

# Topics in Water Chemistry and Water Technology

# 2017

Annual Report of the Chair of Water Chemistry and Water Technology  
and the DVGW Research Center at the Engler-Bunte-Institut

## ENGLER-BUNTE-INSTITUT



Dear colleagues and friends,

As every year, the last 12 months have been accompanied by a certain fluctuation within the group. Three PhD studies have been finished with a defense. Three new PhD students started to work at the Institute on biofilm mechanics, membrane technology and decision support systems for the water management in the North of Chile. The latter is a new topic for us and we cooperate with the Institute for Technology Assessment and Systems Analysis (ITAS) at KIT.

The next pages will show the research conducted in Water Chemistry and Water Technology at the Engler-Bunte-Institut. Without no doubt, the measurement of the concentration boundary layer thickness in a fully operated reverse osmosis/nanofiltration cell with Raman microspectroscopy is one of the major breakthroughs of this year. The use of the liquid chromatography coupled with different online detection systems (C, N, UV) for water quality on the one side and waste water treatment on the other side has been intensified as you can see from the two PhD projects. Especially in waste water treatment, the method has been underestimated within the last decade. We really made a step forward in identifying on how antibiotic resistance genes (ARG) do find their way into the river sediments. This research is possible thanks to a very fruitful cooperation with Thomas Schwartz (Institute of Functional Interfaces, IFG).

Senior researchers from Australia and China joined our group in 2017. Both colleagues will stay until 2018. Prof. Guihua Xu is using optical coherence tomography (OCT) for the characterization of activated sludge flocs and the Alexander von Humboldt scholarship holder Dr. Yiwen Liu is working on the enrichment of ammonium oxidizing archaea (AOA) in biomass from different waste water treatment systems.

The Advanced Biofilm Course (ABC) took place at the University of Copenhagen in Helsingør this year. It was combined with an autumn school for the EU project EVOBLISS. The number of applicants is still very high and we cannot provide enough places for all.

Since October 2016, I have been appointed as Dean of the Faculty of Chemical and Process Engineering. The work is accompanied by more or less fruitful meetings at the KIT level and with an insight into a research institution, which is still debating the best way to make the merge of the Universität Karlsruhe (TH) and the Helmholtz Research Center a successful story.

As you can see from the report of Ulrike Scherer, the Water Research Network organized several workshops and meetings in 2017. The intention of the network, to bring together researchers from different research institutions in Baden-Württemberg, proved to be a good idea as we could experience extremely valuable discussions.

I wish you all the best for 2018

Harald Horn

## Water Research Network Baden-Württemberg



Ulrike Scherer



Tanja Stahlberger

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University of Applied Sciences  
Offenburg University of Applied Sciences  
Stuttgart University of Applied Sciences

For further details see:

[www.wassernetzwerk-bw.de](http://www.wassernetzwerk-bw.de)

In 2017, several workshops and events were organized, covering a broad range of topics in water research:

In February, a two-day workshop on 'Ground Water Management in Natura-2000 Areas' was held at KIT with participants from research institutions, water suppliers, authorities and nature conservation associations. A collaborative workshop on 'Joint Environmental Monitoring' took place in May, aiming at the agreement on a common environmental monitoring strategy among universities in Baden-Württemberg. During the year, a series of workshops was launched on the topic 'Rhine River Hydrosystems – Past, Present, Future' to enhance the cooperation in research and teaching between scientists from German and French universities located in the Upper Rhine Region.

A 'Future Workshop' was organized in July on 'Water Technology in the Context of Global Water Resources Problems'. The workshop included three phases led by a trained moderator. First, critical questions concerning innovations in water technology were framed. Then, future ideas were envisaged and finally, the most attractive ideas were evaluated with regard to implementation. This event was followed by a workshop for early career scientists on the 'Urban Water-Energy Nexus', focusing on aspects of sustainability.

In September, a one-week delegation trip to Canada (Ontario/Quebec) was organized by Baden-Württemberg International. Eleven participants visited the Universities of Waterloo, Queen's and McGill and the Canadian Centre for Inland Waters, exploring cooperation opportunities in water research.

Two international workshops were held in October. The scope of the workshop 'Drought in the Anthropocene' was to initiate comparative studies on drought risk management. The workshop on 'Water Ages in the Hydrological Cycle' brought together the experience of scientists in the field of measuring and simulating water ages and transit times with a focus on the interlinkages between the atmosphere, vegetation, soils, ground and surface waters.



