matrix proteomes will aid in identifying the mechanisms underlying emergent properties in the four-species biofilm.

How to cite: Amador, C., L. Røder, H., Kuhlicke, U., Neu, T., and Burmølle, M.: Decrypting the matrix: how interspecies interactions influence matrix in multispecies biofilm?, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-98, https://doi.org/10.5194/biofilms9-98, 2020

biofilms9-91
https://doi.org/10.5194/biofilms9-91
biofilms 9 conference

Extracellular polysaccharides (EPS) secreted by a model marine bacterium (Alteromonas spp.)

Carla Perez-Cruz[1], Monica Estupiñan[1], Mª Goretti Llamas-Arriba[2], Oier Etxebeste[2], Anders Lanzen[1], and Laura Alonso-Saez[1]
[1]AZTI, Marine Research Division, Sukarrieta, Spain
[2]University of Basque Country (UPV/EHU), San Sebastian, Spain

Alteromonas are model copiotrophic marine bacteria that are able to produce highly hydrated extracellular biopolymers mainly composed of polysaccharides (i.e., extracellular polysaccharides, EPS), which have a role in biofilm formation in oceans. Some of the functions of EPS are related to protection against environmental stressors, adhesion to particles, carbon storage and nutrient acquisition. Microbial EPS are largely heterogeneous in composition and structure, and some strains produce different types of EPS in response to different conditions. This study aimed at characterizing the synthesis of polysaccharides secreted from an *Alteromonas* spp. marine strain isolated from the Biscay Bay, targeting the genes involved in its synthesis. First, the genome of this strain was sequenced and different gene clusters related to the synthesis of EPS were identified. Then, a transcriptomic study was carried out to analyse the expression of EPS synthesis related genes in response to glucose and the EPS composition was preliminary characterized. The long-term objective is to increase our understanding of the patterns of EPS secretion in *Alteromonas*, which may have a key role in their association with phytoplankton blooms and adaptation to different environmental conditions.

How to cite: Perez-Cruz, C., Estupiñan, M., Llamas-Arriba, M. G., Etxebeste, O., Lanzen, A., and Alonso-Saez, L.: Extracellular polysaccharides (EPS) secreted by a model marine bacterium (Alteromonas spp.), biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-91, https://doi.org/10.5194/biofilms9-91, 2020

biofilms9-84
https://doi.org/10.5194/biofilms9-84
biofilms 9 conference

Pharmaceuticals and biofilms in a fresh-water stream in the south of Sweden

Aleksandra Hagberg[1], Shashank Gupta[2], Olena Rzhepishevska[1], Jerker Fick[1], Mette Burmølle[2], and Madeleine Ramstedt[1]
[1]Umeå University , Department of Chemistry , Umeå Center for Microbial Research, Sweden
[2]Section of Microbiology, Department of Biology, University of Copenhagen, Denmark

Pharmaceuticals and biofilms in a fresh-water stream in the south of Sweden

Aleksandra Hagberg[1], Shashank Gupta[2], Olena Rzhepishevska[1], Jerker Fick[1], Mette Burmølle[2], Madeleine Ramstedt[1]

- 1) Department of Chemistry, Umeå Center for Microbial Research, Umeå University, 901 87 Umeå, Sweden
- 2) Section of Microbiology, Department of Biology, University of Copenhagen, 2100, Copenhagen, Denmark

Pharmaceuticals have been detected in the aquatic environment all around the globe. The usage of medicine is growing every year, increasing the number of pharmaceutical residues released into the environment. Chronic exposure creates a significant threat to aquatic organisms. For this reason, it is crucial to investigate how pharmaceuticals can affect inhabitants of the aquatic ecosystem. In our study, we aimed to investigate how pharmaceuticals influenced the sessile bacterial species pattern in the Knivsta river in the south of Sweden. By placing the four sampling points before and after contamination (upstream and downstream), we aimed to see differences between locations that were chronically exposed to pharmaceuticals from a local sewage treatment plant and those that remained unexposed. Sampling was made three times in one year. Bacterial populations were analyzed by sequencing 16S RNA. Water chemistry with respect to pharmaceutical content was determined with LC-MS. Bacterial isolates were also collected and showed a range of phenotypes.

How to cite: Hagberg, A., Gupta, S., Rzhepishevska, O., Fick, J., Burmølle, M., and Ramstedt, M.: Pharmaceuticals and biofilms in a fresh-water stream in the south of Sweden, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-84, https://doi.org/10.5194/biofilms9-84, 2020

biofilms9-87
https://doi.org/10.5194/biofilms9-87
biofilms 9 conference

Probing the topography and mechanical properties of biomaterials with atomic force microscopy

Torsten Mueller, André Koernig, and Tanja Neumann
JPK BioAFM, Bruker Nano GmbH, Berlin, Germany

The composition, topography, adhesiveness and nanomechanical properties of biomaterials are all factors that affect biological processes, e.g. biofilm formation, cell differentiation, and morphogenesis [1-4]. Atomic Force Microscopy (AFM) is a highly versatile tool, ideal for the characterization of samples properties ranging from single molecules to complex biological systems, on the nm scale.

JPK BioAFMs, like the NanoWizard® ULTRA Speed 2, enable fast imaging of challenging biological samples and the visualization of dynamic processes with high spatio-temporal resolution under near physiological conditions, e.g. the kinetics of collagen type I fibrillogenesis was imaged in situ revealing the formation of the 67 nm D-banding hallmark.

Our distinctive Quantitative Imaging mode (QI™) measures various sample properties such as topography, nanomechanics and adhesion on the nanometer scale. Complex data like contact point, Young´s modulus or recognition images can also be extracted at the same resolution. To demonstrate the capability and flexibility of the QI™ mode, various biological samples like living cells have been investigated and their topographical and mechanical properties determined. QI™, based on fast force mapping, can also be used to determine the mechanical properties of different bacterial strains. We will discuss the application of HighSpeed AFM for the characterisation of dynamic biofilms with high spatiotemporal resolution, information which can then be directly correlated with advanced optical microscopy for immuno-characterisation of the sample.

Investigating large, sticky and rough samples such as tissues and hydrogels using AFM has always been a challenge due to the limited z-axis of the AFM. The HybridStage™, equipped with an extended xyz scanner unit up to 300x300x300 μm^3, an additional motorized unit for large sample movements in the mm range and optical tiling, is ideal for investigating such samples. This combination enables multi-region AFM probing over a large, uneven sample area and provides additional correlative optical data sets.

Adhesion dynamics between cells and biomaterials play a crucial role in, e.g. the applicability of potential implant materials. The AFM based Single Cell Force Spectroscopy platform enables quantitative measurement of the interactions between individual cells and any substrate.

A number of important research topics in the field of biomedicine, relate directly to the increased antimicrobial resistance of various biofilms to commonly prescribed drugs, and have identified adhesion, as a leading factor in biofilm formation, colony progression, and pathogenesis of microbial agents. JPK BioAFM has developed novel techniques for studying single-molecule forces and adhesion profiles at the cell/cell or cell/substrate interface. We will provide an overview of how to functionalize various surface substrates for the attachment of bacteria.

We will also provide information on working with AFM under Biosafety Level L2/L3 conditions.

[1] Elter, P. et al., Eur Biophys J, **2011**, 40(3):317-27

[2] Engler AJ. Et al., Cell; **2006**, 126(4):677-89

[3] Cisneros, DA. et al., Small, **2007**, 3(6):956-63

[4] Koser DA. Et al., Nat. Neurosci.; **2016**, 19:1592-1598

How to cite: Mueller, T., Koernig, A., and Neumann, T.: Probing the topography and mechanical properties of biomaterials with atomic force microscopy, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-87, https://doi.org/10.5194/biofilms9-87, 2020

biofilms9-42
https://doi.org/10.5194/biofilms9-42
biofilms 9 conference

Staphylococcus aureus biofilm matrix under bone environment influence

Fabien Lamret, Jennifer Varin-Simon, Sophie Gangloff, and Fany Reffuveille
Université de Reims Champagne-Ardenne, EA 4691 BIOS "Biomatériaux et Inflammation en Site Osseux", Pôle Santé, Reims, France (fany.reffuveille@univ-reims.fr)

Bone and joint infections linked to implanted materials are mostly due to Staphylococcus aureus. Deciphering the biofilm structure appears to be a promising strategy to develop antibiofilm molecules in order to curb infection occurrence and the bacterial recurrence. Indeed, the characterization of biofilm architecture and physiology could help to find new therapeutic targets through notable quantification of the matrix main components. Our hypothesis is that the very complex and interconnected bone microenvironment influences the bacterial adhesion and biofilm maturation and so its composition.

To identify the main factors influencing biofilm formation in the bone microenvironment, we determined biofilm biomass and the number of live adhered bacteria in a static model, completed with microscopy approaches to support our results. Different factors of bone microenvironment were tested: starvation, low oxygen rate, excess of magnesium, and presence of bone cell products. Our first results showed that MSSA or MRSA strains did not have the same behaviors under the tested conditions. However, for both types of strains, excess of magnesium combined to paucity of amino acids and oxygen increased the most the proportion of adhered Staphylococcus aureus (a 6 to 43 fold-increase, $p = < 0.01$). But biofilm biomass quantification and bacterial adhesion results showed divergent profiles leading us to think that matrix could be involved in such contrasts. Scanning electron microscopy highlighted several structures of matrix produced by these bacteria: well-known slime aspect, but also fibrous appearance, and no matrix production was revealed under some conditions. Indeed, all strains produced few matrix when cultured with control medium and oxygenated condition. Only CIP 53.154 strain built a strong slime-like matrix in response to oxygen depletion. However, both MSSA CIP 53.154 and SH1000 strains developed fibrous structures under anaerobic conditions associated with amino acid starvation, high magnesium concentration with or without glucose. MRSA USA300 strain did not seem to produce a matrix under our conditions, which is supported by the literature. Further investigations of the biofilm matrix are needed to conclude on the matrix nature, which surrounds bacteria under our conditions.

The bone microenvironment is complex but our results show that the parameters that mimicked this specific environment influenced the bacterial adhesion and probably the biofilm matrix composition of several strains of Staphylococcus aureus. Further investigations will help to understand how the different factors influence biofilm formation through quantification of the matrix main components by fluorescence microscopy and enzyme digestion. Our final aim is to develop an in vitro model mimicking this specific microenvironment in order to screen different antimicrobial molecules, which could target the biofilm matrix.

How to cite: Lamret, F., Varin-Simon, J., Gangloff, S., and Reffuveille, F.: Staphylococcus aureus biofilm matrix under bone environment influence, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-42, https://doi.org/10.5194/biofilms9-42, 2020

biofilms9-93
https://doi.org/10.5194/biofilms9-93
biofilms 9 conference

Surface Functionalization-Dependent Physicochemical Interactions between Nanoparticles and the Biofilm EPS Matrix

Dishon Hiebner, Caio Barros, Laura Quinn, Stefania Vitale, and Eoin Casey
School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland (dishon.hiebner@ucdconnect.ie)

The contribution of the biofilm extracellular polymeric substance (EPS) matrix to reduced antimicrobial susceptibility in biofilms is widely recognised. As such, directly targeting the EPS matrix is a promising biofilm control strategy that allows for efficient disruption of the matrix to allow an increase in susceptibility to antibiofilm agents. To this end, engineered nanoparticles (NPs) have received considerable attention. However, the fundamental understanding of the physicochemical interactions occurring between NPs and the EPS matrix has not yet been fully elucidated. An insight into the underlying mechanisms involved when a NP interacts with molecules in the EPS matrix will aid in the design of more efficient systems for biofilm control. The use of highly specific fluorescent probes in confocal laser scanning microscopy (CLSM) to illustrate the spatial distribution of EPS macromolecules within the biofilm is demonstrated. Three-dimensional (3D) colocalization analysis was used to assess the affinity of differently functionalized silica NPs (SiNPs) for specific EPS macromolecules from *Pseudomonas fluorescens* biofilms. Results show that both the charge and surface functional groups of SiNPs dramatically affect the extent to which SiNPs interact and localize with EPS macromolecules, including proteins, polysaccharides, and DNA. This research not only develops an innovative strategy for biofilm-nanoparticle interaction studies but also provides a platform on which to build more efficient NP systems for biofilm control.

How to cite: Hiebner, D., Barros, C., Quinn, L., Vitale, S., and Casey, E.: Surface Functionalization-Dependent Physicochemical Interactions between Nanoparticles and the Biofilm EPS Matrix, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-93, https://doi.org/10.5194/biofilms9-93, 2020

biofilms9-70
https://doi.org/10.5194/biofilms9-70
biofilms 9 conference

The role of divalent ions in cariogenic biofilm formation

Monika Astasov-Frauenhoffer[1], Elena Steiger[2], Julia Muelli[2], Olivier Braissant[3], and Tuomas Waltimo[2]

[1]University of Basel, University Center for Dental Medicine (UZB), Department Research, Switzerland (m.astasov-frauenhoffer@unibas.ch)

[2]University of Basel, University Center for Dental Medicine (UZB), Department for Oral Health & Medicine, Switzerland

[3]University of Basel, c/o Department of Biomedical Engineering (DBE), Center of Biomechanics and Biocalorimetry, Switzerland

The aim of the study was to investigate the effect of calcium, magnesium, and zinc on cariogenic biofilm formation and their interaction with bacterial EPS. This was evaluated using two *S. mutans* strains and different carbohydrates (glucose, sucrose and fructose).

Different combinations of carbohydrates and ions were investigated for their effect on the biofilm formation on hydroxyapatite disks by confocal laser scanning microscopy. Moreover, exopolysaccharides were purified and their affinity to the ions was measured by isothermal titration calorimetry.

The biofilm formation of *S. mutans* clinical isolate was almost eliminated in the presence of Zn^{2+} and promoted by Ca^{2+}, while adhesion seems to be more inhibited by Ca^{2+} and Mg^{2+} for *S. mutans* type strain. The EPS of cilincal isolate had a higher binding affinity towards calcium and magnesium than the type strain.

There seems to be a fine balance between these ions that needs to be maintained as excessive concentrations of one or another destroy the balance between the three.

How to cite: Astasov-Frauenhoffer, M., Steiger, E., Muelli, J., Braissant, O., and Waltimo, T.: The role of divalent ions in cariogenic biofilm formation, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-70, https://doi.org/10.5194/biofilms9-70, 2020

biofilms9-99
https://doi.org/10.5194/biofilms9-99
biofilms 9 conference

Unique effects of predation on heterotrophic and nitrifying membrane-aerated biofilm reactors (MABRs)

Bumkyu Kim, Mengfei Li, Patricia Perez-Calleja, and Robert Nerenberg
University of Notre Dame, CEEES, United States of America (bkim13@nd.edu)

The membrane-aerated biofilm reactor (MABR) is an emerging wastewater treatment technology that uses O_2-supplying membranes as a biofilm support. Because O_2 is supplied from the biofilm base instead of the bulk liquid, MABR biofilms have distinct microbial community structures and behavior. Past research showed that protozoan predation in MABR biofilms can create a unique void layer at the base of the biofilm. We hypothesized that the void layer could weaken the biofilm and promote sloughing, and investigated this with heterotrophic and nitrifying MABR biofilms.

Biofilms were grown in flat-sheet MABRs ("Base Case"). As a control, a reactor was supplied with cycloheximide in the media to suppress protozoa ("Suppressed Predation"). Each condition was run in triplicate. A rheometer was used to measure biofilm mechanical strength, and MABR flow cells were used to explore detachment. The biofilms were imaged using optical coherence tomography (OCT) (Ganymede, Thorlabs, Germany), and the images were digitally processed to quantify the biofilm thickness and internal void areas. In all tests, the biofilm was first grown to steady-state, as determined by effluent substrate concentrations and biofilm thicknesses.

In the heterotrophic biofilms, predation increased the internal void ratio from 6 ± 7 % to 50 ± 16 %. The storage modulus was 1,780 ± 1,180 Pa for the Base Case, compared to 9,800 ± 4,290 Pa for Suppressed Predation. Similarly, the loss modulus was 1,580 ± 729 Pa for the Base Case and 363 ± 189 Pa for Suppressed Predation. When subjected to an increased flow, the biofilm loss was 44 ± 24 % for the flow cell with predation, while only 7 ± 9 % for the control.

In the nitrifying biofilms, predation resulted in a greater fraction of internal voids, at 69 ± 6 % for the Base Case vs. 54 ± 5 % for Suppressed Predation. Also, the increased void ratio by predation reduced the biofilm viscosity and elasticity, resulting in greater detachment.

The loss modulus with Base Case and Suppressed Predation was 242 ± 135 Pa and 3640 ± 1860 Pa, respectively. The storage modulus was 1650 ± 853 Pa with Base Case and 23300 ± 11500 Pa with Suppressed Predation. The relative detached area of biofilm with Base Case and Suppressed Predation were 18 ± 12 and 4 ± 5 %, respectively. Thus, the greater detachment for the Base Case was consistent with the weaker mechanical properties. Predation also decreased the nitrification fluxes and promoted partial nitrification. The selective loss of NOB, as confirmed by fluorescence in-situ hybridization (FISH) and qPCR, may be due to the larger size of AOB clusters, providing greater resistance to predation.

These findings suggest that the effects of protozoa may need to be considered to predict the behavior of heterotrophic and nitrifying MABRs. Also, a better understanding of the microbial ecology of protozoa may lead to more effective MABR operational strategies.

How to cite: Kim, B., Li, M., Perez-Calleja, P., and Nerenberg, R.: Unique effects of predation on heterotrophic and nitrifying membrane-aerated biofilm reactors (MABRs), biofilms 9 conference,

Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-99,
https://doi.org/10.5194/biofilms9-99, 2020

Topic 4
Productive Biofilms (syngas, bio-electrochemical)

We define productive biofilms as microbial communities utilised in biotechnological processes as biocatalysts for the production of value-added chemicals. For successful implementation, it is essential to merge engineering and natural sciences to equally address biological aspects like biofilm growth, structure and physiology, as well as technical challenges like reactor configuration, mass transfer issues and scale up. Productive biofilms growing on active substrate like membranes (delivering gaseous substrates) or electrodes (acting as electron donor or acceptor) are perfect model systems to study the benefits, challenges and limitations of continuous productive biofilm systems. Hence, we welcome contributions that present new processes and/or biocatalysts thriving on active substrata, describe new solutions for reactor design or highlight the impact of the biofilm matrix and heterogeneity on productivity.

Oral Presentations

biofilms9-54
https://doi.org/10.5194/biofilms9-54
biofilms 9 conference

Optimization of PolyHydroxyAlkanoate Bioelectrosynthesis by the thermophilic bacterium Kyrpidia spormannii

Guillaume Pillot, Soniya Sunny, Victoria Comes, and Sven Kerzenmacher
University of Bremen, Center for Environmental Research and Sustainable Technology, Research Group Environmental Process Engineering , Germany (pillot@uvt.uni-bremen.de)

The electrosynthesis of valuable compounds by biofilms on electrodes is intensively studied since few years. However, the actual biofilms growing so far on cathode produce mainly small inexpensive compounds such as acetate or ethanol. A novel Knallgas bacteria, *Kyrpidia spormannii* have been recently described to grow on cathode in thermophilic and microaerophilic conditions, producing significant amount of PolyHydroxyAlkanoates (PHAs) (Reiner et al., 2018). These PHA are promising sustainable bioplastic polymers with the potential to replace petroleum-derived plastics in a variety of applications. However, the effect of culture conditions and electrode properties on the growth of *K. spormannii* biofilm and PHA production is still unclear.