Participants of the workshop on 'Water Ages in the Hydrological Cycle' (top left), impressions from the 'Future Workshop' on 'Water Technology in the Context of Global Water Resources Problems' (bottom left and center) and participants of the delegation trip to Canada at Queen's University Biological Station (right).

## Standard Methods for the Examination of Water, Waste Water and Sludge

Validation of the analytical protocol by an external interlaboratory trial is an important step for establishing a method for water examination in the format of a standard. Such trials were successfully performed for several drafted standards. For validation of ISO 20236 on determination of TOC, DOC,  $TN_p$  and  $DN_p$  by high-temperature oxidative combustion, samples were distributed to about 80 laboratories in 11 countries. If an analyte cannot be stabilized properly, all participants meet at the organizer's lab with their respective equipment and perform the measurements at the same time under comparable conditions. In this way, the colorimetric determination of free chlorine and total chlorine for routine control purposes has been validated for revision of DIN EN ISO 7393-2. As chlorine is often determined on-site, also requirements for ready-to-use reagents are specified in the standard.

In the field of bioeffect testing, three reporter gene assays for determination of the estrogenic potential of water and waste water have been validated (future ISO 19040-1, -2, -3). Besides evaluation of estrogenic effects in order to report the lowest ineffective dilution, the standards describe the quantification of estrogenic ingredients as estradiol equivalents at levels that are below the limits of determination of chemical instrumental analysis. The concept of biological equivalence concentrations, expressing a biological activity measured in a biotest in terms of a defined reference concentration, and how to derive them, is subject of a new work item proposal currently being launched in ISO.

Methods for water examination are being standardized in the technical committees ISO/TC 147 'Water quality', CEN/TC 230 'Water analysis' and DIN NA 119-01-03 Arbeitsausschuss 'Wasseruntersuchung'. Most of the standards are becoming part of the loose-leaf collection 'Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung', jointly edited by the German Water Chemistry Society and the DIN Standards Committee 'Water Practice'.



Birgit C. Gordalla

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*Setting up an ISO standard for water examination. The full members of the committee, represented by their national standards bodies, are obliged to cast their votes in several inquiries and have the opportunity to comment on different document stages. Methods on continuously measurable parameters have to be validated by a round robin test.*

## Handling Guidelines for Operating Drip Irrigation Systems



Jueying Qian



Michael Wagner

In arid regions, application of treated waste water (TWW) in drip irrigation systems may offer the most efficient way to cope with water shortages. However, utilization of TWW promotes biofilm growth causing clogging of irrigation systems. A drip irrigation system is considered malfunctioning if the coefficient of variation (CV) of the irrigation rates is  $> 7\%$ . This study investigated on-line monitoring of biofilm accumulation in drip irrigation pipes by DEPOSENS<sup>®</sup> biofilm sensors (LAGOTEC GmbH, Germany) and automation of cleaning procedures based on the sensor signal. The advantages of sensor controlled cleaning strategies are the saving of time and chemicals as well as the increase of cleaning efficiency and reduction of disinfection by-products.

To predict the irrigation performance based on the input parameters (water quality, concentration of cleaning agent), a biofilm model was set-up and calibrated using data from lab-scale experiments. The sensor signal correlated well with the CV and exceeded 47 arbitrary units (a.u.) when  $CV \geq 7\%$ . Thus, sensor values  $\geq 47$  a.u. indicated failure of the irrigation system. Values  $< 40$  a.u. were considered as safe operation. Sensor signals between 40 – 47 a.u. marked a warning level. The results of one out of 25 simulations are presented in the Figure. Biofilm development was simulated using a typical secondary TWW (biochemical oxygen demand over 5 days = 12.4 mg/L and  $PO_4^{3-}\text{-P} = 2$  mg/L). The irrigation time was specified with 6 hours per day during the simulation. As can be seen, the simulated sensor signal (white curve) reached 40 a.u. (warning level) within 7.7 days and 47 a.u. (failure level) within 9.5 days, meaning that without biofilm control, the drip irrigation system failed after approx. 1.5 weeks.

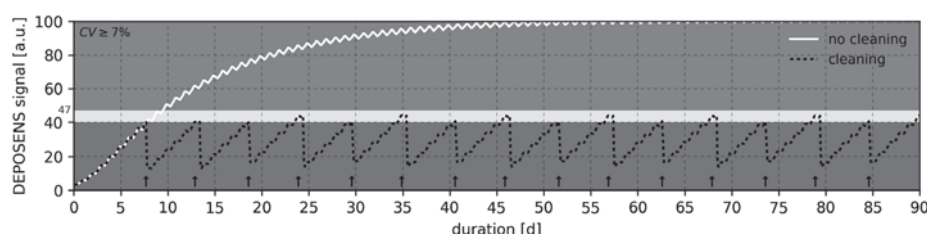
The idea of an automated cleaning is simple: The control system checks the sensor signal at the beginning of each irrigation cycle. If it is  $\geq 40$  a.u., the cleaning agent is dosed during this particular irrigation cycle (1 h). Hence, the model was applied to optimize the frequency and concentration of the cleaning agent. For sodium hypochlorite at a concentration of 20 mg/L free  $Cl_2$ , one treatment every 5 to 6 days was sufficient to keep the sensor signal below 40 a.u. (see dotted curve in the Figure). The model further revealed that even low concentrations of free  $Cl_2$  (0.5 mg/L) can allow for an unaltered drip irrigation if cleanings are applied more frequently.

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*Simulation of biofilm development (white curve) in a drip irrigation system operated using secondary TWW and effect of frequent cleaning with sodium hypochlorite (dotted curve, concentration of free  $Cl_2 = 20$  mg/L). Arrows indicate when the DEPOSENS<sup>®</sup> sensor signal triggered cleaning (levels of failure: no failure: signal  $< 40$  a.u., warning:  $40 < \text{signal} < 47$  a.u., failure: signal  $\geq 47$  a.u.).*



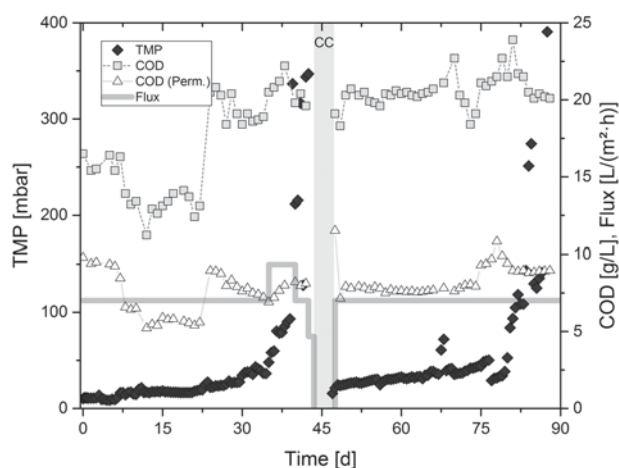
## Autogenerative Two-Phase High Pressure Fermentation – AG-HiPreFer

The two-stage high-pressure fermentation process enables the production of methane at high operating pressure. Higher pressure significantly reduces the energy needed for injecting the produced biogas into the national gas grid. In order to achieve this goal, a methane reactor (MR) was operated under high pressures up to 100 bar. The feed to the MR (percolate) was produced by a thermophilic hydrolysis stage (55 °C) fed with energy crops.

The high pressure MR consists of a fixed bed so that particulate matter can cause severe problems (e.g., clogging) in long-term operation. A microfiltration step could therefore enhance the methane production and protect the fixed bed of the MR against particulate matter. Research activities in the framework of the joint project AG-HiPreFer included membrane process selection and long-term behavior studies of the filtration process.

Ceramic membranes, either crossflow or submerged, proved to be the most suitable option to treat the percolate due to their high chemical and temperature resistance. A range of pore sizes (0.2 – 0.8 µm) was tested. For all of the different membrane types, a similar rejection rate of approx. 55 % chemical oxygen demand (COD) and up to 30 % acetic acid was observed. With crossflow filtration, we achieved sustainable fluxes between 14 and 22 L/(m<sup>2</sup>h), which could be kept stable over several weeks. Fouling could be controlled over the induced shear by operating the system under high crossflow velocities of 1.6 m/s. Long-term experiments with the submerged membrane system (see Figure) showed that the sustainable flux was approximately 7 L/(m<sup>2</sup>h).