We present in this study the successful development and operation of autotrophic biocathode whereby the electroactive biofilm was able to grow by utilizing CO_2 and a cathode as the sole carbon and electron source, respectively. We report for the first time, the effect of operating conditions of the Bioelectrochemical system (BES), cathode materials and cathode surface modification on current consumption, biofilm formation, PHA productivity and overall coulombic efficiency of a *K. spormannii* culture growing on electrodes. In particular, the focus of this study lies on optimization of three main operating conditions, which are the applied cathode potential, pH buffer and the oxygen concentration in the feed gas. Increased biofilm formation and PHA production was observed at an applied potential of -844mV vs. SCE, pH 6.5, O_2 saturation of 2.5%, and for a graphite cathode modified by CO_2 activation. The PHA concentration in the biofilm reached a maximum of ≈40 $\mu g \cdot cm^{-2}$ after optimization. The resultant PHA yield reported after optimization is increased by 12.2 times in comparison to previous results. In conclusion, these findings take microbial electrosynthesis of PHA a step forward towards practical implementation.

How to cite: Pillot, G., Sunny, S., Comes, V., and Kerzenmacher, S.: Optimization of PolyHydroxyAlkanoate Bioelectrosynthesis by the thermophilic bacterium Kyrpidia spormannii, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-54, https://doi.org/10.5194/biofilms9-54, 2020

biofilms9-55
https://doi.org/10.5194/biofilms9-55
biofilms 9 conference

Monitoring and quantification of bioelectrochemical biofilms by means of OCT in novel and customized reactor-setups

Max Hackbarth[1], Tobias Jung[2], Johannes Eberhard Reiner[2], Andrea Hille-Reichel[1], Michael Wagner[1,3], Johannes Gescher[2], and Harald Horn[1,4]

[1]Karlsruhe Institute of Technology (KIT), Engler-Bunte-Institut, Water Chemistry and Water Technology, Germany
[2]Karlsruhe Institute of Technology (KIT), Department of Applied Biology, Institute for Applied Biosciences, Germany
[3]Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces 1 (IBG-1), Institute for Biological Interfaces (IBG), Germany
[4]DVGW Research Centre, Water Chemistry and Water Technology, Germany

In the last 40 years, bioelectrochemical systems (BESs) have been increasingly discussed within the scope of debates about sustainable energy sources and production of value added chemicals independent of fossil sources. Since the produced current in microbial fuel cells as well as the turnover rates in microbial electrosynthesis cells are dependent on the biocatalysts´ activity, control of the growing biofilm plays a major role in BESs. Moreover, the knowledge about the interplay between biofilm development and electrochemical parameters is crucial for optimizing these sytems.

In the last 3 years, various electroactive biofilms (anodic and cathodic) were cultivated and characterized in a versatile and house made lab-scale flow cell system as well as in a rotating disc biofilm contactor (RDBC). Both systems allow for control of substrate (liquid and gaseous), and nutritional conditions as well as hydrodynamics and other physical parameters. The monitoring of biofilm development was conducted non-invasively by means of optical coherence tomography (OCT). For cathodic biofilms, quantitative analysis of generated 3D OCT data sets revealed a correlation between substratum coverage and measured current density. The increase of substratum coverage led to a decrease of measured current density due to less abiotic redox processes on the cathode surface. A stable current density was achieved when a substratum coverage of 99 % was reached. Furthermore, calculated biofilm accumulation rates could also be correlated with the substratum coverage. The overall biofilm accumulation rate decreased when the substratum was fully covered. Both correlations support the hypothesis that the availability of electrons from the cathode surface is a limiting factor in microbial electrosynthesis.

A 10-liter RDBC was designed to continuously harvest biomass from the electrode to extract intracellularly stored products. In future, this approach could be applied for biotechnological processes. Additionally, the RDBC can be used to obtain reliable mass balances and turnover rates because of its larger scale.

How to cite: Hackbarth, M., Jung, T., Reiner, J. E., Hille-Reichel, A., Wagner, M., Gescher, J., and Horn, H.: Monitoring and quantification of bioelectrochemical biofilms by means of OCT in novel and customized reactor-setups, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-55, https://doi.org/10.5194/biofilms9-55, 2020

biofilms9-65, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-65
biofilms 9 conference

Recombinant Protein Production and Plasmid Stability in *Escherichia coli* Biofilms

Luciana C. Gomes[1], Gabriel A. Monteiro[2], and Filipe J. Mergulhão[1]
[1]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal (luciana.gomes@fe.up.pt)
[2]iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Escherichia coli biofilms have a great biotechnological potential since this organism has been one of the preferred hosts for recombinant protein production for the past decades and it has been successfully used in metabolic engineering for the production of high-value compounds.

In a previous study, we have demonstrated that the non-induced enhanced green fluorescent protein (eGFP) expression from *E. coli* biofilm cells was 30-fold higher than in the planktonic state without any optimization of cultivation parameters [1]. The aim of the present work was to evaluate the effect of chemical induction with isopropyl β-D-1-thiogalactopyranoside (IPTG) on the expression of eGFP by planktonic and biofilm cells of *E. coli* JM109(DE3) transformed with a plasmid containing a T7 promoter.

It was shown that induction negatively affected the growth and viability of planktonic cultures, and eGFP production did not increase. Recombinant protein production was not limited by gene dosage or by transcriptional activity. Results suggest that plasmid maintenance at high copy number imposes a metabolic burden that precludes high level expression of the recombinant protein. In biofilm cells, the inducer avoided the overall decrease in the amount of expressed eGFP, although this was not correlated with the gene dosage. Higher specific production levels were always attained with biofilm cells and it seems that while induction of biofilm cells shifts their metabolism towards the maintenance of recombinant protein concentration, in planktonic cells the cellular resources are directed towards plasmid replication and growth [2].

It is expected that this work will be of great value to elucidate the mechanisms of induction on recombinant protein production, especially in biofilm cells which have shown potential to be used as protein factories.

References:

[1] Gomes, L.C., & Mergulhão, F.J. (2017) Heterologous protein production in *Escherichia coli* biofilms: A non-conventional form of high cell density cultivation. *Process Biochemistry, 57, 1-8*. https://doi.org/10.1016/j.procbio.2017.03.018

[2] Gomes, L., Monteiro, G., & Mergulhão, F. (2020). The Impact of IPTG Induction on Plasmid Stability and Heterologous Protein Expression by *Escherichia coli* Biofilms. *International Journal of Molecular Sciences, 21(2), 576*. https://doi.org/10.3390/ijms21020576

How to cite: Gomes, L. C., Monteiro, G. A., and Mergulhão, F. J.: Recombinant Protein Production and Plasmid Stability in Escherichia coli Biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-65, https://doi.org/10.5194/biofilms9-65, 2020

biofilms9-80
https://doi.org/10.5194/biofilms9-80
biofilms 9 conference

Mixed-trophies two species biofilms driven by Cyanobacteria for biotechnnological applications

Katja Bühler[1], Anna Hoschek[2], Andreas Schmid, Ingeborg Heuschkel, and Rohan Karande
[1]Department of Solar Materials, Helmholtzcenter for Environmental Research – UFZ, Leipzig, Germany (katja.buehler@ufz.de)
[2]BASF SE, RBW/OB – A030, Carl-Bosch-Strasse 38, 67056 Ludwigshafen am Rhein, Germany

Despite photo-biocatalysis developing remarkably and the huge potential of photoautotrophic microorganisms for eco-efficient production scenarios, photo-biotechnology is still in its infancy. The lack of scalable photo-bioreactors that provide efficient light transmission, CO_2 supply, and O_2 degassing and thus enable high cell densities (HCD), constitutes a key bottleneck, especially if cost-sensitive bulk chemicals are the product of choice. Commercialized tubular photo-bioreactors with 100 to 600 mm inner diameter offer a surface area to volume ratio (SA/V) of over 100 m^2 m^{-3} enabling the efficient capturing of incident solar radiation.[1] Here we introduce a new generation of photo-bioreactors based on capillary biofilm reactors. The biofilm is composed of two strains, namely the photoautotrophic strain *Synechocystis* sp. PCC 6803 and the chemoheterotrophic strain *Pseudomonas taiwanensis* VLB120, which serves as a biofilm supporter strain. *Pseudomonas* sp. is lowering the pO_2 in the system, which otherwise would toxify the Cyanobacteria. Furthermore, it produces extrapolymeric substances (EPS) and produces a kind of seeding layer promoting the attachment of *Synechocystis* sp.. *Synechocystis* sp. on the other hand produces organic compounds and oxygen consumed by *Pseudomonas* sp. The system is run completely without any organic carbon source.

Depending on the functionalities engineered into the biofilm forming organisms, these systems can be used for biotechnological applications. Here, we will present data on the physiology of the mixed trophies biofilm, and the challenging conversion of cyclohexane to caprolactone, and further on to 6-hydroxyadipic acid, both being important monomers for Nylon production.

References

- [1] Posten, C. Eng. In Life Science. (2009) 9:165-177
- [2] Hoschek, A. et al Bioresource Technology. (2019) 282: 171-178

tract HTML here.

How to cite: Bühler, K., Hoschek, A., Schmid, A., Heuschkel, I., and Karande, R.: Mixed-trophies two species biofilms driven by Cyanobacteria for biotechnnological applications, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-80, https://doi.org/10.5194/biofilms9-80, 2020

biofilms9-158
https://doi.org/10.5194/biofilms9-158
biofilms 9 conference

Biotechnological production of platform chemicals through anode assisted fermentation by using an artificial biofilm of S. oneidensis

Miriam Edel[1] and Johannes Gescher[1,2]
[1]Institute for Applied Biosciences - Department of Applied Biology, Karlsruhe Institute of Technology, Karlsruhe, Germany
[2]Institute for Biological Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

A shift from petrochemical processes to a bio-based economy is inevitable to establish a sustainable industry. Bioelectrochemical systems (BESs) are a future technology for the environment-friendly production of platform chemicals. In BESs exoelectrogenic bacteria such as *Shewanella oneidensis* can directly transfer respiratory electrons to the anode, which serves as a non-depletable electron acceptor. So far, the main limiting factor in BESs is the achievable current density which correlates to some extend with the density, thickness and metabolic activity of anode biofilms composed of exoelectrogenic microorganisms. This is especially true for *S. oneidensis* as the organism forms rather thin biofilms under anoxic conditions on anode surfaces.

In order to enhance the organisms' biofilm formation capabilities Bursac *et al*. deleted the *λ*-prophage from the genome. The deletion of the *λ*-prophage led to a 2.3-fold increased cell number on the anode ongoing with a 1.34-fold increased mean current density (Bursac *et al*., 2017). Furthermore, we just recently discovered that exogenous riboflavin enhances biofilm formation by the upregulation of the Ornithine-decarboxylase *speC*. This is probably based on a quorum sensing effect of riboflavin. Taken together the upregulation of *speC* ongoing with the deletion of the *λ*-prophage leads to a 4-fold increase in current density ongoing with a 6.1-fold increased biofilm formation on the anode.

However, to ensure an optimal performance of the biofilm in BESs, biofilm thickness itself is not sufficient. The biofilm also needs to be conductive. Our aim is to establish the Spytag-/Spycatcher-tool to synthetically steer biofilm conductivity. Spytag and Spycatcher are two protein residues from the fibronectin binding protein of *Streptococcus pyogenes* (Spy). These two protein residues form a spontaneous isopeptide bond under a variety of temperatures, pH values and buffers (Zakeri et al., 2012). By coupling Spytag and Spyctacher to different outer membrane *c*-type cytochromes of *S. oneidensis* the cells are covalently bound to each other while the biofilm remains conductive. In a first application the production of acetoin as one of the top 30 platform chemicals world-wide is desired (US Department of Energy, 2004).

In order to render *S. oneidensis* producing acetoin instead of the native end product acetate, Bursac *et al*. deleted the key genes for acetate production and introduced the acteoin production pathway (Bursac *et al*., 2017). To broaden the substrate spectrum of *S. oneidensis* further genes for glucose metabolism were introduced. Through a long term adaption, the glucose degradation, the biofilm formation abilities and the bioelectrochemical performance were significantly enhanced.

Merging all genetic optimizations into one production strain will enable us to produce acetoin from glucose as a platform chemical with high space-time yields. This will give rise to a production process that is competitive with existing oxic process routines without being dependent on expensive aeration.

References:

Bursac, T., Gralnick, J.A.,Gescher, J. (2017) Acetoin production via unbalanced fermentation in *Shewanella oneidensis*. Biotechnol Bioeng 114: 1283–1289.

Zakeri, B., Fierer, J.O., Celik, E., Chittock, E.C., Schwarz-Linek, U., Moy, V.T., Howarth, M. (2012) Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. Proc Natl Acad Sci U S A 109: E690.

How to cite: Edel, M. and Gescher, J.: Biotechnological production of platform chemicals through anode assisted fermentation by using an artificial biofilm of S. oneidensis, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-158, https://doi.org/10.5194/biofilms9-158, 2020

Poster Presentations

biofilms9-97
https://doi.org/10.5194/biofilms9-97
biofilms 9 conference

A membrane-based biofilm photobioreactor for enhanced algal growth rates

Meenu Garg[1], Patricia Perez-Calleja[1], **J. Saul Garcia-Perez**[1], Aura Ontiveros-Valencia[2], Cristian Picioreanu[3], Roberto Parra-Saldivar[4], and Robert Nerenberg[1]

[1]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, Notre Dame, United States of America (nerenberg.1@nd.edu)

[2]Department of Environmental Sciences, Instituto Potosino de Investigación Científica y Tecnológica AC, SLP, México

[3]Department of Biotechnology, Faculty of Applied Sciences, Delft University of Technology, Delft, The Netherlands

[4]Tecnologico de Monterrey, School of Sciences and Engineering, Monterrey, México

Biofilm-based algal processes are increasingly used for wastewater treatment, carbon capture, and production of biofuels and other valuable products. They provide high cell densities, are more robust, and are easier to harvest and concentrate than suspended algae. However, algal biofilms are more likely to experience carbon limitation, O_2 inhibition, and pH limitations, especially when thick and exposed to high light intensities. To address these limitations, we studied a novel photobioreactor based on CO_2-supplying hollow-fiber membranes, where the algal biofilms grow directly on the membranes. We used modelling and experiments to study our membrane biofilm photobioreactor (MB-PBR) system and to compare it to a control with atmospheric CO_2 and bicarbonate supplied in the bulk liquid.

Mathematical models of the MB-PBR and the control were developed using COMSOL Multiphysics®. The models included phototrophic growth, diffusion of gases (CO_2, O_2, N_2) across the membrane, nutrient diffusion from the bulk liquid, pH-dependent carbonate speciation, and light attenuation. Experimentally, we compared the MB-PBR and control using bench-scale photobioreactors with hollow-fiber membranes attached to them, 10% BG-11 media and white light from an LED lamp. The MB-PBR membranes were supplied with 5% CO_2 and 95% N_2. The control system had sealed membranes, to prevent gas exchange. We measured the biomass dry weight gravimetrically and the biofilm growth rates by daily measurement of the thicknesses using optical coherence tomography (OCT).

Both modeling and experiments suggested that MB-PBR biofilms grow significantly faster than the control. Using our model, we studied the effect of light intensity, pH, buffer concentration and light and oxygen inhibition on MB-PBR behavior. Growth was inhibited by excessively high levels of light and O_2. By providing CO_2 through the membrane, the carbon limitation was minimized, O_2 was stripped from the biofilm, and pH shifts were attenuated. These results suggest the MB-PBR may provide a more efficient platform for algal biofilm processes.

How to cite: Garg, M., Perez-Calleja, P., Garcia-Perez, J. S., Ontiveros-Valencia, A., Picioreanu, C., Parra-Saldivar, R., and Nerenberg, R.: A membrane-based biofilm photobioreactor for enhanced algal growth rates, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-97, https://doi.org/10.5194/biofilms9-97, 2020

biofilms9-45, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-45
biofilms 9 conference

Application of hollow fibre membrane reactor for biological removal of H_2S

Jewel Das[1,2], Harish Ravishankar[1], and Piet Lens[1]
[1]School of Natural Sciences, and Ryan Institute for Environmental, Marine and Energy Research, National University of Ireland Galway, University Road, H91 TK33, Galway, Ireland
[2]Bangladesh Council of Scientific and Industrial Research (BCSIR), BCSIR Laboratories Chattogram, Chattogram 4220, Bangladesh

Hydrogen sulfide (H_2S) is a toxic pollutant and harmful to human health. Industries such as pulp and paper manufacturing, rayon production, natural gas extraction and refining, and crude petroleum refineries generate waste gas streams with high H_2S concentrations. Both physico-chemical and biological methods are used for H_2S removal from the gas stream. Biological methods offer several advantages such as environmental friendly, less expensive and require simple operation and maintenance compared to physico-chemical methods. In this study, a hydrophilic hollow fibre membrane (HFM) based bioreactor configuration has been tested for biological H_2S removal. Three reactors were fabricated and operated for ~ 3 months where two reactors were used for biological conversion process and the third reactor was used for abiotic process. The effective membrane area of a HFM module used in each reactor was 0.0138 m^2. The bioreactors demonstrated efficient gas-liquid mass transfer through the HFM module and achieved ~ 99% removal efficiency with an elimination capacity of ~ 17.0 g m^{-3} h^{-1}. The H_2S flux of the bioreactor was ~ 0.20 g m^{-2} day^{-1} which was ~ 9 times higher than the abiotic reactor for an inlet H_2S concentration of ~ 0.90 g m^{-3}. The overall mass transfer coefficient value for the biotic process was 17.2 µm s^{-1} which was ~ 25 times higher than the abiotic process. The bioreactors demonstrated both microbial attached growth on the membrane surface and suspended growth in the liquid phase. Microbial community analysis confirmed the presence of diverse sulfur-oxidizing bacteria at genus level including *Acinetobacter*, *Dechloromonas*, *Hydrogenophaga*, *Rhodopseudomonas* and *Sulfurospirillum*. Moreover, the enrichment of other bacterial genera such as ammonia-oxidizing (e.g. *Nitrosospira*), organic matter degrading (e.g. *Trichococcus*) and methanogenic (e.g. *Methanosaeta*) microorganisms demonstrate the diverse microbial ecology of the sludge growing in the bioreactor.

How to cite: Das, J., Ravishankar, H., and Lens, P.: Application of hollow fibre membrane reactor for biological removal of H2S, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-45, https://doi.org/10.5194/biofilms9-45, 2020

biofilms9-156
https://doi.org/10.5194/biofilms9-156
biofilms 9 conference

Bacterial nanotubes and their role as bacterial nanowires in Pseudomonas aeruginosa biofilms

luyan ma
Institute Of Microbiology Chinese Academy of Sciences, State Key Laboratory of Microbial Resources (SKLMR), Chile
(luyanma27@im.ac.cn)

Microbial nanowires are nanofilaments that could offer an extracellular electron transfer (EET) pathway linking the bacterial respiratory chain to external surfaces, such as oxidized metals in the environment and engineered electrodes in renewable energy devices. Filaments proposed to function as nanowires have been reported in multiple bacteria, yet it remains largely unclear about the composition and electron transfer mechanism of bacterial nanowires. Pseudomonas aeruginosa is an environmental and electrochemically active bacterium. In this study, we found nanotube-like extracellular filaments in P. aeruginosa biofilms, which were bacterial membrane extensions similar to the nanowires reported in Shewanella oneidensis. Remarkably, conductive probe atomic force microscope showed measurable conductivity of these extracellular filaments, suggesting that they may function as nanowires in P. aeruginosa. Our results also indicated that the electron shuttle pyocyanin significantly affected the conductivity of P. aeruginosa nanowires, suggesting that the electron transfer mechanism of P. aeruginosa nanowires was different from S. oneidensis. Furthermore, factors that impact biofilm formation, such as flagella, type IV pili, and exopolysaccharides, were not essential for nanowires formation, while affect the formation and length of nanowires of P. aeruginosa. Taken together, this is the first report that investigated the role of electron shuttle on the conductivity of nanowires and factors that affected nanowires formation.

How to cite: ma, L.: Bacterial nanotubes and their role as bacterial nanowires in Pseudomonas aeruginosa biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-156, https://doi.org/10.5194/biofilms9-156, 2020

biofilms9-126
https://doi.org/10.5194/biofilms9-126
biofilms 9 conference

Biofilm Engineering Approaches for Improving the Performance of Bioelectrochemical Systems

Rajesh Sani
SOUTH DAKOTA SCHOOL OF MINES AND TECHNOLOGY, Chemical and Biological Engineering, United States of America (navanietha.rathinam@sdsmt.edu)

Biofilm Engineering Approaches for Improving the Performance of Bioelectrochemical Systems

ABSTRACT:

Bio-electrochemical devices are realized as promising technologies for a wide range of applications such bioenergy, and in biocommodity engineering. Bioelectrochemical systems make use of electroactive biofilms as electrocatalysts for converting chemical energy to electrical energy and vice versa. In this presentation, surface engineering of electrodes (using biopolymers such as chitosan/alginate, nanomaterials such as reduced graphene oxide), extremophilic bioprocessing, and biofilm engineering strategies for enhancing the biofilm formation and performance of bio-electrochemical systems will be discussed. This talk will also cover the applications of biofilm for energy and environment.

Keywords: Electrochemical devices, Electrode materials, Bioelectricity, Biofilm Engineering, Biopolymers

How to cite: Sani, R.: Biofilm Engineering Approaches for Improving the Performance of Bioelectrochemical Systems, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-126, https://doi.org/10.5194/biofilms9-126, 2020

biofilms9-17
https://doi.org/10.5194/biofilms9-17
biofilms 9 conference

Biofilm and productivity-associated community changes in serial-transfer experiments in heterogeneous liquid microcosms

Robyn Jerdan, Emily Donaldson, Scott Cameron, and Andrew Spiers
Abertay University, School of Applied Sciences, United Kingdom of Great Britain and Northern Ireland (r.jerdan@abertay.ac.uk)

Static incubation of liquid microcosms results in a physically heterogeneous environment, where depletion of O_2 in the lower region creates a relatively high-O_2 niche directly below the air-liquid (A-L) interface. This has been investigated using the model bacterium *Pseudomonas fluorescens* SBW25 and the biofilm-forming adaptive mutant known as the Wrinkly Spreader. In this system, colonisation of the A-L interface by the Wrinkly Spreader provides a fitness advantage over non-biofilm-forming competitors, including the ancestral SBW25, due to better access to O_2 in an otherwise O_2-growth limiting environment. Our current research seeks to understand how the ecological interactions of this simple system applies in more complex communities, where biofilms can be produced by multiple competing or co-operative strains and the low-O_2 region colonised by a range of strains capable of micro-aerobic growth. Here we report the effect of selection on the productivity of A-L interface biofilm-forming communities initiated by soil-wash (SW) inocula, which were serially transferred across ten microcosms and sixty days with mixed-community or biofilm-only samples. Initial analysis of the serial transfer experiments shows a decrease in community productivity which is explained by the accumulation of toxic metabolites, though small increases in community biofilm strength and attachment were also observed. Isolate-level analysis revealed a decrease in community diversity and a biofilm-associated phenotypic shift between the SW inocula and final-transfer communities, and these changes provide evidence of selection within our system.

Cell-localisation experiments confirm enrichment at the top of the liquid column in the high-O_2 region, but also show high cell densities in the low-O_2 region, even within the biofilm-only final-transfer communities. Samples taken from the biofilm and lower region of these communities were able to re-colonise both in fresh microcosms, indicating that community members were capable of migration within the liquid column. Despite the over-all decrease seen in community productivity in the serial transfer experiments, we suggest that communities maximised productivity by colonising both regions of the liquid column, with a resource trade-off between fast growth in the highly competitive high-O_2 region and slower growth in the less-competitive low-O_2 region. Many isolates from the final-transfer communities could occupy both regions and were capable of migration, with almost all isolates capable of flagella-mediated motility, and we interpret this ability to move between regions as a fitness advantage in A-L interface biofilm-forming communities. Although we have not been able to test this directly using the final-transfer communities or isolates, we have been able to demonstrate a fitness advantage in the less complex *P. fluorescens* SBW25 system, where biofilm-forming mutants capable of colonising both regions had a greater competitive fitness advantage over those with a poor ability to colonise the liquid column.

How to cite: Jerdan, R., Donaldson, E., Cameron, S., and Spiers, A.: Biofilm and productivity-associated community changes in serial-transfer experiments in heterogeneous liquid microcosms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-17, https://doi.org/10.5194/biofilms9-17, 2020

biofilms9-25
https://doi.org/10.5194/biofilms9-25
biofilms 9 conference

Climate-friendly production of renewable resources by the innovative use of diazotrophic, terrestrial cyanobacteria

Dorina Strieth, Judith Stiefelmaier, Sarah Di Nonno, Daniel Kronenberger, and Roland Ulber
TU Kaiserslautern, Maschinenbau und Verfahrenstechnik, Bioverfahrenstechnik, Germany (strieth@mv.uni-kl.de)

Renewable raw materials from agriculture help to reduce greenhouse gases, as they expand the CO_2 cycle and release less greenhouse gases than fossil raw materials when used for energy purposes. However, since high levels of nitrous oxide are released, especially by organic fertilization, no emission-free production of renewable raw materials is possible so far. Accordingly, there is an urgent need to replace organic and mineral fertilizers with nitrogen-binding systems. Here, diazotrophic (atmospheric nitrogen fixing), terrestrial cyanobacteria provide a way to fix atmospheric nitrogen and pass it on to plants for the production of renewable raw materials. Especially terrestrial cyanobacteria grow embedded in a thick matrix of extracellular polymeric substances which can contribute to a desirable soil stabilization and thus protection against soil erosion and to promoting water retention in the soil.

The main goal of this research is the sustainable establishment of nitrogen-fixing terrestrial cyanobacteria, which are immobilized on biodegradable carriers, in the ground in agriculture. Based on the aerosol-based photobioreactors constructed and established at the chair of bioprocess engineering, a new reactor system for the cultivation of cyanobacteria on carriers was developed and characterized. In addition, a screening for potential diazotrophic terrestrial cyanobacteria including the formation from vegetative cells to heterocysts (nitrogen fixing cells), nitrogen uptake and release rates for the use in the German agricultural economy was performed. A proof-of-principle to prove the use of terrestrial cyanobacteria as fertilizer for a climate-friendly production of renewable resources was tested by sowing seeds of Arabidopsis thaliana on agar plates with and without nitrogen as well as with and without a diazotrophic phototrophic biofilm.

This project is financially supported by the def DFG (UL 170/16-1) and the "Landesforschungsschwerpunkt NanoKat".

How to cite: Strieth, D., Stiefelmaier, J., Di Nonno, S., Kronenberger, D., and Ulber, R.: Climate-friendly production of renewable resources by the innovative use of diazotrophic, terrestrial cyanobacteria, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-25, https://doi.org/10.5194/biofilms9-25, 2020

biofilms9-135
https://doi.org/10.5194/biofilms9-135
biofilms 9 conference

Electrode assisted production of platform chemicals in Rhodobacter sphaeroides.

Ferdinand Schmid and Johannes Gescher
Karlsruhe Institute of Technology, Institute for Applied Biosciences, Applied Biology, Germany (ferdinand.schmid@kit.edu)

The aim of this study was to establish cathodic biofilms of the photosynthetic non sulfur purple bacterium *Rhodobacter sphaeroides* as biocatalyst for the production of platform chemicals from carbon dioxide as carbon source and an electrical current as energy and electron source. Therefore, *R. sphaeroides* was cultivated in a bioelectrical system (BES) in which light, CO_2 and a stable current were provided. Chronopotentiometric measurements revealed the cathode potential necessary to maintain the applied current of I = 22,2 µA/cm². Interestingly, exposure of *R. sphaeroides* to the antibiotic kanamycin lead to increased biofilm production on the cathode although the organism expressed the necessary resistance marker. This enhanced biofilm production raised the potential by 170 mV to E = -1 V compared to the wildtype (E = -1,17 V) and hence increased the efficiency of the process. To date, the molecular basis of this effect remains unclear and is under investigation using a proteomic approach. To elucidate, if the productivity of *R. sphaeroides* as a production strain is also enhanced, the production of acetoin was established as proof of principle. After the confirmation of the acetoin production under autotrophic conditions, various approaches to increase the space-time yields of the process were conducted and their effect will be presented.

How to cite: Schmid, F. and Gescher, J.: Electrode assisted production of platform chemicals in Rhodobacter sphaeroides., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-135, https://doi.org/10.5194/biofilms9-135, 2020

biofilms9-13
https://doi.org/10.5194/biofilms9-13
biofilms 9 conference

First steps for the scale up of a dual trophies microtubular biofilm reactor - preventing biofilm detachment

Amelie Kenkel, Andreas Schmid, Rohan Karande, and Katja Bühler
Helmholtz Centre for Environmental Research, Solar Materials, Germany

The use of phototrophic cyanobacteria in biotechnology is highly interesting as they represent a carbon neutral production platform, relying mainly on carbon dioxide, light and water for growth. However, one key bottleneck for utilizing cyanobacteria as production hosts is that in the currently established cultivation systems like tube or flatpanel reactors only cell densities of 2 to 4 g_{CDW}/L are possible, which is at least 20 times too low for most applications. One promising concept to solve this shortcoming is the cultivation of such microbes as dual trophies biofilms in microtubular systems in a segmented flow fashion with air bubbles, as recently reported in [1]. According to the aspects mentioned in Posten et. al [2], it becomes clear that the concept fulfils most requirements for photo-bioreactors. Firstly, the surface area to volume ratio is increasing with decreasing tube diameter. Hence, the path of the light through the reactor is reduced, leading to an optimal light supply. Secondly, using air segments increases the mixing within the reactor leading to a better supply of the cells with a carbon source as well as a better extraction of oxygen. Apart from that, the attached biofilm provides continuous cell regeneration and thus a continuous production system. All these aspects lead to a biomass concentration in this reactor system of up to 60 g_{CDW}/L [1].

The microtubular system was successfully applied in the challenging conversion of cyclohexane to cyclohexanol [1]. The reaction was conducted in a small lab scale system utilizing capillaries of 20 cm length, with a total volume of 1.4 mL. Here, we are evaluating the impact of larger scale on biofilm performance. Experiments were conducted in 1 m capillaries with 3 mm inner diameter. First, the impact of different flow rates was investigated. Results show, that a total minimal flow rate of 104 µL/min (52 µL air and 52 µL medium /min) leads to a significant biofilm detachment in various positions in the tube after one week of cultivation. A total flow rate of 520 µL/min (260 µL air and 260 µL medium /min) prevents detachment, however, it seems to hinder full surface coverage of the tube. An optimal condition turned out to be a cultivation of the biofilm with a starting flowrate of 520 µL/min for the initial attachment of the cells and a consecutive decrease of the flow to 104 µL/min after one week of cultivation. Thereby biofilm detachment was prevented and full surface coverage was achieved, while scaling the system by 5 fold. Respective data will be presented and discussed.

[1] Hoschek, Heuschkel, Mixed-species biofilms for high-cell-density application of *Synechocystis* sp. PCC 6803 in capillary reactors for continuous cyclohexane oxidation to cyclohexanol, Bioresource Technology, 2019

[2] Posten, Design principles of photo-bioreactors for cultivation of microalgae, Engineering in Life Sciences, 2009

How to cite: Kenkel, A., Schmid, A., Karande, R., and Bühler, K.: First steps for the scale up of a dual trophies microtubular biofilm reactor - preventing biofilm detachment, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-13, https://doi.org/10.5194/biofilms9-13, 2020

biofilms9-24
https://doi.org/10.5194/biofilms9-24
biofilms 9 conference

Genomic screening for novel peptide antibiotics in biofilm cyanobacteria by in-silico analysis and PCR

Selina Lenz
Chair of Bioprocess Engineering, TU Kaiserslautern, Kaiserslautern, Germany (lenz@mv.uni-kl.de)

Cyanobacteria are a group of phototrophic prokaryotes commonly known as blue-green algae. They grow embedded as biofilms in a thick matrix of extracellular polymeric substances (EPS) and can produce a highly diverse range of secondary metabolites, which are interesting in terms of their antimicrobial activity. Among these components, polyketide and polypeptide molecules are dominating. Antimicrobial polypeptide molecules are usually post-translational-modified or synthesised by non-ribosomal peptide synthetase (NRPS). Standard screening for antibiotics by inhibition tests is very time consuming and expression of antimicrobic activity highly depend on cultivation conditions. Therefore, they can vary between different cultivations. On a genomic level existing, but in this cultivation not synthesized, antibiotics are completely neglected. Due to the increasing amount of available genomic sequence data, screening for novel antibiotics can also be done in-silico. Highly homologous sequences to known antibiotic gen clusters can be determined in cyanobacterial genomes and eventually be detected in-vivo through PCR analysis. Compared to inhibition tests, a major advantage of PCR is the little amount of biomass needed. As the growth of cyanobacteria is slow, e.g. Trichocoleus sociatus (0.44 d^{-1}) compared to bacteria like Escherichia coli (2.08 h^{-1}), this leads to significant shorter cultivation and screening time. In addition, qPCR can be used to determine gene expression quantity of the considered genes. PCR with degenerated primers for specific gen cluster like NRPS, polyketide synthetases, lanthipeptides etc. can also be used to screen non-sequenced cyanobacteria for the possible origin of an unidentified antibiotic.

The following work is part of the iProcess project, whose overall scientific goal is to develop the process engineering fundamentals for using fungi and cyanobacteria as production organisms for pharmaceutically active substances. As part of the iProcess project, a semi-continuous process for the production of antibiotics from cyanobacteria biofilms in aerosol reactors shall be developed. Aim of the following work is the in-silico search for new polypeptide antibiotics, as well as the subsequent in-vivo detection to discover promising cyanobacteria as production strains. In the first instance, the screening is focusing on the intern cyanobacteria strain collection of the TU Kaiserslautern. Subsequently the new strains will be cultivated as biofilms in an aerosol reactor and the resulting extracellular polymeric substances can be analysed for their antimicrobial activity.

This project is financially supported by Ministry of Science, Further Education and Culture of Rhineland-Palatinate (mwwk.rlp) (iProcess intelligent process development – from modelling to product).

How to cite: Lenz, S.: Genomic screening for novel peptide antibiotics in biofilm cyanobacteria by in-silico analysis and PCR , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-24, https://doi.org/10.5194/biofilms9-24, 2020

biofilms9-18
https://doi.org/10.5194/biofilms9-18
biofilms 9 conference

Increased biofilm in Shewanella oneidensis MR-1 leads to higher current generation in METs

Ana V. Silva[1], Miriam Edel[2], Johannes Gescher[2,3], and Catarina M. Paquete[1]
[1]ITQB-NOVA, Portugal (anavsilva@itqb.unl.pt)
[2]Institute for Applied Biology, Department of Applied Biology, Karlsruhe Institute of Technology, Karlsruhe, Germany
[3]Institute for Biological Interfaces, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Biofilm formation is a central process in the function of Microbial Electrochemical Technologies (METs). These technologies have emerged in recent years as a promising alternative green source of energy, in which microbes consume organic matter to produce energy or valuable by-products. It is the ability of performing extracellular electron transfer that allows these microbes, called electroactive organisms, to exchange electrons with an electrode in these systems. The low levels of current achieved have been the set-back for the large-scale application of METs. *Shewanella oneidensis* MR-1 is one of the most studied electroactive organisms, and it has been demonstrated that its increased biofilm formation can lead to higher current generation. The *bolA* gene has been identified as a central player in biofilm formation in different organisms, with its overexpression leading to increased biofilm production. In this work, we explored the effect of this gene in biofilm formation and current production by *S. oneidensis* MR-1. Our results demonstrate that this gene is involved in the biofilm formation by this organism, with its over expression leading to an increased biofilm formation. We could also show that this increase in biofilm formation lead to a consequent higher current generation. This information is a relevant step for the optimization of electroactive organisms towards their practical application in METs.

How to cite: V. Silva, A., Edel, M., Gescher, J., and M. Paquete, C.: Increased biofilm in Shewanella oneidensis MR-1 leads to higher current generation in METs, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-18, https://doi.org/10.5194/biofilms9-18, 2020

biofilms9-110
https://doi.org/10.5194/biofilms9-110
biofilms 9 conference

In situ probing and evaluation of two different electrode materials in bio electrochemical systems by means of Optical Coherence Tomography on automated robotic platform

Jinpeng Liu[1], Harald Horn[1,2], and Michael Wagner[1,3]
[1]Engler-Bunte-Institut, Water Chemistry and Water Technology, Karlsruhe Institute of Technology (KIT), Engler-Bunte-Ring 9a, 76131 Karlsruhe, Germany
[2]Deutscher Verein des Gas und Wasserfaches (DVGW) Research Center at the Engler-Bunte-Institut of Karlsruhe Institute of Technology (KIT), Engler-Bunte-Ring 1, 76131 Karlsruhe, Germany
[3]Institute for Biological Interfaces 1 (IBG-1), Institute for Biological Interfaces (IBG), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

Carbon-based and stainless steel-based materials are widely utilized as anode/cathode electrodes in bio electrochemical systems (BESs) due to its low capital cost, high conductivity and large specific surface area. Carbon-based materials such as carbon veil are mostly applied in lab-scale reactors because of its versatile shape and configuration. Moreover, stainless steel type materials show higher strength and are easier to incorporate within flow field. Optical coherence tomography (OCT) as an image technique is a suitable method to monitor biofilm growth and fluid-structure interactions at the meso-scale. In BESs, investigating bulk-biofilm interface (fluid-structure interactions) is of particular interest to optimize the mass transfer under suitable hydrodynamic condition and enhances the overall effectivity of BESs systems. To extend the knowledge about the influence of different anode electrodes as substratum on OCT monitoring and quantification, the biofilm structural properties analyzed by OCT image processing and bioelectrochemical systems performance were compared.