The application of membrane filtration led to a reduction of hydraulic retention time from more than 4 to 1.5 days in the high pressure MR (25 bar). Additionally, the methane content of the produced biogas generated with permeate feed was increased by almost 9 vol.-%. The results of the project show an overall improvement of the two-stage biogas production. The AG-HiPreFer concept is a good basis for the production of compressed high-calorific biogas and allows for integrating of power-to-gas via biological methanation without any gas compression.



Long-term experiment with the submerged membrane system (TMP = transmembrane pressure, COD = chemical oxygen demand).



Marc Tuczinski



Florencia Saravia

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## Resource Recovery from Concentrates Arising from Industrial Water Reuse – HighCon



Annika Bauer  
Florenca Saravia

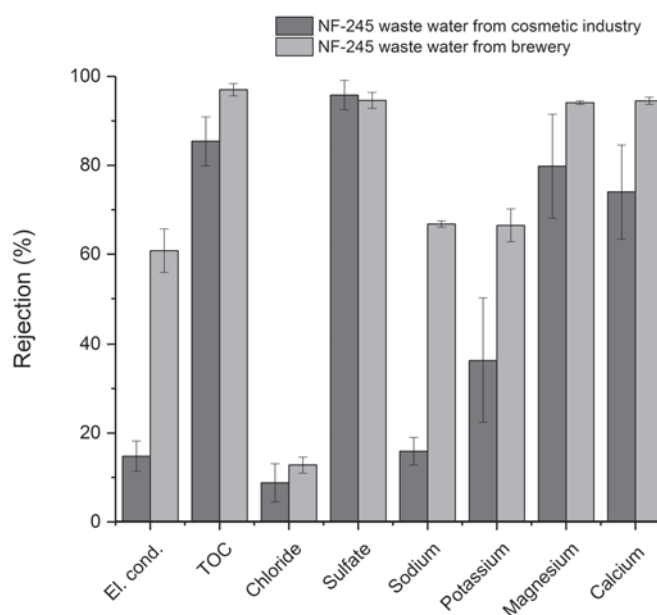
The aim of the HighCon project is the further development, optimization and combination of specific processes (e.g., reverse osmosis, membrane distillation, crystallization, etc.) for the reuse of industrial waste water. Main goals of the joint project are: i) the complete closed loop circulation of waste water streams and ii) the further utilization of the concentrate contents. The tasks of the DVGW Research Center at the Engler-Bunte-Institut, Water Chemistry and Water Technology include investigations of the fouling and rejection behavior of nanofiltration (NF) and membrane distillation (MD) as well as the accurate measurement of different water parameters in high concentrated solutions.

In the project, NF is considered as one of the first processes of the zero liquid discharge chain. NF is expected to principally remove organic and divalent ions. Evaluation of NF was done using two different waste waters (from a brewery and cosmetic industry) and three different NF membranes. The NF membranes achieved a similar removal of organic components (up to 98 %) and divalent ions (80 – 94 %) despite different water compositions. The rejection of monovalent ions strongly depended on the feed water matrix (see Figure). The permeability during the experiment was also dependent on the type of applied waste water.

Additionally, first MD experiments were carried out. A severe problem is fouling formation on the feed side of the membrane, leading to a flux decrease as well as possible membrane wetting and, thus, restricting the membrane separation performance. Current investigations focus on the fouling layer detection by the application of different optical as well as analytical methods. The results should allow an early quantitative evaluation of the fouling layer on the feed side of the membrane and a better understanding of the fouling formation mechanisms.

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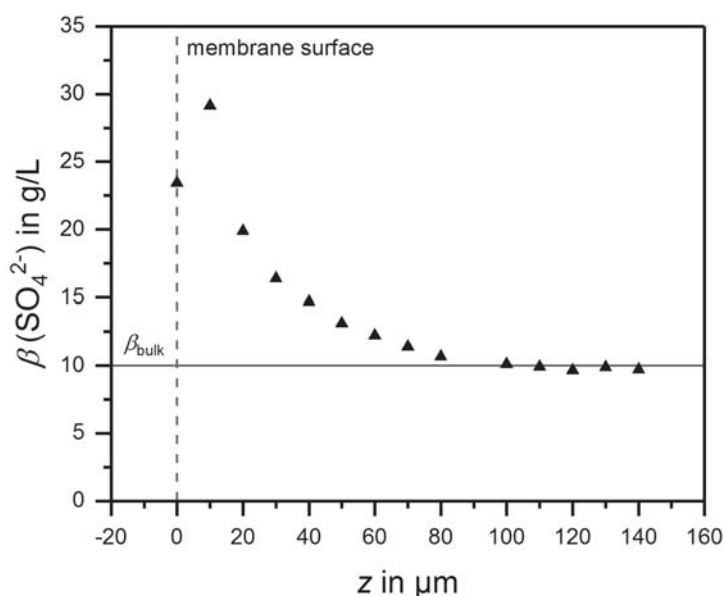
Rejection of electrical conductivity (el. cond.), organic compounds (TOC) and mono- and divalent ions (raw waste water properties: cosmetic industry: TOC = 635 mg/L, el. cond. = 17.2 mS/cm; brewery: TOC = 45 mg/L, el. cond. = 4.2 mS/cm).

## Making Concentration Polarization Visible? – A Study Using Vibrational Spectroscopy

Scaling is a major drawback in membrane desalination and often determines the limit of recovery of desalination systems. One of the main factors contributing to scaling formation is the phenomenon of concentration polarization (CP). That is why reverse osmosis/nanofiltration operation tries to minimize CP by introducing feed spacers, using high cross-flow velocities and addition of chemicals (antiscalant) designed to prevent nucleation. However, CP has never been measured in these practical conditions.

Measuring CP is difficult as it occurs in a closed system under pressure involving dissolved species in a transparent sample. Confocal Raman microspectroscopy (CRM) has the ability to non-destructively measure concentrations of Raman active species spatially resolved in solution and, thus, providing the opportunity to visualize CP under operational conditions for the first time. CRM is a method of optical vibrational spectroscopy, commonly used to identify and characterize molecules. The Raman activity of sulfate and its role in membrane scaling formation make it a very suitable test compound. The symmetric S-O vibrational mode has a distinct Raman band at  $981\text{ cm}^{-1}$ . The calibration curves show a linear correlation of intensity and concentration. Sulfate in solution can also be differentiated from sulfate which has precipitated.

In this study, spatially resolved visualization of CP using a flat sheet membrane filtration unit suitable for Raman microspectroscopy was successfully achieved. The determined sulfate concentration distribution above the membrane is depicted in the Figure and was corrected using background measurements. Moreover, optical restrictions were compensated, allowing for the determination of important CP parameters such as the concentration boundary layer thickness. Recently, the experimental setup as well as data post-processing routines have further been improved.



Concentration profile of sulfate at 10 bar, measured with confocal Raman microspectroscopy ( $\beta$  = mass concentration,  $z$  = distance from membrane surface).



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Florencia Saravia

Michael Wagner

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The Raman microscope is provided by Prof. Thomas Leisner from the Institute of Meteorology and Climate Research (IMK-AAF), KIT, Germany.

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The University of Jordan, Amman, Jordan

Palestine Water Authority, Ramallah, Palestinian Territories

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and other partners from Israel, Jordan and Germany

## Magnetic Resonance Imaging (MRI) on Partially Water Saturated Porous Media



Florian Ranzinger



Gisela Guthausen

Andrea Hille-Reichel

The dynamics of water in soil is an important factor, which determines the soil properties with respect to, for example, wettability and bioavailability of water and nutrients.

In a first study, artificial porous media were investigated using magnetic resonance imaging (MRI) and compared to micro-computed tomography ( $\mu$ -CT) data (left Figure) with the aim to determine the structure of the packed bed. In addition, the water content was spatially resolved during processes of drying (right Figure) and water exchange using MRI.