A custom-designed dual-chamber setup was constructed by two transparent optical flow cells and fixed in the automated monitoring platform (Evobot). Herein, we applied OCT to in-situ characterize and quantify the biofilm structure properties on two different anode electrodes (carbon veil-CV and porous stainless steel-SS) as substratum in microbial fuel cell (MFC) mode. 3D OCT dataset analysis presented 3 structural parameters for biofilm-carbon veil interface and 5 structural parameters for biofilm-stainless steel interface, separately. Biofilm volume (BioV) was calculated to compare CV and SS anode electrodes.

In this study, a time-series of biofilm development was performed on both CV and SS materials. At the fourth day, the biofilm almost covered the entire anode surface and achieved 97% substratum coverage. Afterwards the biofilm grew mostly in vertical direction. With the further biofilm growth along height the electric resistance increased and power production gradually reached the equilibrium. Nevertheless, both materials did not show predominant advantage on power production. Furthermore, a relatively small error appeared on quantitative analysis of Biofilm volume using stainless steel. Whereas, the predictability of biofilm volume on the carbon veil anodes was hindered by the appearance of shadowing effects. Thus, it can be concluded that stainless steel flat plate electrode is preferable as anode material to investigate the interaction between biofilm structure, hydrodynamic conditions and mass transfer in BESs via OCT.

How to cite: Liu, J., Horn, H., and Wagner, M.: In situ probing and evaluation of two different electrode materials in bio electrochemical systems by means of Optical Coherence Tomography on

automated robotic platform, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-110, https://doi.org/10.5194/biofilms9-110, 2020

biofilms9-28
https://doi.org/10.5194/biofilms9-28
biofilms 9 conference

Novel photobioreactor for moving bed biofilm cultivation of terrestrial cyanobacteria

Jakob Walther, Niklas Erdmann, Katharina Wastian, Dorina Strieth, and Roland Ulber
TU Kaiserslautern, Institute for Bioprocess Engineering, Mechanical and process Engineering, Germany (walther@mv.uni-kl.de)

Terrestrial cyanobacteria grow quite poorly as suspension culture. This is one of the reasons why they have not yet been considered as producers of interesting metabolites such as antibacterial substances. Previous work in our group have shown that surface-associated growth can significantly increase productivity [1]. Moving bed bioreactor technology, which is already established in wastewater treatment, offers a possibility to carry out such growth on a larger scale. In these reactors, the bacteria grow on the surface of solid structured carrier particles in areas protected from mechanical abrasion (protected surface). These particles are usually about 1-5 cm in size and are made of high-density polyethylene (HDPE). Moving bed processes for microalgae have only been described for fabric as a solid substrate [2] whereby only 30% of the biomass was actually immobilized on the carrier particles. For this reason, different HDPE carrier particles and different cyanobacteria were investigated. Three different cyanobacteria could be successfully cultivated on two different particles in a 1.5-liter photobioreactor in a moving bed. As an up-scale step, a larger reactor was developed, which provided a larger cultivation surface in combination with a sufficient illumination.

Photobioreactor

The design of the reactor is similar to Zhuang et al. [2]. Based on an 80x35x40 cm tank, the reactor has a working volume of 65 liters. At a particle filling degree of 27 %, the reactor has a protected cultivation surface area of 11.26 m² within the particles. This corresponds to 173 m² per m³ reactor volume. Their circulation is generated by a gassing unit on the ground. An inclined plate is installed beside the gassing unit, to avoid a flow dead zone at the bottom of the reactor. The reactor is illuminated by LEDs located outside the reactor. The growth is monitored offline by the determination of the dry biomass (bdm) and the measurement of the biofilm thickness by optical coherence tomography (OCT).

Results

Cultivations with the cyanobacterium Trichocoleus sociatus were carried out. The inoculum was added to the reactor as suspended biomass with a concentration of 0.035 g_{bdm}/L. After two weeks, the complete biomass was immobilized as a thin biofilm on the carrier particles. Between day 18 and day 45, an increase in the median biofilm thickness from 36 µm to 65 µm could be measured with an increase of the dry biomass from 0.44 to 1.56 g/L. This volume-specific yield is similar to cultivations in the 1.5-liter photobioreactors with carrier particles.

Funding

The project is financially supported by the DFG (Project number UL 170/16-1) and the Ministry of Education of Rhineland-Palatinate (bm.rlp) (iProcess intelligent process development – from

modelling to product)

References

How to cite: Walther, J., Erdmann, N., Wastian, K., Strieth, D., and Ulber, R.: Novel photobioreactor for moving bed biofilm cultivation of terrestrial cyanobacteria, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-28, https://doi.org/10.5194/biofilms9-28, 2020

biofilms9-102
https://doi.org/10.5194/biofilms9-102
biofilms 9 conference

Online Analysis of CO2 Production in Electroactive Biofilms by Differential Electrochemical Mass Spectrometry

Fabian Kubannek[1], Uwe Schröder[2], and Ulrike Krewer[3]
[1]TU Braunschweig, Institute of Energy and Process Systems Engineering, Germany (f.kubannek@tu-braunschweig.de)
[2]TU Braunschweig, Institute of Environmental and Sustainable Chemistry, Braunschweig, Germany (uwe.schroeder@tu-braunschweig.de)
[3]KIT, Institute for Applied Materials - Materials for Electrical and Electronic Engineering, Karlsruhe, Germany (ulrike.krewer@kit.edu)

Electroactive biofilms are routinely characterized in-operando by dynamic electrochemical measurement techniques such as cyclic voltammetry or electrochemical impedance spectroscopy. Since electrical signals can be recorded and processed very quickly, these techniques allow to investigate slow and fast electron transfer processes.

In contrast, the dynamics of species production rates are usually not addressed because standard measurement techniques for the quantification of reaction products such as gas chromatography are slow. Instead it is often assumed that species production rates are either directly proportional to the current - under so called turnover conditions - or equal zero - under so called non-turnover conditions.

To challenge this assumption, we measured species production rates of a biofilm electrode with a high time resolution by differential electrochemical mass spectrometry (DEMS). An acetate oxidizing biofilm electrode was placed just micrometers away from the mass spectrometer inlet in which enabled us to observe CO_2 production directly at the electrode during cyclic voltammetry (CV) and potential steps.

The measurement results showed that the CO_2 production deviates significantly from the expected value calculated from the current by Faraday's law under certain operating conditions. We analyze this effect in detail and show that it can be explained with biofilm storage capacities for charge and substrate. These capacities are quantified by deconvoluting the faradaic and non-faradaic currents. [1]

Also, the onset of the complete oxidation of acetate to CO_2 during CVs was determined to be just 22 mV above the standard potential for acetate oxidation. Determining this value by directly measuring CO_2 instead of current is advantageous because capacitive effects can be excluded. [1]

In conclusion, we demonstrate that electrical current and CO_2 production can be partly decoupled in biofilm electrodes and that DEMS is a valuable technique for analyzing processes in such electrodes.

[1] Kubannek, F., Schröder, U., Krewer, U. (2018). Revealing metabolic storage processes in electrode respiring bacteria by differential electrochemical mass spectrometry. Bioelectrochemistry, 121, 160–168, doi: 10.1016/j.bioelechem.2018.01.014

How to cite: Kubannek, F., Schröder, U., and Krewer, U.: Online Analysis of CO2 Production in Electroactive Biofilms by Differential Electrochemical Mass Spectrometry, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-102, https://doi.org/10.5194/biofilms9-102, 2020

biofilms9-159
https://doi.org/10.5194/biofilms9-159
biofilms 9 conference

Power output enhancement in ceramic, mL-scale Microbial Fuel Cell

Iwona Gajda[1,2], Buddhi Arjuna Mendis[2], John Greenman[2,3], and Ioannis Ieropoulos[2]
[1]Bristol BioEnergy Centre, University of the West of England (iwona.gajda@uwe.ac.uk)
[2]Bristol BioEnergy Centre, Bristol Robotics Laboratory, University of the West of England, Bristol, BS16 1QY, UK
[3]Biological, Biomedical and Analytical Sciences, University of the West of England, Bristol, BS16 1QY, UK

A microbial fuel cell (MFC) is a renewable energy converter, which transforms organic biomass directly into electricity, using biofilm-electrode metabolic interaction within a bioelectrochemical cell. Efficiency of this transformation can be enhanced through miniaturisation. Miniaturisation of MFCs offers higher surface-area-to-volume ratio and improved mass transfer.

The development of mL-scale; power dense and low cost MFCs, are of great interest in diverse areas of research, ranging from modern bio-robotics, internet-of-things devices, electrical energy generation, remote sensing to wastewater treatment and mineral recovery. The biofilms increased ability in converting organic pollutants into electric power more efficiently, makes mL-sized MFCs attractive for the development of multi-modular stacks and usable off-grid power sources with an ability of enhanced wastewater treatment. This work focuses on small scale MFCs; i) minimising the distance between feeding stream and the biofilm, ii) construction and analysis of a millilitre scale prototype, using a low cost ceramic separator for higher energy recovery efficiency and sensitivity enhancement to substrates and pollutants. The study aims to test efficient cathode modifications, using graphene ink and magnetite (Fe_3O_4); in order to improve the oxygen reduction reaction (ORR). This in turn is envisioned in an increase of the output, reaching comparable power levels to the larger MFC prototypes tested so far. The additives are chosen such that, both graphene and iron–based oxides are known from the literature to be catalysts for electrochemical processes, this work focusses on their incorporation into the open-to air cathode in novel, low cost MFC bioreactors.

The miniaturised MFC construction constituted of an in-house fabricated small scale ceramic cylinder of internal volume of 3.88 mL. An anode, made of carbon veil fibre with a coating of activated carbon powder, was placed inside the ceramic cylinder, while the cathode was attached to the outer surface of the structure. Three types of cathodes were tested: i) activated carbon as the control (AC), ii) AC with a graphene ink coating (AC+G) and iii) AC with graphene ink and magnetite powder blend (AC+G+M). Experiments were conducted in triplicate using activated sludge and urine inoculum and thereafter continuously supplemented with 100% real human urine. The results show that the control produced up to 0.85 mW (219 W/m^3), while AC+G produced 1.22 mW (312 W/m^3), and AC+G+M 1.12 (288 W/m^3) which is a 44 % and a 32 % increase respectively in comparison to the control. Comparison of linear sweep voltammetry (LSV) showed superior performance of both modified electrodes against the unmodified AC cathode; further resulting in an enhancement of ORR reaction rate. Power outputs from this work show over 14 times improvement in power density levels in comparison to larger reactors of 20 times the volume, as well as comparable raw (actual) power levels. This makes these novel small-scale bioreactors particularly attractive for use in numerous practical applications such as energy autonomous robots (e.g. EcoBots) and multi-modular stacks for off-grid energy sources.

How to cite: Gajda, I., Mendis, B. A., Greenman, J., and Ieropoulos, I.: Power output enhancement in ceramic, mL-scale Microbial Fuel Cell , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-159, https://doi.org/10.5194/biofilms9-159, 2020

biofilms9-79
https://doi.org/10.5194/biofilms9-79
biofilms 9 conference

Surface-associated plant cell culture

Alexander Mehring, Judith Stiefelmaier, and Roland Ulber
Technische Universität Kaiserslautern, Bioverfahrenstechnik, Germany (mehring@mv.uni-kl.de)

Biofilms are typically characterized as a consortium of microorganisms, which adhere to each other and often to surfaces. This adhesion is realized by extracellular polymeric substances (EPS), which are secreted by the microorganisms and mainly consist of water, polysaccharides, proteins and lipids as well as nucleic acids and lysis products [1]. Although cultured plant cells are not typically considered biofilms, parallels can be found in the properties of plant calli. These callus cells tend to form cohesive aggregates, owing to their extracellular matrix, and often strongly adhere to the agar plates they are kept on. The extracellular matrix of plant cells is mainly composed of structural polysaccharides, such as xyloglucans, arabinogalactans [2], homogalacturonan and extensins [3] among others. Cultured plant cells were found to adhere to surfaces before [4]. Surface-associated plant cell culture may have potential in a (semi□)continuous cultivation including product secretion, as was shown in principle for alginate-embedded plant cells [5]. For cyanobacterial biofilms, an efficient strategy for EPS extraction was recently developed [6]. The transferability of these protocols to biofilm-like growing plant calli of Ocimum basilicum is currently being investigated. Subsequently, the composition of the extracellular matrix extracted from cultured O. basilicum cells is of interest. Furthermore, the adhesive properties of O. basilicum suspension cultures to microstructured surfaces and the potential role of the extracellular matrix are under investigation. An investigation of culture properties in an aerosol photobioreactor [7] is planned as well.

This project is financially supported by the German research foundation (DFG, project number SFB 926-C03).

References:

[1] H. C. Flemming, T. R. Neu, and D. J. Wozniak, "The EPS matrix: The 'House of Biofilm Cells,'" J. Bacteriol., vol. 189, no. 22, pp. 7945–7947, 2007.

[2] I. M. Sims, K. Middleton, A. G. Lane, A. J. Cairns, and A. Bacic, "Characterisation of extracellular polysaccharides from suspension cultures of members of the Poaceae," Planta, vol. 210, no. 2, pp. 261–268, Jan. 2000.

[3] M. Popielarska-Konieczna, K. Sala, M. Abdullah, M. Tuleja, and E. Kurczyńska, "Extracellular matrix and wall composition are diverse in the organogenic and non-organogenic calli of Actinidia arguta," Plant Cell Rep., no. 0123456789, 2020.

[4] R. J. Robins, D. O. Hall, D. □J Shi, R. J. Turner, and M. J. C. Rhodes, "Mucilage acts to adhere cyanobacteria and cultured plant cells to biological and inert surfaces," FEMS Microbiol. Lett., vol. 34, no. 2, pp. 155–160, 1986.

[5] Y. Kobayashi, H. Fukui, and M. Tabata, "Berberine production by batch and semi-continuous cultures of immobilized Thalictrum cells in an improved bioreactor," Plant Cell Rep., vol. 7, no. 4, pp. 249–252, 1988.

[6] D. Strieth, J. Stiefelmaier, B. Wrabl et al., "A new strategy for a combined isolation of EPS and pigments from cyanobacteria," J. Appl. Phycol., no. Fromme 2008, Feb. 2020.

[7] S. Kuhne, D. Strieth, M. Lakatos, K. Muffler, and R. Ulber, "A new photobioreactor concept enabling the production of desiccation induced biotechnological products using terrestrial cyanobacteria," J. Biotechnol., vol. 192, no. Part A, pp. 28–33, 2014.

How to cite: Mehring, A., Stiefelmaier, J., and Ulber, R.: Surface-associated plant cell culture, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-79, https://doi.org/10.5194/biofilms9-79, 2020

biofilms9-37
https://doi.org/10.5194/biofilms9-37
biofilms 9 conference

Surface adhesion of phototrophic biofilms

Judith Stiefelmaier[1], Dorina Strieth[1], Susanne Schaefer[2], Daniel Kronenberger[1], Björn Wrabl[1], Ulrich Bröckel[2], and Roland Ulber[1]

[1]Chair of Bioprocess Engineering, TU Kaiserslautern, Kaiserslautern, Germany
[2]Institute of Micro-Process-Engineering and Particle Technology, University of Applied Sciences Trier, Environmental Campus Birkenfeld, Birkenfeld, Germany

Cyanobacteria belong to the oldest known microorganisms and are capable of oxygenic photosynthesis. Depending on their habitat aquatic and terrestrial cyanobacteria are distinguished. Terrestrial cyanobacteria grow embedded in a matrix of extracellular polymeric substances (EPS) as phototrophic biofilms. Those EPS serve as nutrient storage, protection from desiccation and play an important role in surface adhesion. For cultivation of phototrophic biofilms different biofilm reactors have been developed in the last years. One interesting parameter when cultivating biofilms is the surface material and structure, since it can influence the surface adhesion and thus biofilm formation. Therefore, different materials as cultivation surfaces were investigated as well as the strain specific behavior of different cyanobacteria and the impact on EPS formation. In this work the adhesion of the terrestrial cyanobacteria *Coleofasciculus chthonoplastes* and *Trichocoleus sociatus* to different materials was investigated. For characterization of materials measurements concerning surface roughness were conducted using atomic force microscopy. Biofilms were cultivated in an aerosol and the development of surface adhesion in connection with biofilm age was analyzed using two different methods. In the first set-up biofilms were placed in a specially designed flow-through chamber and overflown with medium at increasing flow speed. The detachment of the biofilm was documented with optical coherence tomography (OCT). Additionally, the experiments were supplemented with CFD-simulation for quantification of shear forces. The second method analyzed adhesion forces using rotational rheometry. Hereby, differences between cyanobacteria strains and surface materials could be observed as well as an increasing adhesion with increasing cultivation time. The developed flow-through chamber, which could as well be utilized with a camera instead of OCT, offers a simple low-priced possibility for investigation of surface adhesion.

This project is financially supported by the German Research Foundation (DFG; Project number: UL 170/16-1; MU 2985/3-1 and SFB 926) and the Landesförderung Rheinland-Pfalz (Project: iProcess).

How to cite: Stiefelmaier, J., Strieth, D., Schaefer, S., Kronenberger, D., Wrabl, B., Bröckel, U., and Ulber, R.: Surface adhesion of phototrophic biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-37, https://doi.org/10.5194/biofilms9-37, 2020

biofilms9-47
https://doi.org/10.5194/biofilms9-47
biofilms 9 conference

Viability of mono-specie biofilm formed by the solvent producer Clostridium beijerinckii during continuous fermentation in packed bed bioreactor.

Maxime Carrié[1], Hélène Velly[2], Jean-Christophe Gabelle[1], and Fadhel Ben-Chaabane[2]
[1]IFP Energie Nouvelles, Rond-point de l'échangeur de Solaize, BP3, 69360 Solaize
[2]2 IFP Energie Nouvelles, 1 et 4 avenue de Bois préau 92852 Rueil-Malmaison.

Butanol and Isopropanol are naturally produced by the bacteria C. beijerinckii. Those products are used in large field of applications such as fuel and bulk chemicals. Since butanol is toxic at small concentration for cells, bacterial growth and metabolism are inhibited during classical batch fermentation (1). These phenomena lead to the production of low solvent concentration (around 7 $g.L^{-1}$) and a low volumetric productivity (0,13 $g.L^{-1}.h^{-1}$) (2). Continuous fermentation can be performed in order to avoid product inhibition by a continuous removal of fermentation broth. However, the solvent productive biomass is easily washout at high dilution rate because of the low maximum growth rate of the strain in this metabolism phase (0,05 h^{-1}) (3). To overcome this issue, cell immobilization of C. beijerinckii by biofilm formation on solid support is the best solution. As a result, the biomass residence time can be uncorrelated from the hydraulic residence time leading to a higher viable biomass concentration in the bioreactor and consequently a higher volumetric productivity (up to 5 $g.L^{-1}$.h-1) (4). Our study aimed at evaluating biofilm viability which is an important parameter that is linked to process productivity and has been little studied in the case of the IBE fermentation (5).