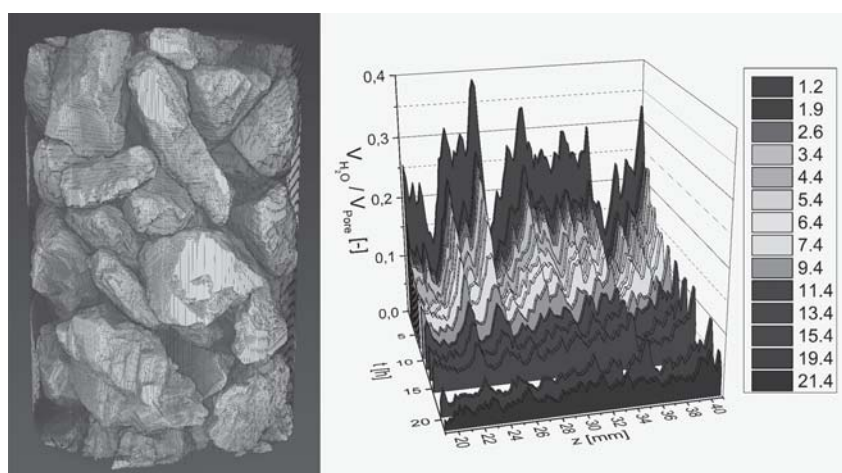
As model systems, packed beds exhibiting different degrees of heterogeneity were created inside glass columns with an inner diameter of 14 mm. The columns were packed with glass spheres with a narrow or broader particle size distribution (diameter:  $2 \text{ mm} \pm 0.1 \text{ mm}$  or  $2.5 - 3 \text{ mm}$ , respectively) or heterogeneous and irregularly shaped gravel particles (diameter:  $2 - 4 \text{ mm}$ ).

For data acquisition, several MRI sequences were investigated as they show different sensitivities to NMR relaxation. It was found that the classic sequence 'rapid acquisition with relaxation enhancement' (RARE) led to the highest reliability of the data obtained.

Solid, liquid and gaseous phases were identified via reference images for mask creation and numerical segmentation. This approach allowed the spatially and temporally resolved quantification of water content in the columns.

Both processes, drying as well as water exchange, were shown to depend on the geometry of the particles and, thus, the corresponding distribution and geometry of the pores: Depending on the width of the particle size distribution, either an evaporation frontier was observed for the homogenous glass sphere packing or, in the case of the more irregularly shaped gravel packing, a successive reduction of the water fraction along the column's height was determined (right Figure).

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Left: 3D reconstruction of the packed bed of sand particles, based on  $\mu$ -CT data (diameter of column: 14 mm). Right: Drying was detected via MRI techniques over time (see color bars in hours) for the partially unsaturated packed bed – as the change of the ratio of water volume to pore volume.



## Sunlight-Driven Catalytic Degradation of Sulfamethoxazole Using Pt- and Pd-Doped $\text{TiO}_2$

In the era of highly polluted aquatic environment, the search for innovative methods of water treatment is one of the crucial scientific tasks. A relatively easy access to sunlight and proven potential of catalytic processes for micropollutants removal from water triggered the establishment of a new research area – the application of sunlight-driven catalysis for environmental purposes.

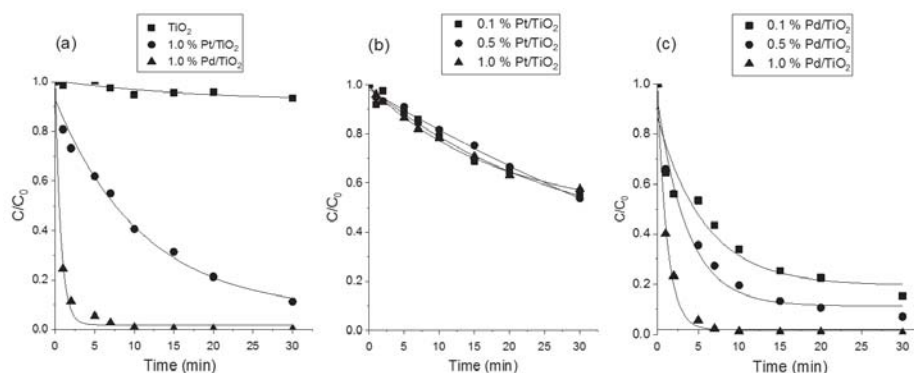
The aim of the study was to investigate the efficiency of photocatalytic degradation of a popular pharmaceutical, sulfamethoxazole (SMX), using titanium dioxide modified with platinum ( $\text{Pt}/\text{TiO}_2$ ) or palladium ( $\text{Pd}/\text{TiO}_2$ ) and natural sunlight. The experiments were performed in Karlsruhe on a cloudless sunny day in June 2017.