In this study we developed two techniques to monitor biofilm viability during immobilized cell fermentation: Flow cytometry (FC) and PMA qPCR. After FC analysis, a high background noise due to the biofilm extra polymeric substance is obtained. Consequently, an enzymatic sequential enzymatic biofilm deconstruction using Dnase I and Proteinase K was developed . This pre-treatment successfully lowered the background noise of this analysis. The suspensions obtained were stained with carboxyfluoresceine diacetate (cFDA) and propidium iodide (PI) which are indicators of cellular activity and alteration of membrane integrity, respectively, and analyzed by flow cytometry. The percentage of viable cells obtained after pre-treatment compared to the control sample is increased from 2.6 ± 0.9 % to 22.8 ± 8.6% because of the background noise decrease. PMA-qPCR confirmed the results obtained by flow cytometry without using enzymatic pre-treatment. Although FC is less accurate than PMA-qPCR, this technique is less time-consuming, cheaper and reliable to study biofilm viability.

References

How to cite: Carrié, M., Velly, H., Gabelle, J.-C., and Ben-Chaabane, F.: Viability of mono-specie biofilm formed by the solvent producer Clostridium beijerinckii during continuous fermentation in packed bed bioreactor., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-47, https://doi.org/10.5194/biofilms9-47, 2020

Topic 5
Biofilm Lifecycle and its Regulation

Biofilms form and disperse following complex regulatory regimes, which are the consequence of both endogenous and exogenous signals. These signals are sensed and translated into the regulation of the expression of a number of target genes. It seems that the type of the natural environment has formed the complex response of the microorganisms. Consequently, one signal can inhibit biofilm formation in one organism while it promotes biofilm formation for others. Although elements like flagellar motility, carbon metabolism, quorum sensing molecules or c-di-GMP have over the last years been established as major players for biofilm regulation, we are also aware that we are still far away from completely understanding the full regulatory networks or the impact of cellular heterogeneity on the regulation of biofilm formation. Also, the development of molecules that interfere with the regulatory machinery and can hence be used for the dispersal or enhanced formation of biofilms is still in its infancy. Hence, we welcome contributions that would address the above mentioned emerging fields in the regulation of the biofilm lifecycle.

Oral Presentations

biofilms9-71, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-71
biofilms 9 conference

Diversity in regulatory regions of *icaADBCR* and *fnbAB* genes among *Staphylococcus aureus* strains isolated from periprosthetic joint infections

Liliana Morales[1], Maite Echeverz[1], Margarita Trobos[2], Cristina Solano[1], and Iñigo Lasa[1]
[1]Laboratory of Microbial Pathogenesis. Navarrabiomed, Complejo Hospitalario de Navarra (CHN)-Universidad Pública de Navarra (UPNA), IDISNA, Pamplona-31008, Spain.
[2]Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg. Gothenburg, Sweden.

Introduction: The ability of bacteria to colonize implant surfaces and tissues as a biofilm plays a relevant role in medical-device-associated infections. *Staphylococcus aureus* strains can produce a biofilm matrix made of the poly-N-acetylglucosamine (PIA/PNAG) exopolysaccharide and/or proteins. PIA/PNAG is synthesised by enzymes encoded by the *icaADBC* operon whose expression is repressed by the transcriptional regulator IcaR, while the protein-dependent biofilm is commonly associated to fibronectin-biding proteins, FnBPA and FnBPB, encoded by *fnbA* and *fnbB* genes. The aim of this work was to identify common genetic features in the regulatory regions of biofilm-related genes among clinical *S. aureus* strains derived from periprosthetic joint infections (PJI).

Material and Methods: Genomes of 45 *S. aureus* strains from PJI were sequenced. Firstly, the sequence comprising the entire *icaADBC* regulatory region (5'UTR of *icaADBC* and *icaR*, the *icaR* coding sequence and its 3'UTR region) and secondly, the sequence of the promoter region of *fnbAB* were compared to those of *S. aureus* MW2 strain. Regulatory regions containing distinctive features were identified, fused to a reporter gene and introduced in a reference strain to analyze differences in gene expression.

Results: In the case of the *icaADBC* operon, single nucleotide polymorphisms (SNPs) in the *icaADBC* regulatory region allowed clustering of the strains in five groups from which a representative strain was chosen for further studies: *S. aureus* MIC 6924 (20% of isolates), MIC 6934 (13%), MIC 6936 (7%), MIC 6948 (2%) and MIC 7018 (4%). Of note, MICs 6948 and 7018 contained mutations in the *icaR* coding sequence. In this respect, a single nucleotide mutation in *icaR* (Val176Glu) caused a significant increase in *icaADBC* transcription and thus, in PIA/PNAG production and biofilm formation. In contrast, none of the rest of the SNPs found in the *icaADBC* regulatory region modified the transcription levels of the reporter gene. With respect to fnBPA and fnBPB genes and in agreement with previous studies, 100% of the strains contained the *fnbA* gene whereas only 69% contained the *fnbB* gene. The promoter region of *fnbA* was found to be highly conserved. SNPs in the promoter region of *fnbB* allowed clustering the strains in five groups. From these, the most frequently identified pattern was represented by *S. aureus* MIC 6948 (53%) and correlated with a lower level of reporter expression, whereas the group containing SNPs in the LexA binding sites was represented by MIC 7014 (4%) and correlated with higher expression levels.

Conclusion: Our results suggest that *S. aureus* isolates from periprosthetic joint infections do not share specific features in cis regulatory regions of *icaADBC* and *fnbB* genes that may help to predict a higher expression levels of biofilm matrix compounds.

Acknowledgement: This project has received funding from the European Union's H2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 801586.

How to cite: Morales, L., Echeverz, M., Trobos, M., Solano, C., and Lasa, I.: Diversity in regulatory regions of icaADBCR and fnbAB genes among Staphylococcus aureus strains isolated from periprosthetic joint infections, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-71, https://doi.org/10.5194/biofilms9-71, 2020

biofilms9-32
https://doi.org/10.5194/biofilms9-32
biofilms 9 conference

Antibiofilm and antivirulence effect of stilbenes on clinically relevant staphylococci

Petra Kašparová and Olga Maťátková
University of Chemistry and Technology in Prague, Faculty of Food and Biochemical Technology, Department of Biotechnology, Technická 5, 166 28, Prague 6 - Dejvice, Prague, Czechia (kasparop@vscht.cz)

Genus *Staphylococcus* comprises many greatly pathogenic species like *S. aureus*, *S. epidermidis* or *S. saprophyticus*. The great pathogenicity of stated species is often facilitated by their capability to form thick complex biofilms on various biotic or abiotic surfaces. Biofilm formation together with extracellular hydrolases or toxins represents important virulence factor, which increases persistence of staphylococci in host via enhancing their ability to evade host immune system and further promote the infection development. With an increased emergence of antibiotic resistance among pathogenic bacteria including staphylococci the search for novel antibiotic compounds with antivirulence effect is sought. Such substances might be stilbenes, phenolic compounds isolated from various plants (*Vitis* spp., *Vaccinium* spp., *Pterocarpus* spp., *Pinus* spp.). They possess strong antioxidant activity and a wide spectrum of beneficial pharmacological effects (antitumor, hypolipidemic, hypoglycemic). Apart from that, they also have great antimicrobial activity with a potent ability to enhance antibiotics action in combination.

Presented work focused on resveratrol, pterostilbene (PTE) and pinosylvine and their effect on *S. aureus* and *S. epidermidis* biofilm formation. The effect of stilbene representatives on production of other virulence factors (proteases, phospholipases, haemolysins), cell surface hydrophobicity and morphology was also observed.

PTE was found to be the most effective among studied stilbenes against *S. aureus* and *S. epidermidis* biofilm with minimum biofilm inhibitory concentrations ($MBIC_{80}$) ranging from 40 to 130 mg/l. Its effect on mature staphylococcal biofilm eradication was even greater with 80% eradication rate achieved by 40-75 mg/l. PTE (49 mg/l) was found to have a potent combinatory antibiofilm activity with erythromycin or tetracycline (5 mg/l both) causing more than 80% inhibition in metabolic activity of biofilm cells. It was able to permeabilize cytoplasmic membrane, thus probably enabling antibiotic uptake by the cell. PTE also altered cell surface hydrophobicity and production of haemolysin.

PTE might be the solution to increasing biofilm-related resistance problem and a promising candidate with antibiofilm and antivirulence potential for future antibiotic treatment of staphylococcal infections.

This work was supported by the grant of Specific university research – grant No. A2_FPBT_2020_004.

How to cite: Kašparová, P. and Maťátková, O.: Antibiofilm and antivirulence effect of stilbenes on clinically relevant staphylococci, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-32, https://doi.org/10.5194/biofilms9-32, 2020

biofilms9-20
https://doi.org/10.5194/biofilms9-20
biofilms 9 conference

Discovery and characterization of NO-responsive hemoproteins that regulate bacterial biofilms

Elizabeth Boon
Stony Brook University, Chemistry, United States of America (elizabeth.boon@stonybrook.edu)

Bacteria colonize most surfaces, forming multicellular, antibiotic-resistant, communities known as biofilms. Biofilms cause chronic infections and persistent biofouling of medical implants, marine vessels, and environmental sensors. Biofilm dispersal by nanomolar nitric oxide (NO) appears to be a general phenomenon, but fundamental questions remain concerning the identity of the NO sensor and mechanism of signal transduction. NO has been reported to disperse bacterial biofilms through regulation of intracellular cyclic-di-guanosine monophosphate concentrations. C-di-GMP is a tightly regulated second messenger-signaling molecule that is tightly correlated with biofilm formation. H-NOX proteins are well known NO sensors conserved in many bacteria. Indeed, we have shown that NO/H-NOX signaling disperses bacterial biofilms through a mechanism consistent with c-di-GMP signaling. However, H-NOX proteins are not conserved in most human pathogens. Therefore, an alternate NO sensor must also exist. We have identified a potential alternate NO sensor, a novel hemoprotein we named NosP (nitric oxide sensing protein). NosP domains are conserved in many bacterial genomes, they bind NO, but not molecular oxygen, as expected for a NO-specific sensor, and they are encoded as fusions with, or in close chromosomal proximity to, proteins annotated as c-di-GMP synthesis or hydrolysis enyzmes. We hypothesize that NO generally disperses bacterial biofilms through regulation of intracellular c-di-GMP concentrations, but the sensor varies; both NosP and H-NOX can fill this role.

How to cite: Boon, E.: Discovery and characterization of NO-responsive hemoproteins that regulate bacterial biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-20, https://doi.org/10.5194/biofilms9-20, 2020

Poster Presentations

biofilms9-40
https://doi.org/10.5194/biofilms9-40
biofilms 9 conference

CRISPRi enables studies of enterococcal biofilm initiation, maturation and maintenance.

Irina Afonina, Timothy Lu, and Kimberly Kline
Singapore-MIT Alliance for Research and Technology, Antimicrobial Resistance Interdisciplinary Research Group, Singapore (irina@smart.mit.edu)

Among the Enterococci, *Enterococcus faecalis* is most frequently associated with human infections ranging from the urinary tract and wound infection to endocarditis and bacteraemia. These infections are often multidrug-resistant and, hence, life-threatening. Moreover, *E. faecalis* are often co-isolated with other pathogenic bacteria from polymicrobial biofilm-associated infections contributing to disease progression and poorer patient outcomes. Genetic tools to dissect complex interactions in biofilms and mixed microbial communities are largely limited to transposon mutagenesis and traditional allelic exchange methods requiring time- and labour-intensive two-step integration and excision screening that can take a week or more to make a single mutant. We built upon the well-characterized CRISPR interference system using streptococcal dCas9 to develop an easily-modifiable, inducible system for *E. faecalis* that can efficiently silence single and multiple genes in a matter of hours. We show that this system can silence genes involved in biofilm formation, antibiotic resistance, and can be used to interrogate gene essentiality. Uniquely, this tool is optimized to study genes important for biofilm initiation, maturation, and maintenance, and can be used to perturb pre-formed biofilms. This inducible CRISPRi system will be valuable to rapidly and efficiently investigate a wide range of aspects of complex enterococcal regulation networks within the biofilms, including polymicrobial biofilms.

How to cite: Afonina, I., Lu, T., and Kline, K.: CRISPRi enables studies of enterococcal biofilm initiation, maturation and maintenance. , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-40, https://doi.org/10.5194/biofilms9-40, 2020

biofilms9-127
https://doi.org/10.5194/biofilms9-127
biofilms 9 conference

A SIR2 family protein impacts biofilm formation by post-translational modifications in Acinetobacter baumannii

Brandon Robin[1,2], Sebastien Massier[1,2], Anaïs Potron[3], Jean-Baptiste Vuillemenot[3], Salomé Sauvage[1,2], Thierry Jouenne[1,2], Valérie Perrot[1], Emmanuelle Dé[1,2], and Julie Hardouin[1,2]
[1]PBS Laboratory UMR 6270 CNRS, University of Rouen, F-76821 Mont-Saint-Aignan cedex, France (thierry.jouenne@univ-rouen.fr)
[2]PISSARO Proteomic Facility, IRIB, F-76820 Mont-Saint-Aignan, France
[3]French National Reference Center for Antibiotic Resistance, University Hospital of Besançon, UMR CNRS 6249 ChronoEnvironnement, Bourgogne Franche-Comté University, France

Acinetobacter baumannii is one of the most problematic opportunist pathogen responsible for many infections worldwide (1). Besides its high capacities to acquire antibiotic resistance mechanisms, it also presents high adhesion abilities on any types of abiotic or living surfaces leading to biofilm development, a mode of growth conferring an additional protection against various treatments and allowing the infection relapse (2). *A. baumannii* has been recently ranked on the global priority pathogens list established by the World Health Organization for which there is an urgent need for new treatments. One interesting way to identify new therapeutic targets to eradicate this pathogen is the characterization of its post-translational modifications (PTMs) (3). The functions and extents of PTMs remain largely unknown in prokaryotic cells compared to eukaryotic cells. Lysine acetylation is an attractive and prevalent PTM in bacteria. An increasing number of investigations have been dedicated to identify acetylated proteins by proteomics. Some studies have shown that acetylation can play a pivotal role in bacterial virulence, resistance, or biofilm (4). Enzymes involved in acetylation addition (lysine acetyltranferase KAT) or removal (lysine deacetylase KDAC) would provide a better mechanistic understanding of bacterial physiology and therefore could be considered as potential therapeutic targets. So far, little information is available on these enzymes in *A. baumannii* (5). Recently, in a global dynamic proteome study of *A. baumannii* ATCC 17978 strain grown in sessile mode, we highlighted the highest protein fold change for a protein belonging to the Sir2-like family which may possess a KDAC activity (6). The aim of the current study was to evaluate the involvement of this protein in *A. baumannii* physiology. For this purpose, a gene deletion approach was carried out to perform different phenotype tests (drugs and oxidative stress resistance, virulence assays, motility and biofilm formation) on wild-type and mutant strains. We compared, in biofilm mode of growth, acetylomes of the WT and the mutant. Our results demonstrated more than twice acetylated proteins in mutant in comparison to the WT. Of interest, biofilm formation in mutant was sensibly decreased. These different results suggest a potential involvement of this protein in *A. baumannii* biofilm formation.

(1) Antunes et al. Acinetobacter baumannii: evolution of a global pathogen. Pathog. Dis. 71(2014), 292-301.

(2) Espinal et al. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. J. Hosp. Infect. 80(2012), 56–60.

(3) Richters. Targeting protein arginine methyltransferase 5 in disease. Future Med. Chem. 9(2017), 2081-2098.

(4) VanDrisse and Escalante-Semerena. Protein acetylation in bacteria. Annu. Rev. Microbiol. 73(2019), 111-132.

(5) Carabetta and Cristea. Regulation, function, and detection of protein acetylation in bacteria. J. Bacteriol. 199(2017), e00107-17.

(6) Kentache et al. Global dynamic proteome study of a pellicle-forming Acinetobacter baumanii strain. Mol. Cell. Proteomics. 16(2017), 100-112.

How to cite: Robin, B., Massier, S., Potron, A., Vuillemenot, J.-B., Sauvage, S., Jouenne, T., Perrot, V., Dé, E., and Hardouin, J.: A SIR2 family protein impacts biofilm formation by post-translational modifications in Acinetobacter baumannii, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-127, https://doi.org/10.5194/biofilms9-127, 2020

biofilms9-147
https://doi.org/10.5194/biofilms9-147
biofilms 9 conference

Antibiofilm activity of Human Milk Oligosaccharides against pathogens isolated from cystic fibrosis patients

Sylwia Jarzynka[1], Kamila Strom[1], Oliwia Makarewicz[2], Anna Minkiewicz-Zochniak[1], Anna Koryszewska-Baginska[1], and Gabriela Oledzka[1]
[1]Medical University of Warsaw, Department of Medical Biology, Poland
[2]Institute for Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany

Background: Human milk oligosaccharides (HMOs) are the third most abundant component of breast milk, after fat and lactose, that promote infant health. Recent studies have shown that HMOs demonstrated antimicrobial and antibiofilm activity against different strains. Cystic fibrosis (CF), it is one of the major respiratory diseases, the clinical management and definitive treatment of CF biofilm-mediated chronic bacterial lung infection remains a challenge.

Objective: In this study, we examine HMOs antibiofilm activity against pathogens isolated from CF patients.

Methods and results: In current work, we investigated the antibiofilm activity of the saccharide fraction obtained from pooled human milk of 9 donors against strains of: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Staphylococcus aureus* and *Burkholderia cenocepacia*, an intrinsically multi-resistant pathogen associated with high mortality in CF patients. We tested the ability of HMOs to inhibit biofilm formation and to eradicate matured biofilms. Live/dead staining of the biofilms and CLSM image acquisition were used.

The pooled HMOs showed a biofilm eradicating effect on most tested pathogens. The HMOs effectively killed the bacteria at high concentration (20 mg/ml, corresponds to the concentration in human milk), but visible reduction of viable bacteria and biofilm mass was observed already at lower concentrations that varied between the species. The biofilm mass was also reduced in almost all pathogenic biofilms.

The data presented in this paper supporting the importance and potential inhibitory effect of HMOs in biofilm formation. HMOs could potentially be used as novel therapeutics to treat or prevent infectious disease in patient with CF.

How to cite: Jarzynka, S., Strom, K., Makarewicz, O., Minkiewicz-Zochniak, A., Koryszewska-Baginska, A., and Oledzka, G.: Antibiofilm activity of Human Milk Oligosaccharides against pathogens isolated from cystic fibrosis patients, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-147, https://doi.org/10.5194/biofilms9-147, 2020

biofilms9-22
https://doi.org/10.5194/biofilms9-22
biofilms 9 conference

Bacteria and biofilms under the influence of shear

Christine Müller-Renno[1], Jürgen Seehase[1], Katharina Huttenlochner[1], Jonas Chodorski[2], Roland Ulber[2], and Christiane Ziegler[1]
[1]TU Kaiserslautern, Department of Physics, Germany (cmueller@physik.uni-kl.de)
[2]Institute of Bioprocess Engineering, University of Kaiserslautern, Germany

In our attempts to improve the biofilm formation for productive bacteria (here the gram-negative seawater bacterium Paracoccus seriniphilus), we focus on the attachment of single bacteria to a solid surface as the first step of the biofilm formation process. Beside adhesion forces and elasticity of the bacteria, we investigate the minimal detachment forces due to lateral shear forces.

In order to investigate the influence of shear forces on already adhered bacteria in the laboratory, the Lateral Force Microscopy(LFM) was used first.The tip is moved laterally towards the adherent cell with different lateral forces until the cell detaches and thus the force required to shear the cell is determined.

By applying LFM, we found a correlation between the applied force and the number of moved bacteria as well as between the number of detached bacteria and the surface energy of the substrate. Further, any structuring of the substrate hinders the detachment substantially [1]. In agreement with the vertical adhesion forces, the bacteria are harder to detach at pH 4 than at pH 7.

In order to get closer to reality, the next step is to examine the (lateral) scanning force microscopic measurements under the influence of a flowing liquid and compare them with the LFM measurements. In combination with digital holography and proteome analysis, a better understanding of biofilm formation under the influence of a flowing liquid is to be achieved.

[1] K. Huttenlochner, N. Davoudi, C. Schlegel, M. Bohley, C. Müller-Renno, J. C. Aurich, R. Ulber, and C. Ziegle "Paracoccus seriniphilus adhered on surfaces: Resistance of a seawater bacterium against shear forces under the influence of roughness, surface energy, and zeta potential of the surfaces." Biointerphases 13.5 (2018)

How to cite: Müller-Renno, C., Seehase, J., Huttenlochner, K., Chodorski, J., Ulber, R., and Ziegler, C.: Bacteria and biofilms under the influence of shear, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-22, https://doi.org/10.5194/biofilms9-22, 2020

biofilms9-138
https://doi.org/10.5194/biofilms9-138
biofilms 9 conference

Better treatment options through a better understanding of Pseudomonas aeruginosa biofilm formation and biofilm-mediated resistance

Jules Valentin[1,2], Adithi R. Varadarajan[3], Christian H. Ahrens[3], Henny van der Mei[2], and Qun Ren[1]
[1]Empa, Laboratory for Biointerfaces, St Gallen, Switzerland (Qun.Ren@empa.ch)
[2]University of Groningen and University Medical Center Groningen, Department of Biomedical Engineering, Groningen, Netherlands (h.c.van.der.mei@umcg.nl)
[3]Agroscope, Research Group Molecular Diagnostics, Genomics & Bioinformatics and SIB Swiss Institute of Bioinformatics, Wädenswil, Switzerland (christian.ahrens@agroscope.admin.ch)

Bacteria living in biofilms tolerate much higher antibiotic concentrations compared to planktonic bacteria and can cause chronic infections. Among the most difficult pathogens to treat, *Pseudomonas aeruginosa* is responsible for many biofilm-related infections and for much of the mortality associated with airway infections in cystic fibrosis. We speculated that there are specific genes responsible for increased antibiotic resistance in biofilms and aimed to identify them in *P. aeruginosa*. By doing so, a better understanding of biofilm-mediated resistance can be achieved and new bacterial targets can be identified. A *P. aeruginosa* transposon mutant library was screened to assess the impact on biofilm formation and the biofilm resistance toward antibiotics. Briefly, the biofilm resistance was estimated by following the re-growth of biofilm cells exposed to different concentrations of antibiotics. A few candidates, e. g. the response regulator CbrB, involved in nutrient uptake, have been identified as crucial for biofilm formation and resistance towards antibiotics. Further characterization of these interesting genes has been carried out to explore the underlying mechanism of resistance. Such knowledge can lead to the identification of susceptibility of *P. aeruginosa* biofilm and help to develop tools to treat persistent infections.

How to cite: Valentin, J., Varadarajan, A. R., Ahrens, C. H., van der Mei, H., and Ren, Q.: Better treatment options through a better understanding of Pseudomonas aeruginosa biofilm formation and biofilm-mediated resistance, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-138, https://doi.org/10.5194/biofilms9-138, 2020

biofilms9-105
https://doi.org/10.5194/biofilms9-105
biofilms 9 conference

CRISPR interference knockdown screen identifies novel proteins involved in formation of structured macrocolonies in Staphylococcus aureus.

Morten Kjos[1], Danae Morales Angeles[1], Marita Torrissen Mårli[1], Maria Victoria Heggenhougen[1], Vincent de Bakker[2], Xue Liu[2], and Jan-Willem Veening[2]
[1]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Norway (morten.kjos@nmbu.no)
[2]Department of Fundamental Microbiology, University of Lausanne, Switzerland

Staphylococcus aureus biofilms play important roles during infection. The main components of these biofilms are well studied; however, we lack the full understanding of factors and genes involved in regulation of biofilm formation. To screen for essential and non-essential biofilm regulatory genes in *S. aureus*, we have created a pooled inducible CRISPR interference library. The pooled library is designed to allow knockdown of every transcriptional unit in the *S. aureus* genome, thus targeting both essential and non-essential genes. We used our library in *S. aureus* Newman, a strain which forms structured macrocolonies on agar plates. We performed an unbiased screen of 1500 macrocolonies and found 10 macrocolonies with stably altered structures. The genotypes of these macrocolonies were determined by sequencing the single guide RNAs of the CRISPR interference system. As a proof of the validity of the approach, we identified several genes previously reported to be implicated in biofilm and macrocolony formation, including *ica*-genes, and metabolic genes of the TCA-cycle and gluconeogenesis. In addition, three new genes (two encoding putative enzymes and one hypothetical genes) whose depletion resulted in completely altered macrocolonies were also identified. The molecular mechanisms explaining the roles of these proteins in biofilm formation are currently under investigation.

How to cite: Kjos, M., Morales Angeles, D., Mårli, M. T., Heggenhougen, M. V., de Bakker, V., Liu, X., and Veening, J.-W.: CRISPR interference knockdown screen identifies novel proteins involved in formation of structured macrocolonies in Staphylococcus aureus., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-105, https://doi.org/10.5194/biofilms9-105, 2020

biofilms9-130
https://doi.org/10.5194/biofilms9-130
biofilms 9 conference

Cellular L-arginine pools modulate c-di-GMP turnover and biofilm formation in Pseudomonas putida

Laura Barrientos-Moreno[1,2], María Antonia Molina-Henares[1], María Isabel Ramos-González[1], and **Manuel Espinosa-Urgel**[1]

[1]Estacion Experimental del Zaidin, CSIC, Granada Spain (manuel.espinosa@eez.csic.es)

[2]School of Life Sciences, Nottingham Biodiscovery Institute, University Park, University of Nottingham, Nottingham, UK

The intracellular second messenger cyclic diguanylate (c-di-GMP) is broadly conserved in bacteria, where it influences processes such as virulence, stress resistance and biofilm development. In the plant-beneficial bacterium *Pseudomonas putida* KT2440, the response regulator with diguanylate cyclase activity CfcR is the main contributor to c-di-GMP levels in the stationary phase of growth. When overexpressed, CfcR increases c-di-GMP levels and gives rise to a pleiotropic phenotype that includes enhanced biofilm formation and crinkly colony morphology. Our group has previously reported that insertion mutants in *argG* and *argH*, the genes that encode the last two enzymes in the arginine biosynthesis pathway, do not display the crinkly colony morphology phenotype and show decreased c-di-GMP levels even in the presence of *cfcR* in multicopy (Ramos-González, M.I. *et al.* 2016. Front. Microbiol. 7, 1093). Here we present results indicating that L-arginine acts both as an environmental and as a metabolic signal that influences the lifestyles of *P. putida* through the modulation of c-di-GMP levels and changes in the expression of structural elements of biofilms. Exogenous L-arginine partially restores c-di-GMP levels in arginine biosynthesis mutants, a response that is transduced through CfcR and possibly (an)other diguanylate cyclase(s). At least three periplasmic binding proteins, each forming part of an amino acid transport system, contribute in different ways to the response to external L-arginine. We propose that the turnover of the second messenger c-di-GMP is modulated by the state of global arginine pools in the cell resulting both from anabolism and from uptake.

How to cite: Barrientos-Moreno, L., Molina-Henares, M. A., Ramos-González, M. I., and Espinosa-Urgel, M.: Cellular L-arginine pools modulate c-di-GMP turnover and biofilm formation in Pseudomonas putida, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-130, https://doi.org/10.5194/biofilms9-130, 2020

biofilms9-108
https://doi.org/10.5194/biofilms9-108
biofilms 9 conference

Cellular and environmental factors influencing biofilm formation and colonization of plant tissue by a beneficial strain of bacteria, Pseudomonas donghuensis P482.

Magdalena Rajewska, Marta Matuszewska, and Sylwia Jafra
University of Gdańsk, Intercollegiate Faculty of Biotechnology UG&MUG, Laboratory of Biological Plant Protection, Poland
(magdalena.rajewska@biotech.ug.edu.pl)

The ability to colonize different environmental niches by bacteria is most often determined by the ability to form biofilms - complex, multicellular communities. This, in turn, depends on both cellular and extracellular factors such as genetic background of the strain, type of surface (biotic or abiotic) to which bacteria attach, availability of nutrients, temperature, *etc*. *Pseudomonas donghuensis* P482 strain is a little-known isolate from tomato rhizosphere, exhibiting antimicrobial activity towards bacterial and fungal plant pathogens. Studies have shown that it efficiently colonizes plant rhizosphere and forms biofilm on artificial surfaces. Which genetic or environmental factors underlie the mechanism of biofilm formation were yet to be elucidated. The presented research aimed at identifying those factors. Basing on the analysis of genome, knock-out mutants of the P482 strain were constructed in the genes potentially involved in biofilm formation and further analyzed for motility, colony morphology, attachment to artificial surfaces in different culture conditions, and colonization of maize and tomato rhizosphere.

How to cite: Rajewska, M., Matuszewska, M., and Jafra, S.: Cellular and environmental factors influencing biofilm formation and colonization of plant tissue by a beneficial strain of bacteria, Pseudomonas donghuensis P482., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-108, https://doi.org/10.5194/biofilms9-108, 2020

biofilms9-133
https://doi.org/10.5194/biofilms9-133
biofilms 9 conference

Evaluation of mesoporous silica nanoparticles-based nanoantibiotics and capsaicin on E. coli and S. aureus biofilms

Anna Slita[1], Prakirth Govardhanam[1], Ida Opstad[2], Didem Sen Karaman[1,3], and Jessica Rosenholm[1]
[1]Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi, Turku, Finland(aslita@abo.fi)
[2]The Arctic University of Norway, Tromsø, Norway
[3]İzmir KatipCelebiUniversity, Faculty of Engineering and Architecture, Department of Biomedical Engineering, İzmir, Turkey

Introduction

Since antibiotics were discovered, bacteria have demonstrated the ability to develop resistance by many different mechanisms. According to WHO reports from 2014, there has been an alarming increase in the antibiotic resistant bacterial strains in most parts of the world[1]. Our previous results showed that a nanoantibiotic (NAB) design created in our laboratory[2], composed of a cerium oxide core, mesoporous silica shell loaded with capsaicin, and a chitosan coating, are effective against planktonic E. coli. However, most of the pathogenic bacteria form biofilms during infections. That is why the next stage of studying NAB is to determine whether they are effective against biofilms of different species. Moreover, the results of NAB efficiency against planktonic E. coli did not clearly show the contribution of the antibiotic drug component of NAB – capsaicin. Hence, the first step of the current study is to determine whether and to what degree, mesoporous silica nanoparticles (MSN) – serving as NAB model in this case - penetrate biofilms as a function of particle shape and surface coating; as well as finding the efficient concentration of capsaicin against E. coli and S. aureus to optimize the NAB dosing against biofilms.

Aim

To check in vitro penetration of MSN on S. aureus biofilm and antibacterial activity of NAB and pure capsaicin on E. coli and S. aureus biofilms.

Methods

To investigate NAB efficiency on biofilms MBEC-high-throughput assay[3] was performed. Equal biofilms formed on peg-lids were incubated with different concentrations of NAB and capsaicin. After different time point biofilms were sonicated and plated on agar plated to perform CFU counting. To determine the efficient concentration of capsaicin, biofilms were formed in 12 well plates and then incubated with different concentrations of capsaicin. To visualize inhibitory effect, plating for CFU counting and Resazurin assay were applied. To evaluate the penetration of particles, labeled and non-labeled particles were added to fully grown St. aureus biofilms, incubated and visualized with confocal microscopy and structured illumination microscopy.

Results

Conclusion

References

How to cite: Slita, A., Govardhanam, P., Opstad, I., Sen Karaman, D., and Rosenholm, J.: Evaluation of mesoporous silica nanoparticles-based nanoantibiotics and capsaicin on E. coli and S. aureus biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-133, https://doi.org/10.5194/biofilms9-133, 2020

biofilms9-12
https://doi.org/10.5194/biofilms9-12
biofilms 9 conference

Functionalising antibiotics with nitroxides as an effective broad-spectrum biofilm eradication strategy.

Anthony Verderosa[1], Kathryn Fairfull-Smith[2], and Makrina Totsika[1]
[1]Queensland University of Technology, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Australia
[2]Queensland University of Technology, School of Chemistry, Physics and Mechanical Engineering, Australia

Background:

The adhesion of planktonic bacteria to a surface (biotic or abiotic), and their subsequent ability to aggregate into multicellular communities called biofilms, is a major driving force of failing antibiotic therapy and persistence in chronic infections caused by a variety of pathogens (e.g., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus)* and plaguing healthcare systems worldwide. Biofilms are estimated to be involved in over 80% of all microbial infections in humans, and commonly exhibit extreme resistance to conventional antimicrobial treatments. Consequently, there is an urgent need for novel antimicrobial agents, which target biofilm residing cells. Here, we present the development and evaluation of a new generation of dual-acting nitroxide functionalised antibiotics with potent biofilm eradication activity.

Methodology:

Synthetic organic chemistry was utilised to produce a new generation of nitroxide functionalised antibiotics with targeted biofilm eradication capabilities. These compounds were tested for biofilm eradication and/or dispersal of several bacterial species using the MBEC^{TM} device, a reproducible high-throughput static biofilm formation system. Mature biofilms were treated with serial dilutions of the specific test agent(s) and recovered bacterial numbers were quantified by absorbance spectroscopy at 600 nm or plating for viable cell counts. Treated biofilms were also stained with Live/Dead (SYTO-9/PI) bacterial viability kit and analysed by fluorescence and confocal laser scanning microscopy.

Results:

Nitroxide functionalised antibiotics exhibit potent biofilm-eradication activity against a variety of medically important pathogens, including *P. aeruginosa*, uropathogenic *E. coli*, and *S. aureus*. In Minimal Biofilm Eradication Concentration (MBEC) assays nitroxide functionalised antibiotics were 64-fold more potent against *S. aureus* biofilms, and at least 2-fold more potent against uropathogenic *E. coli* biofilms than the parent antibiotic ciprofloxacin.

Conclusions:

Currently, antibiotics are often entirely ineffective against biofilm infections. Nitroxide functionalised antibiotics represent a promising new strategy, which could circumvent the resistance of Gram-positive and Gram-negative biofilms to conventional treatments.

How to cite: Verderosa, A., Fairfull-Smith, K., and Totsika, M.: Functionalising antibiotics with nitroxides as an effective broad-spectrum biofilm eradication strategy. , biofilms 9 conference, 29 September–1 Oct 2020, biofilms9-12, https://doi.org/10.5194/biofilms9-12, 2020

biofilms9-119
https://doi.org/10.5194/biofilms9-119
biofilms 9 conference

Identifying biofilm regulators as novel targets for antimicrobial drug design

Melanie Dostert, Corrie R Belanger, Travis M Blimkie, Reza Falsafi, Bhavjinder K Dhillon, Amy HY Lee, and Robert EW Hancock
University of British Columbia, Microbiology and Immunology, Canada (melanie@hancocklab.com)

Antibiotic treatment regularly fails to cure patients suffering from infections caused by adaptively resistant microbial communities, referred to as biofilms. Even though at least two thirds of all clinical infections are associated with biofilms, there are no biofilm-specific therapies on the market or in clinical trials. *Pseudomonas aeruginosa* is a remarkably antibiotic resistant, nosocomial pathogen and biofilm-former that causes morbidity and mortality especially in cystic fibrosis and immunocompromised patients. This project aims to identify regulatory genes associated with drug resistance in *P. aeruginosa* biofilms to provide novel biofilm-specific targets for the design of potent drugs. A genome-wide screen of *P. aeruginosa* burn wound isolate UCBPP-PA14 identified 362 genes involved in biofilm formation, including dozens of regulatory and hypothetical genes. I will discuss regulatory as well as metabolic genes corresponding to the known resistome of antimicrobials.

How to cite: Dostert, M., Belanger, C. R., Blimkie, T. M., Falsafi, R., Dhillon, B. K., Lee, A. H., and Hancock, R. E.: Identifying biofilm regulators as novel targets for antimicrobial drug design, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-119, https://doi.org/10.5194/biofilms9-119, 2020

biofilms9-107
https://doi.org/10.5194/biofilms9-107
biofilms 9 conference

Impact of estradiol on biofilm formation of Pseudomonas aeruginosa clinical isolates from cystic fibrosis patients

Faria Afzal[1], Tony Le Gall[2], Tristan Montier[2], and **Mareike Müller**[1]
[1]Physical Chemistry I & Research Center of Micro and Nanochemistry and Engineering (Cμ), Department of Chemistry and Biology, University of Siegen, Siegen, Germany (m.mueller@chemie-bio.uni-siegen.de)
[2]"Gene Transfer and Gene Therapy" Team, INSERM UMR 1078; IBSAM; Laboratoire de Génétique Moléculaire et Histocompatibilité, CHRU Brest; UFR Médecine et Sciences de la Santé, Brest, France

Women with Cystic Fibrosis (CF) have a significantly lower life expectancy compared to men, which is indicated by an earlier impairment of lung function due to chronic colonization of pathogenic bacteria like *Pseudomonas aeruginosa* (PA). Reasons for this "CF gender gap" until now have not yet been fully clarified and are assumed to be multifactorial.

This study aims to shed light on the contribution of sex hormones to the CF-Gender gap considering microbial endocrinology. Therefor the study investigates whether the sex hormone estradiol, whose blood serum concentrations are significantly fluctuating within the female cycle and during pregnancy, has a regulatory influence on the development of PA biofilms in the context of CF.

For that purpose, biofilms of PA isolates from CF-patients are treated *in vitro* with various estradiol concentrations and are examined in a comparative study quantitatively regarding the total biomass, *e.g.* via crystal violet staining, and qualitatively, *e.g.* via scanning electron microscopy, to characterize the ultrastructure of the biofilm.

We observed that estradiol induces biofilm-forming capacity of CF-PA-isolates with respect to the total biomass and modulates the biofilm structure especially concerning the distribution and clustering of bacteria.

The observed *in vitro* correlation between estradiol concentration and extent of biofilm growth provides a possible microbiological explanation for gender differences in CF disease progression.

These insights and further research on possible underlying mechanisms might be relevant in the long-term for new approaches in personalized treatment for female CF patients.