First results showed that modification of  $\text{TiO}_2$  surface drastically increased the efficiency of the degradation process (Figure a). No SMX decay ( $< 5\%$ ) was observed after 30 min of the catalytic process with non-modified  $\text{TiO}_2$ . On the other hand, in the presence of noble metal on the surface of  $\text{TiO}_2$ , SMX concentration was reduced by 60 % and 100 % after 10 min of irradiation with  $\text{Pd}/\text{TiO}_2$  and  $\text{Pt}/\text{TiO}_2$ , respectively. Moreover, the effect of various doping levels – 0.1 %, 0.5 %, 1 % – was investigated. SMX degradation of 40 % was noted after 30 min of irradiation with any of the tested doping levels of  $\text{Pt}/\text{TiO}_2$  (Figure b). However, the same duration of the sunlight exposition resulted in the removal of 80 %, 90 % and 100 % for 0.1 %, 0.5 % and 1 % Pd-doping, respectively (Figure c).

These preliminary results clearly indicate the potential of sunlight-driven catalysis with Pd/Pt-doped  $\text{TiO}_2$  for micropollutants removal in the aquatic environment. Further investigations will focus on the application of this type of photocatalysis in surface water samples, the explanation of catalytic process mechanism and the identification of formed transformation products.



Ewa Borowska



Decay of sulfamethoxazole during sunlight-driven catalysis using: (a) various catalysts, (b)  $\text{Pt}/\text{TiO}_2$  of various Pt-doping, (c)  $\text{Pd}/\text{TiO}_2$  of various Pd-doping.

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## Photolysis of $\beta$ -Lactam Antibiotics under Environmental Conditions

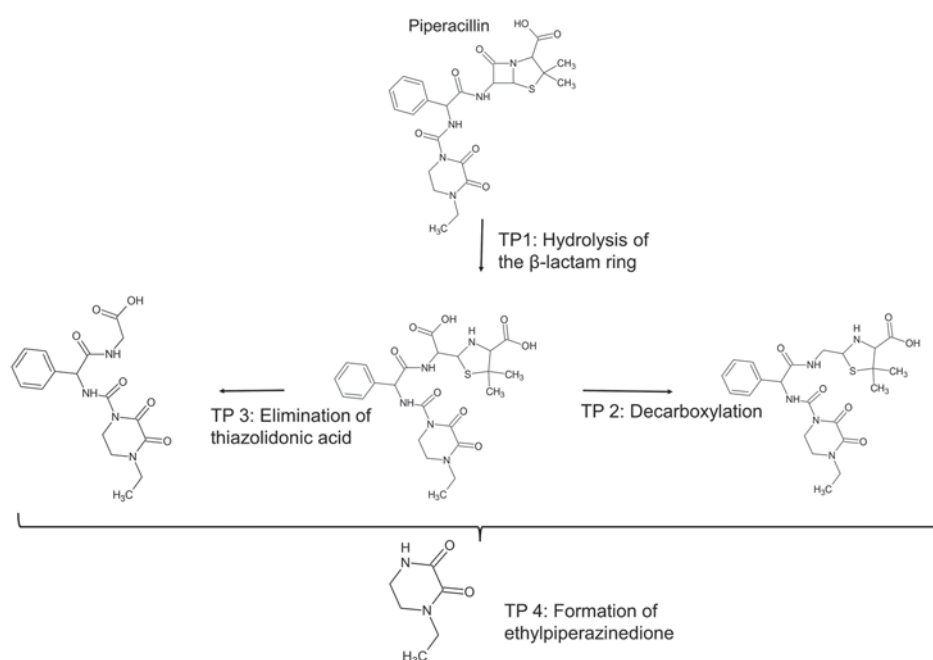


Alexander Timm

Ewa Borowska

$\beta$ -lactam antibiotics belong to the most essential drugs used for the treatment of bacterial infections. Due to their high consumption, there is an interest in exploring their environmental fate, especially their transformation pathways and the effect on bacteria with regard to formation and spreading of antibiotic resistance genes. This study focuses on the photolytical degradation of amoxicillin, ampicillin, piperacillin and penicillin V. Degradation experiments performed with artificial sunlight (Solar Simulator 3A, Newport) showed that all analytes are photolytically degradable with half-lives between 15.7 and 36.5 hours, under middle European summer conditions. Structure elucidation of transformation products using high resolution mass spectrometry revealed that hydrolysis of the  $\beta$ -lactam ring is a primary transformation, followed by vicinal decarboxylation. While both of these transformation pathways have already been reported in the literature, the elimination of thiazolidonic acid was recognized for the first time. For piperacillin, the additional transformation product ethylpiperazinedione and for penicillin V, two epimers were found.