Acknowledgement

This work is supported by a financial grant from Mukoviszidose Institut gGmbH, Bonn, the research and development arm of the German Cystic Fibrosis Association Mukoviszidose e.V., the Christiane Herzog foundation. We further thank the Equal Opportunities Office of the University Siegen as well as the DAAD PPP Frankreich (Project-ID 55976814) for financial support.

How to cite: Afzal, F., Le Gall, T., Montier, T., and Müller, M.: Impact of estradiol on biofilm formation of Pseudomonas aeruginosa clinical isolates from cystic fibrosis patients, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-107, https://doi.org/10.5194/biofilms9-107, 2020

biofilms9-38
https://doi.org/10.5194/biofilms9-38
biofilms 9 conference

Peptides' involvement in Pseudomonas putida biofilm formation

Riho Teras[1], Hanna Ainelo[2], and Marge Puhm[1]
[1]University of Tartu, Institute of Molecular and Cell Biology, Chair of Genetics, Estonia (riho.teras@ut.ee)
[2]Université libre de Bruxelles, Post-Doc

Pseudomonas putida rapidly forms a biofilm, after which its biomass usually disperses to half its initial amount. We have observed different biofilm dynamics of P. putida in a complex medium LB and a minimal medium M9+glc+CAA and inquired about the importance of extracellular factors for the formation of P. putida biofilm.

The proteinaceous component of LB increases the biomass of P. putida biofilm. Supplementation of M9 with tryptone but not CAA increased the biofilm biomass. Proteinase K treatment of LB medium reduced the biomass of P. putida biofilm. At the same time, growth rate or maximum OD of planktic bacteria in used media did not correlate with biofilm biomass of the same media. Thus, peptides appeared to have a positive effect on the biofilm as an extracellular factor and not as a source of C and N.

We replaced tryptone in M9 medium with positively charged poly-L-lysine (MW. 1000-5000 Da), negatively charged poly-L-glutaminic acid (MW. 1500-5500 Da) or neutral poly-LD-alanine (MW. 3000-7000). Poly-lysine and poly-glutamic acid had a slight positive effect on the biomass of P. putida wild type strain PSm biofilm and poly-alanine did not affect the biofilm.

We have previously shown that overexpression of fis in P. putida strain F15 increases biofilm biomass by increasing the lapA expression, the main adhesin gene of biofilm. Using media similar to that used for the wild-type strain for strain F15, we ascertained that only poly-lysine out of these three polypeptides restored the positive effect of fis-overexpression on the biofilm biomass. At the same time, the positive impact of fis-overexpression was absent in lapA deletion mutant strain, but not in lapF deletion mutant strain.

In conclusion, the formation of P. putida biofilm depends on polypeptides in the environment. The enhancing effect of positively charged polypeptides appears to be evident in the presence of LapA, a key factor for P. putida biofilm.

How to cite: Teras, R., Ainelo, H., and Puhm, M.: Peptides' involvement in Pseudomonas putida biofilm formation, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-38, https://doi.org/10.5194/biofilms9-38, 2020

biofilms9-89, updated on 22 Sep 2020
https://doi.org/10.5194/biofilms9-89
biofilms 9 conference

Phosphodiesterase activity of NbdA from *Pseudomonas aeruginosa*

Anna Scherhag, Martina Rüger, Katrin Gerbracht, Jaqueline Rehner, Susanne Zehner, and Nicole Frankenberg-Dinkel
TU Kaiserslautern, Biology, Microbiology, Germany (ascherha@bio.uni-kl.de)

The molecule c-di-GMP is a bacterial second messenger that controls various processes such as motility or biofilm formation in bacteria [1]. To synthesize and degrade c-di-GMP, enzymes called diguanylate cyclases (DGC) containing a GGDEF-domain and phosphodiesterases (PDE) containing an EAL-domain or HD-GYP-domain are important [1, 2]. *Pseudomonas aeruginosa*, a model organism for biofilm formation and dispersion, encodes for 18 GGDEF, 5 EAL, 16 GGDEF / EAL, and 3 HD-GYP-domain-containing proteins [3].
One of the GGDEF / EAL-containing proteins is NbdA. This protein also harbors an N-terminal membrane anchored MHYT-domain, that is predicted to be a sensor for NO, CO or O_2 [4]. In this work, recombinant and affinity purified NbdA was tested for its PDE activity. Three different methods were used to measure the PDE activity of NbdA: a bis-pNPP-assay in which the conversion of the pseudosubstrate bis-pNPP into p-nitrophenol was detected spectroscopically, an HPLC-analysis of an enzymatic assay with the native substrate c-di-GMP, and a MANT-c-di-GMP-assay in which a fluorescently labeled form of the presumed substrate c-di-GMP was utilized.
To establish these methods, the two known phosphodiesterases, PdeH from *Escherichia coli* [5] and RocR from *P. aeruginosa* [6], were also produced and tested. Subsequently, three variants of NbdA were investigated: the full-length version and two truncated versions of the protein. Activity was further assessed using functional complementation of an *E. coli* phosphodiesterase deficient strain with full-length and truncated NbdA variants confirming PDE activity *in vivo*.

[1] Hengge, R. (2009) Nature Rev. Microbiol. 7: 263-273.

[2] Römling, U., Gomelsky, M., Galperin, M.Y. (2005). Mol. Microbiol. 57: 629–639.

[3] Valentini, M., Filloux, A. (2016). J. Biol. Chem. 291: 12547–12555.

[4] Galperin, M.Y., Gaidenko, T.A., Mulkidjanian, A.Y., Nakano, M., und Price, C.W. (2001). FEMS Microbiol. Lett. 205, 17–23.

[5] Pesavento, C., Becker, G., Sommerfeldt, N., Possling, A., Tschowri, N., Mehlis, A., Hengge, R. (2008). Genes Dev. 22: 2434–2446.

[6] Chen et al. (2012) Chen, M.W., Kotaka, M., Vonrhein, C., Bricogne, G., Rao, F., Chuah, M.L.C., Svergun, D., Schneider, G., Liang, Z.-X., Lescar, J. (2012). Signaling. J. Bacteriol. 194: 4837–4846

How to cite: Scherhag, A., Rüger, M., Gerbracht, K., Rehner, J., Zehner, S., and Frankenberg-Dinkel, N.: Phosphodiesterase activity of NbdA from Pseudomonas aeruginosa, biofilms 9

conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-89, https://doi.org/10.5194/biofilms9-89, 2020

biofilms9-122
https://doi.org/10.5194/biofilms9-122
biofilms 9 conference

The SagS sensory protein modulates biofilm formation and c-di-GMP levels by Pseudomonas aeruginosa in response to glucose-6-phosphate.

Soyoung Park[1,2], Jozef Dingemans[1,2], Madison Gowett[1,2], and Karin Sauer[1,2]
[1]Department of Biological Sciences, Binghamton University, Binghamton, New York, USA
[2]Binghamton Biofilm Research Center, Binghamton University, Binghamton, New York, USA

In *Pseudomonas aeruginosa*, the orphan two-component sensor SagS contributes to both, the transition to biofilm formation and to biofilm cells gaining their heightened tolerance to antimicrobials. However, little is known about the identity of the signals or conditions sensed by SagS to induce the switch to the sessile, drug tolerant mode of growth. Using a modified Biolog phenotype assay to screen for compounds that modulate attachment in a SagS-dependent manner, we identified glucose-6-phosphate to enhance attachment in a manner dependent on the glucose-6-phosphate concentration and SagS. The stimulatory effect was not limited to the attachment as glucose-6-phosphate likewise enhanced biofilm formation. We show that exposure to glucose-6-phosphate results in decreased swarming motility but increased cellular c-di-GMP levels in biofilms. Genetic analysis indicated that the diguanylate cyclase NicD is an activator of biofilm formation and is not only required for enhanced biofilm formation in response to glucose-6-phosphate but also interacts with SagS. Our findings indicate glucose-6-phosphate to likely mimic a signal or conditions sensed by SagS to activate its motile-sessile switch function. Additionally, our findings provide new insight into the interfaces between the ligand-mediated TCS signaling pathway and c-di-GMP levels.

How to cite: Park, S., Dingemans, J., Gowett, M., and Sauer, K.: The SagS sensory protein modulates biofilm formation and c-di-GMP levels by Pseudomonas aeruginosa in response to glucose-6-phosphate., biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-122, https://doi.org/10.5194/biofilms9-122, 2020

biofilms9-118
https://doi.org/10.5194/biofilms9-118
biofilms 9 conference

The bacterial lifecycle in cotton and polyester textiles

Andreas Moellebjerg[1,2] and Rikke Meyer[1]
[1]Aarhus University, Science and Technology, Interdisciplinary Nanoscience Center, Denmark (andreasmoellebjerg@inano.au.dk)
[2]Novozymes A/S

Colonization of textiles and subsequent metabolic degradation of sweat and sebum components by axillary skin bacteria cause the characteristic sweat malodor and discoloring of dirty clothes. Once inside the textile, the bacteria can form biofilms that are hard to remove by conventional washing. When the biofilm persists after washing, the textiles retain the sweat odor. In addition to posing a huge industrial problem, textile biofilms constitute an interesting case study of bacterial behavior in periodically wetted and dried substrates with varying surface hydrophobicity. Here we aim to study the bacterial behavior in each of the four stages of the bacterial lifecycle in textiles: adhesion, growth, drying and washing. To accomplish this, we designed a novel in vitro model to mimic physiological sweating while wearing cotton and polyester textiles. The hydrophobic polyester adhered bacteria more strongly and absorbed more sebum, the bacteria's primary nutrient source. Bacteria were therefore initially more active in polyester textiles than in cotton. However, polyester did not bind water as well as cotton. The increased water content of cotton allowed the bacteria to retain a higher activity after the textile had dried. However, neither of the textiles retained enough water upon drying to prevent the bacteria from irreversibly adhering to the textile fibers by capillary action. This demonstrates that bacterial colonization depends on the hydrophobic and hygroscopic properties of the colonized material while highlighting the possibility of controlling bacterial behavior by either changing the surface properties or the surrounding environment.

How to cite: Moellebjerg, A. and Meyer, R.: The bacterial lifecycle in cotton and polyester textiles, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-118, https://doi.org/10.5194/biofilms9-118, 2020

biofilms9-63
https://doi.org/10.5194/biofilms9-63
biofilms 9 conference

The phycobiota-derived proteins Pμ84 and Pμ19 suppress bacterial biofilm formation in gram-negative pathogens

Yuchen Han, Wolfgang R. Streit, and Ines Krohn
University of Hamburg, Department of Microbiology & Biotechnology, Germany (yuchen.han@uni-hamburg.de)

Microalgae are typically found in freshwater and marine systems and they harbor a mostly a beneficial growth promoting microbiota. We have recently isolated several small proteins from the microbiomes of microalga (*Scenedesmus quadricauda*, *Microasterias crux-melintensis*, *Chlorella saccherophilia*) and have tested them for their role in either inhibition of biofilm formation and/or biofilm degradation. Thereby we have identified two candidate proteins that showed promising activities on biofilm inhibition and degradation. These proteins were designated Pμ84 and Pμ19 and strongly affected biofilm formation in several human- and plant-pathogenic bacteria. Recombinant and purified Pμ84 and Pμ19 were applied in biofilm assays in microtiter plates and reduced biofilms formed by *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. If expressed in the different hosts, biofilms were reduced by a factor of 40% and if applied as exogenous proteins, biofilms were reduced up to 20%. Pμ84 application also resulted in a delayed biofilm formation and biofilm formation was affected by a factor of 17%. The microprotein Pμ19 consist of 57 aa and Pμ84 consists of 49 aa. Ongoing work elucidates the mechanism of Pμ84 and Pμ19 on the reduction of biofilm in order to achieve the optimal activity.

How to cite: Han, Y., Streit, W. R., and Krohn, I.: The phycobiota-derived proteins Pμ84 and Pμ19 suppress bacterial biofilm formation in gram-negative pathogens , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-63, https://doi.org/10.5194/biofilms9-63, 2020

Topic 6
Synthetic, Artificial Biofilm Development and its Optimisation

The synthetic engineering of biofilms can lead to processes with increased productivities, biofilm matrices with better properties or the tailor-made interaction of microorganisms. Meanwhile, we have molecular tools to engineer the synthetic interaction of organisms by designing the share of labour or by precipitating organisms with each other. Moreover, bioprinting gives us the possibility to print organisms in defined mixtures and densities and with specifically adapted inks. Nevertheless, the question on the long-term applicability of synthetic biofilms or organisms in synthetic matrices has not been conclusively answered yet. At biofilms 9, we hope to discuss results and maybe answer fundamental questions with respect to the synthetic development of biofilms like how can we achieve long-term interactions in biofilms, what defines an advanced synthetic biofilm matrix, or what are the benefits that we can gain by the synthetic development of tailored biofilm communities and structures.

Oral Presentations

biofilms9-128
https://doi.org/10.5194/biofilms9-128
biofilms 9 conference

Synthetic gene circuits for programmable Pseudomonas catalytic biofilms

Daniel Christoph Volke[1], Ingeborg Heuschkel[2], Katja Bühler[2], and Pablo Iván Nikel[1]
[1]DTU Denmarks Technical University, Biosustain, Systems Environmental Microbiology, Germany (chdavo@biosustain.dtu.dk)
[2]Department Solar Materials, Helmholtz Centre for Environmental Research - UFZ

Nowadays, industrial fermentations rely almost entirely on the use of planktonic cells. However, biofilms (the most common form of bacterial growth in nature), offer several advantages to be exploited in modern fermentation processes. Bacteria in biofilms are more tolerant to several stresses than free cells, including toxic chemicals and shear stress. Furthermore, the adhesion of cells to surfaces can be exploited to operate a continuous fermentation process without excessive loss of biomass, thereby facilitating downstream processing. A programmable switch between planktonic and biofilm lifestyle is desirable to harness the advantages of both lifestyles. On this premise, we constructed a genetic gene circuit for biofilm formation in the platform strains *Pseudomonas putida* and *Pseudomonas taiwanensis*. Both *P. putida* and *P. taiwanensis* are robust, non-pathogenic soil bacteria and promising chassis for biotechnology as they can thrive under harsh operating conditions, displaying high tolerance towards several chemicals and can metabolize a broad range of substrates. These characteristics make them ideal for the production of a wide spectrum of chemicals. The synthetic circuit initiates biofilm formation upon detection of substrate or intermediate metabolites of the desired biotransformation, thus no additional inducer is needed. The circuit also allows for the propagation of cells in planktonic state prior employment in the bioreactor, which facilitates handling and speed up expansion of the culture. The design proposed herein employs a feedback-resistant diguanylate cyclase (DGC) from *Caulobacter crescentus*, which increases the concentration of DGC and therefore triggers biofilm formation. The resulting engineered strains were utilized for the biotransformation and degradation of chemicals (cyclohexanol) in continuous cultivation systems. This approach led to a ~300-fold increase in biofilm formation in microtiter plates, and was successfully used in diverse fermentation systems displaying increased catalytic efficiency.

How to cite: Volke, D. C., Heuschkel, I., Bühler, K., and Nikel, P. I.: Synthetic gene circuits for programmable Pseudomonas catalytic biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-128, https://doi.org/10.5194/biofilms9-128, 2020

biofilms9-56
https://doi.org/10.5194/biofilms9-56
biofilms 9 conference

Enhanced erosion resistance of biopolymer-enriched B. subtilis NCIB 3610 biofilms

Elif Nur Hayta and Oliver Lieleg
Munich School of Bioengineering and Department of Mechanical Engineering, Technical University of Munich, Garching, Germany (elifnur.hayta@tum.de)

Erosion resistance is one of the advantages bacteria gain by producing biofilms. While it is undesirable for us humans when biofilms grow on medical devices or industrial pipelines, biofilms with a high erosion resistance can be advantageous for biotechnological applications. Here, we demonstrate how the erosion resistance of *B. subtilis* NCIB 3610 biofilms can be enhanced by integrating foreign (bio)polymers such as γ-polyglutamate (PGA), alginate and polyethylene glycol (PEG) into the matrix during biofilm growth.

Artificial enrichment of the NCIB 3610 biofilms with these biopolymers causes a significant increase in the erosion resistance by slightly changing the surface topography: A decreased cavity depth on the surface results in an alteration in the mode of surface superhydrophobicity, and we obtain a state that is located somewhere between rose-petal like and lotus-like wetting resistance. Surprisingly, the viscoelastic and microscopic penetration properties of the biofilms are not affected by the artificial incorporation of (bio)polymers. As we obtained similar results with all the biopolymers tested (which differ in terms of charge and molecular weight), this indicates that a variety of different (bio)polymers can be employed for a similar purpose.

The method introduced here may present a promising strategy for engineering beneficial biofilms such, that they become more stable towards shear forces caused by flowing water but, at the same time, remain permeable to nutrients or other molecules.

How to cite: Hayta, E. N. and Lieleg, O.: Enhanced erosion resistance of biopolymer-enriched B. subtilis NCIB 3610 biofilms, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-56, https://doi.org/10.5194/biofilms9-56, 2020

biofilms9-72
https://doi.org/10.5194/biofilms9-72
biofilms 9 conference

The effect of synthetic microbial spatial self-organization on the fate of antibiotic resistance genes

Yinyin Ma[1,2] and David Johnson[2]
[1]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland (yinyma@student.ethz.ch)
[2]Department of Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland (david.johnson@eawag.ch)

Biofilms are considered as hotspots for the transfer of antibiotic resistance genes (ARGs), but very few studies have investigated the fate of ARGs (e.g. proliferation or elimination) in situ given different microbial spatial self-organization (SSO). SSO refers to a pervasive process during biofilm formation when microbes arrange themselves non-randomly across surfaces. So far the causes of SSO have been uncovered in a sense, however, the consequences of SSO were largely overlooked. Here, I hypothesize that the magnitude of inter-species intermixing, as one fundamental character of SSO, will determine the fate of ARG-carrying conjugative plasmid in both absence and presence of antibiotic selection. I evaluated this by performing range expansion experiments on agar plates to develop an artificial biofilm using a synthetic microbial community consisting of two isogenic Pseudomonas Stutzeri *A1501* who are facultative denitrifiers in anaerobic condition. By knocking out different functional genes responsible for different steps of denitrification I am able to modify the metabolic interactions between these two strains from competing (without trophic interaction) to cross-feeding (with trophic interaction), which will further result in different magnitude of inter-species intermixing. Competing group has lower magnitude due to demixing of two, while cross-feeding group has higher magnitude due to mixing. I observed that in the absence of antibiotic selection plasmid experienced faster pace of elimination in competing group than cross-feeding group, whereas in the presence of antibiotic selection plasmid proliferated more efficiently in cross-feeding group than competing group. These results suggest that SSO is a determining factor of the fate of ARGs in biofilms, which provides a novel perspective of better understanding ARGs-related pressing problems facing our society.

How to cite: Ma, Y. and Johnson, D.: The effect of synthetic microbial spatial self-organization on the fate of antibiotic resistance genes, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-72, https://doi.org/10.5194/biofilms9-72, 2020

Poster Presentations

biofilms9-100
https://doi.org/10.5194/biofilms9-100
biofilms 9 conference

Bidirectional alterations in antibiotics susceptibility in Staphylococcus aureus - Pseudomonas aeruginosa dual-species biofilm

Elena Trizna[1], Diana Baidamshina[1], Maria Yarullina[1], Anna Mironova[1], Alsu Khabibrakhmanova[2], Almira Kurbangalieva[2], and Airat Kayumov[1]

[1]Kazan Federal University, Institute of Fundamental Medicine and Biology, Genetics, Russian Federation (trizna9144@gmail.com)
[2]Kazan Federal University, A. Butlerov Institute of Chemistry, Biofunctional Chemistry Laboratory, Russian Federation (almira99@mail.ru)

While in biofilms bacteria are embedded into an extracellular matrix which forms inaccessible barrier for antimicrobials thereby drastically increasing the concentrations of antibiotics required for treatment. Here we show that the susceptibility of *S. aureus* and *P. aeruginosa* to antibiotics in mixed biofilms significantly differs from monoculture biofilms depending on both conditions and chosen antimicrobial agents. While *S. aureus* could completely avoid vancomycin, ampicillin and ceftriaxone by embedding into the biofilm of *P. aeruginosa*, the very same consortium was characterized by 10–fold increase in susceptibility to broad-spectrum antimicrobials like ciprofloxacin and aminoglycosides compared to monocultures. These data clearly indicate that efficient treatment of biofilm-associated mixed infections requires antimicrobials active against both pathogens, since the interbacterial antagonism would enhance the efficacy of treatment. Moreover, similar increase in antibiotics efficacy was observed when *P. aeruginosa* suspension was added to the mature *S. aureus* biofilm, compared to *S. aureus* monoculture, and vice versa. These findings open promising perspectives to increase the antimicrobial treatment efficacy of the wounds infected with nosocomial pathogens by the transplantation of the skin residential microflora.

This work was supported by the Russian Science Foundation (Project №20-64-47014)

How to cite: Trizna, E., Baidamshina, D., Yarullina, M., Mironova, A., Khabibrakhmanova, A., Kurbangalieva, A., and Kayumov, A.: Bidirectional alterations in antibiotics susceptibility in Staphylococcus aureus - Pseudomonas aeruginosa dual-species biofilm, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-100, https://doi.org/10.5194/biofilms9-100, 2020

biofilms9-46
https://doi.org/10.5194/biofilms9-46
biofilms 9 conference

Finding the comfort zone: Online-monitoring of electroactive bacteria colonising electrode surfaces with different chemical properties

Hanna Frühauf[1], Markus Stöckl[1], and Dirk Holtmann[2]
[1]Industrielle Biotechnologie, DECHEMA Forschungsinstitut, Frankfurt a.M., Germany (hanna.fruehauf@dechema.de)
[2]Institut für Bioverfahrenstechnik und Pharmazeutische Technologie, Technische Hochschule Mittelhessen, Gießen, Germany

Mechanisms of electron transfer vary greatly within the diverse group of electroactive microorganisms and so does the need to attach to the electrode surface, e.g. by forming a biofilm.

Electrochemical impedance spectroscopy (EIS) and confocal laser scanning microscopy (CLSM) are well established methods to monitor cell attachment to an electrode surface and have therefore been combined in a flow cell as a screening system. The flow cell, equipped with a transparent indium tin oxide working electrode (ITO WE), allows monitoring of attachment processes in real time with minimal needs for additional biofilm preparation. In preliminary experiments the flow cell was successfully used as microbial fuel cell (MFC) with a potential of +0.4 V vs. Ag/AgCl using *Shewanella oneidensis* as electroactive model organism. [1]

Commonly, graphite-based electrode materials are used in bioelectrochemical systems due to their low costs and high conductivity. However, the hydrophobic and negatively charged surface is not yet optimal for microbial attachment. There are numerous attempts on electrode surface engineering in order to overcome this problem. In the majority of studies the biofilm analysis and evaluation of the attachment takes place at the end of the experiment, neglecting the impacts of the chemical surface properties and initial electrode conditioning during the very beginning of biofilm formation.

To investigate initial attachment and biofilm formation in real-time, the transparent ITO-electrode is coated with polyelectrolytes differing in hydrophobicity and polarity to evaluate their effects on the initial surface colonisation by different electroactive microorganisms. Combining CLSM and EIS, both, surface coverage and electrochemical interaction of electrode-associated bacteria can be assessed.

With this we aim to understand and ease initial steps of biofilm formation to improve efficiency of bioelectrochemical applications, e.g. with regards to start-up time.

[1] Stöckl, M., Schlegel, C., Sydow, A., Holtmann, D., Ulber, R., & Mangold, K. M. (2016). Membrane separated flow cell for parallelized electrochemical impedance spectroscopy and confocal laser scanning microscopy to characterize electro-active microorganisms. *Electrochimica Acta*, 220, 444-452.

How to cite: Frühauf, H., Stöckl, M., and Holtmann, D.: Finding the comfort zone: Online-monitoring of electroactive bacteria colonising electrode surfaces with different chemical properties, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-46, https://doi.org/10.5194/biofilms9-46, 2020

biofilms9-139
https://doi.org/10.5194/biofilms9-139
biofilms 9 conference

Interfacing anoxic Shewanella oneidensis biofilms with electrically conducting nanostructures

Edina Klein[1], René Wurst[1], David Rehnlund[2], and Johannes Gescher[1,3]
[1]Karlsruhe Institute of Technology, Applied Biology, Department of Applied Biology, Germany (edina.klein@kit.edu)
[2]Department of Chemistry - Ångström Laboratory, Uppsala University, Sweden
[3]Karlsruhe Institute of Technology, Institute for Biological Interfaces I, Eggenstein-Leopoldshafen, Germany

Shewanella oneidensis MR1 is the best understood model organism with regards to dissimilatory metal reduction and extracellular electron transfer onto carbon electrodes in bioelectrochemical systems (BES)[1]. However, under anoxic conditions *S. oneidensis* is known to form very thin biofilms resulting in low current density output. In contrast, another exoelectrogenic model organism *Geobacter surfurreduscens* can form electroactive biofilms up to 100 µm in thickness. This organism is known for its ability to transport electrons over a long range (> 10 µm) along a network of protein filaments, called microbial nanowires. Although still controversial, it was recently reported that OmcS has a special importance for the conductivity of these nanowires[2]. One of the key differences between *G. surfurreduscens* and *S. oneidensis* lies in how cell-to-cell electronic communication occurs, which dictate the range of electronic communication between distant cells. *S. oneidensis* relies on direct cell-to-cell communication via electron transfer between outer membrane cytochromes or via soluble redox active flavins that are secreted by the cells[3]. Our research is based on the question, what if the *S. oneidensis* biofilm formation could be improved by introducing an artificial electronic network, similar to the native microbial nanowires for *G. sulfurreducens*?

We hypothesize that synthetic biofilms containing conductive nanostructure additives would allow *S. oneidensis* to build multilayer thick biofilms under anoxic conditions on solid electron acceptors. To answer this question of how conductive materials affect the formation of anoxic *S. oneidensis* biofilms, we integrated both biological and synthetic conductive nanostructures into these biofilms. As biological additive, the *c*-type cytochrome OmcS purified from *G. sulfurreducens* was utilized. As synthetic additives, both commercially available biotinylated gold nanorods and in-house electrochemically synthesized metal nanostructures were added to anoxic *S. oneidensis* biofilms.

Cultivation and characterization of the biofilms was performed using our newly developed microfluidic bioelectrochemical platform. Microbial cultivation with the aid of microfluidic flow chambers has a great potential to form biofilms on an easy to handle laboratory scale with simultaneously ongoing multianalytical analysis[4]. In our bioelectrochemical microfluidic, system *S. oneidensis* biofilms can be grown under anoxic conditions using an anode as sole electron acceptor. The growth behavior and bioelectrochemical performance was evaluated by a combination of electrochemical techniques (chronoamperometry, electrochemical impedance spectroscopy, cyclic voltammetry) and optical analyses (confocal laser scanning microscopy and optical coherence tomography). The strategy of conductive nanostructured additives for improved electroactive biofilm formation could be an important tool for other exoelectrogenic microorganisms in order to exploit their physiological abilities for biotechnology.

References:

How to cite: Klein, E., Wurst, R., Rehnlund, D., and Gescher, J.: Interfacing anoxic Shewanella oneidensis biofilms with electrically conducting nanostructures, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-139, https://doi.org/10.5194/biofilms9-139, 2020

biofilms9-29
https://doi.org/10.5194/biofilms9-29
biofilms 9 conference

Melt electro written three-dimensional scaffolds engineered as oral microcosm models-an in vitro study.

Srinivas Ramachandra[1], Abdulla Abdal-hay[2], Pingping Han[3], Ryan Lee[4], and Saso Ivanovski[5]
[1]PhD student, The University of Queensland, Faculty of Health and Behavioural Sciences, School of Dentistry, Australia (s.sulugoduramachandra@uq.net.au)
[2]Post doctoral researcher, The University of Queensland, Faculty of Health and Behavioural Sciences, School of Dentistry, Australia
[3]Post doctoral researcher, The University of Queensland, Faculty of Health and Behavioural Sciences, School of Dentistry, Australia
[4]Senior Lecturer, The University of Queensland, Faculty of Health and Behavioural Sciences, School of Dentistry, Australia
[5]Professor, The University of Queensland, Faculty of Health and Behavioural Sciences, School of Dentistry, Australia

Introduction: Biofilms are 3-dimensional (3D) aggregates of microorganisms that are associated with a wide range of diseases. Although there have been several studies investigating biofilm formation on two-dimensional substrates, the use of 3D substrates may result in more representative and clinically relevant models. Accordingly, the aim of this study was to compare the growth of biofilms in the 3D substrates against biofilms grown in 2D substrates.
Material and Methods: Two grams of medical grade polycaprolactone (PCL) were loaded into a plastic Luer-lock 3 ml syringe and a 23G needle was used as a spinneret. The syringe was placed in a melt electro-writing (MEW) device to obtain fine fibers under controlled parameters. The 3-dimensional MEW PCL scaffolds were manufactured and characterised with an overall thickness of ~ 0.8 mm, with ~ 15 µm diameter fibers and ordered pore sizes of either 100 or 250 µm. PCL films employed as 2D substrates were manufactured by dissolving 10 gms of PCL in 100 ml chloroform and stirred for 3 h to obtain a transparent solution. Then, the solution was cast in glass petri dishes and dried to remove all organic solvents. In addition, commercial hydroxyapatite discs were also used as 2D controls. Unstimulated saliva from six healthy donors (gingival health) were used to grow biofilms. The formed biofilms were assessed at day 4, day 7 and day 10 using crystal violet assay, confocal microscopy, scanning electron microscopy and next-generation 16s sequencing.
Results: The results demonstrates that 3D PCL scaffolds dramatically enhanced biofilm biomass and thickness growth compared to that of the 2D controls. Confocal microscopy of biofilms at day 4 stained with SYTO 9 and propidium iodide showed thickness of biofilms in 2D substrates were 39 µm and 81µm for hydroxyapatite discs and PCL films, respectively. Biofilms in 3D substrates were 250 µm and 338 µm for MEW PCL 100µm pore size and MEW PCL 250 µm pore size, respectively. Similar results were noticed at day 7 and day 10. Scanning electron microscopy showed biofilm bridges formed over the fibers of the MEW scaffolds. Pilot trials of next generation sequencing detected similar taxa in biofilms formed in 3D scaffolds compared to that of 2D substrates.
Discussion: We have successfully investigated a 3D biofilm growth model using 3D medical grade PCL scaffolds. Thicker biofilms can be conveniently grown using this inexpensive static model. This will facilitate 3D microbial community studies that are more clinically relevant and improve our understanding of biofilm-associated disease processes.

How to cite: Ramachandra, S., Abdal-hay, A., Han, P., Lee, R., and Ivanovski, S.: Melt electro written three-dimensional scaffolds engineered as oral microcosm models-an in vitro study., biofilms

9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-29, https://doi.org/10.5194/biofilms9-29, 2020

biofilms9-82
https://doi.org/10.5194/biofilms9-82
biofilms 9 conference

Modelling Staphylococcus aureus biofilms on infected chronic wounds

Yanyan Cheng, Paul De Bank, and Albert Bolhuis
Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom of Great Britain and Northern Ireland

Chronic wounds, for instance venous, pressure, arterial and diabetic ulcers, are a major health problem throughout the world. Compared with normal wounds, those that take more than four weeks to heal are defined as chronic. Interestingly, the numbers of patients suffering from chronic wounds and the cost for treatment have been increasing during the past two decades. There is increasing evidence that suggests that bacteria infect those chronic wounds and there exist as a biofilm, which affects wound healing and success of treatment. To study biofilms in infected wounds, both in vitro and in vivo biofilm models are important to be developed.

In this project, a dynamic ex vivo chronic wound biofilm model for Staphylococcus aureus using a 3D printed chamber and porcine skin was developed. This dynamic model then used to determine antibiotic treatment by using poly(ε□caprolactone) (PCL) electrospun fibrous mats containing different antibiotics, e.g. tetracycline, gentamicin and fusidic acid. Furthermore, electrospun PCL/silk fibroin scaffolds were also used as carrier of gentamicin. The killing effect of mature S. aureus MRSA 252 growing in the wound model was tested by both viable count and qPCR.

The results indicated that this newly designed dynamic model was successful in mimicking single-strain biofilm on infected chronic wounds. Compared with traditional biofilm assays, the flow system generates an air-liquid-solid interface, which more closely approaches to real conditions. Furthermore, results from using electrospun fibrous scaffolds provided strong evidence for their potential in clinical applications to treat infected skin.

How to cite: Cheng, Y., De Bank, P., and Bolhuis, A.: Modelling Staphylococcus aureus biofilms on infected chronic wounds, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-82, https://doi.org/10.5194/biofilms9-82, 2020

biofilms9-96
https://doi.org/10.5194/biofilms9-96
biofilms 9 conference

Nucleating biofilms for biotechnology using synthetic polymers

Pavan Adoni[1], Tim Overton[1], and Paco Fernandez-Trilli Fernandez-Trillo[2]
[1]University of Birmingham, Chemical Engineering, United Kingdom of Great Britain and Northern Ireland (pxa688@student.bham.ac.uk)
[2]University of Birmingham, Chemistry, United Kingdom of Great Britain and Northern Ireland (pxa688@student.bham.ac.uk)

This project is on developing robust biocatalysts in the form of enzymes expressed in biofilms. The aim is to design polymer scaffolds onto which bacteria adhere in a controlled manner, to form biofilms that can be used in biotechnology. Poly(acryloyl hydrazide) has been chosen as the polymer scaffold, due to easy synthesis and post-polymerization modification resulting in highly functional polymers[1] that are predicted to interact and cluster bacteria together. In this study poly(acryloyl hydrazide) with a range of hydrophobic functionalities were used to cluster and interact with two biofilm forming isogenic E.coli K-12 strains; PHL644 containing a point mutation ompR234 resulting in the overexpression of curli (a biofilm adhesin) and its parental wild type MC4100, with data showing correlations between polymer hydrophobicity, bacterial clustering and biofilm intensity in both strains.

Data suggests these polymer induced clusters go on to develop many of the traits of a biofilm; crystal violet staining, lectin staining, and the use of reporter genes have confirmed the presence of extracellular polymeric substances, with their expression levels being directly linked to polymer hydrophobicity. Furthermore our polymers are able to boost biofilm intensity of MC4100 to the levels of PHL644, thereby opening up a potential avenue for non-biofilm forming but industrially relevant strains to experience the benefits of being in biofilm form (robustness, chemical and mechanical resistance).

Biocatalytic ability of our polymer induced biofilms has also been tested, again with polymer properties dictating biotransformation yields.

Finally, preliminary results have shown that we are able to selectively disperse our polymer induced biofilms, and then re-induce them by simply altering polymer properties in situ, providing a potentially useful reversible platform.

How to cite: Adoni, P., Overton, T., and Fernandez-Trillo, P. F.-T.: Nucleating biofilms for biotechnology using synthetic polymers , biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-96, https://doi.org/10.5194/biofilms9-96, 2020

biofilms9-61
https://doi.org/10.5194/biofilms9-61
biofilms 9 conference

Using elastomeric materials to model biofilm physico-mechanical properties and the associated drag penalty

Alexandra Jackson[1], ShiQi An[2], Simon Dennington[1], Paul Stoodley[1,3], Julian Wharton[1], Jennifer Longyear[4], and Jeremy Webb[2]

[1]National Centre for Advanced Tribology, Faculty of Engineering and Physical Sciences, Southampton University, United Kingdom
[2]National Biofilm Innovations Centre, School of Biological Sciences, Southampton University, United Kingdom
[3]Microbial Infection and Immunity and Orthopedics Infectious Diseases Institute, The Ohio State University, OH, Columbus, USA
[4]AkzoNobel/International Paint Ltd., Stoneygate Lane, Gateshead, United Kingdom

The physical structure and mechanical properties of a biofilm are known to respond to external stressors, such as hydrodynamic shear, and are expected to play a vital role in determining biofilm-associated drag. Yet, both, how these structural and mechanical properties interact with one another and with fluid flow, and how these interactions influence drag is poorly understood. In part this is due to a lack of standard methods for studying biofilm physical and mechanical (physico-mechanical) properties and relating them to drag. To date, rigid structures, such as sandpaper, have been typically used to model biofilms. Whilst rigid structures can simulate roughness, they neglect features such as viscoelasticity and heterogeneity. To address this, our novel work demonstrates the practical application of new test methods for biofilm research: the use of elastomeric and gel-like materials to better model biofilm physico-mechanics under controlled flow conditions, and the use of tensile and rheological testing to measure the elastic modulus of marine biofilms. Artificial biofilms were cast / made from materials with mechanical properties comparable to natural biofilms. Marine biofilms were grown in-house, within a recirculating system, using a field-sourced, mixed species inoculate. The elastic modulus of marine and artificial biofilms was measured using tensile and rheological testing. Though elastic modulus has been recorded for biofilms previously, until now, the elastic modulus of marine biofilms had not been recorded; partly due to the complexity of their physical structures and their biological composition (bacterial and microalgal components). Despite biological differences, the elastic modulus of marine biofilms tested sits comfortably within the range recorded for other biofilms studied, at 0.0000098 MPa - 0.0002 MPa. A marine biofilm flow cell was utilised for pressure drop experiments, alongside the use of a non-invasive imaging technique, Optical Coherence Tomography. This experimental set-up enabled real-time visualisation and data collection of the physical response of the elastomers and biofilms grown in the marine environment to different flow rates. We found that for artificial biofilms, elasticity had a greater impact on biofilm-associated drag than roughness ($P < 0.05$). Biofilms are a unique and complex material, and therefore to better understand their physico-mechanical properties in flow, we first need to understand these properties independent of their complex biology. The use of fully artificial biofilms, with controlled properties, based on mechanical properties of marine biofilms, can help achieve this.

How to cite: Jackson, A., An, S., Dennington, S., Stoodley, P., Wharton, J., Longyear, J., and Webb, J.: Using elastomeric materials to model biofilm physico-mechanical properties and the associated drag penalty, biofilms 9 conference, Karlsruhe, Germany, 29 September–1 Oct 2020, biofilms9-61, https://doi.org/10.5194/biofilms9-61, 2020