The growth inhibition of UV-irradiated antibiotic solutions was measured on *Bacillus subtilis* as an exemplary environmental bacterium. Results showed that the growth inhibition decreased proportionally to the concentration loss caused by irradiation for all substances except for penicillin V. The remaining growth inhibition effect of photolytically degraded penicillin V can be explained by the formation of epimers, which include the  $\beta$ -lactam ring as active center of the bactericidal activity. Standardized toxicity tests on *Aliivibrio fischeri* showed that the degraded antibiotics have no or a minor inhibition effect on the bacterial activity.



Photolytical transformation pathway of piperacillin.

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## Dissemination and Spread of Antibiotic Resistance Genes in Benthic Microbial Biofilm Communities

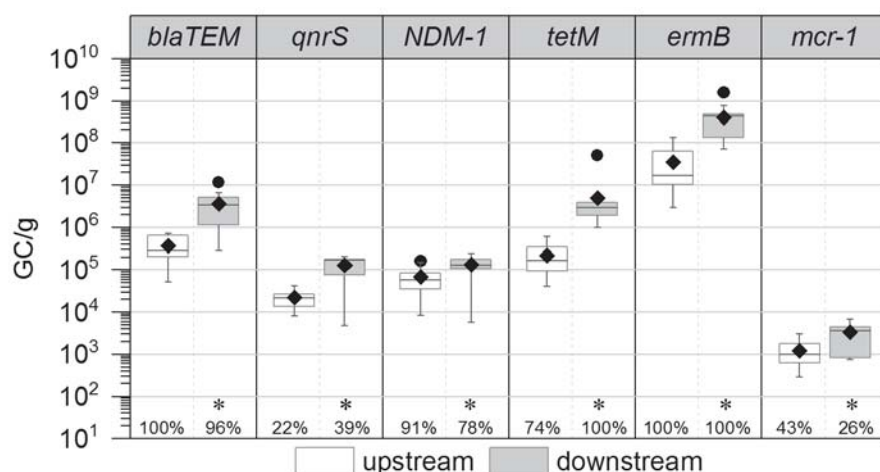
The dissemination, persistence and spread of antibiotics, resistant microbes and resistance genes with potential adverse impacts on public health have become an emerging environmental problem. A variety of studies reported on the occurrence of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) as well as the presence of antibiotics in waste water and surface water. Evidently, waste water treatment plants (WWTPs) have a direct impact on the abundance of ARGs in the environment, but the question of how natural bacterial populations evolve and respond, remains unanswered. Results from this study indicate that ARGs increase, evolve and persist within microbial communities in river sediments downstream of a WWTP effluent (see Figure). Although the reasons are not entirely clear, sub-inhibitory concentrations of antibiotics are repeatedly held responsible because they might exert a selective pressure on microbial populations. Another source seems to be apparent: Untreated municipal waste water, carrying high loads of ARGs and ARB, is flushed into the aquatic environment via combined sewer overflows during heavy rainfall events. Potentially, following this path, particle-bound microorganisms could transport ARGs from municipal waste water into the benthic biofilm through sedimentation.

To test this theory, natural benthic biofilm communities taken from river sediments are exposed to particulate matter from raw municipal waste water in batch reactors. Additionally, sub-inhibitory concentrations of the antibiotic substances erythromycin, roxithromycin, penicillin V, tetracycline and ciprofloxacin are introduced to investigate if they exert a selection pressure on the present biofilm community. ARGs and ARB are measured and quantified over time within whole bacterial communities after DNA-extraction using RT-qPCR (real-time quantitative polymerase chain reaction).



Philip Brown

Ewa Borowska



Abundance (gene copies per g sediment) of each ARG in total DNA extracted from 46 sediment samples upstream and downstream of a WWTP effluent. One-way ANOVA tests were performed to show statistical significance (\*). Positive hits are shown as percentages of total sample number; (*blaTEM* = beta-lactamase, *qnrS* = quinolone resistance gene (RG), *NDM-1* = New Delhi Metallo-beta-lactamase-1, *tetM* = tetracycline RG, *ermB* = erythromycin RG, *mcr-1* = mobilized colistin RG).

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Thomas Schwartz, Institute of Functional Interfaces (IFG), KIT, Germany

## Analysis of Urea and Dissolved Nitrogen Compounds by means of Size Exclusion Chromatography (SEC)



Nico Seeleib



Gudrun Abbt-Braun

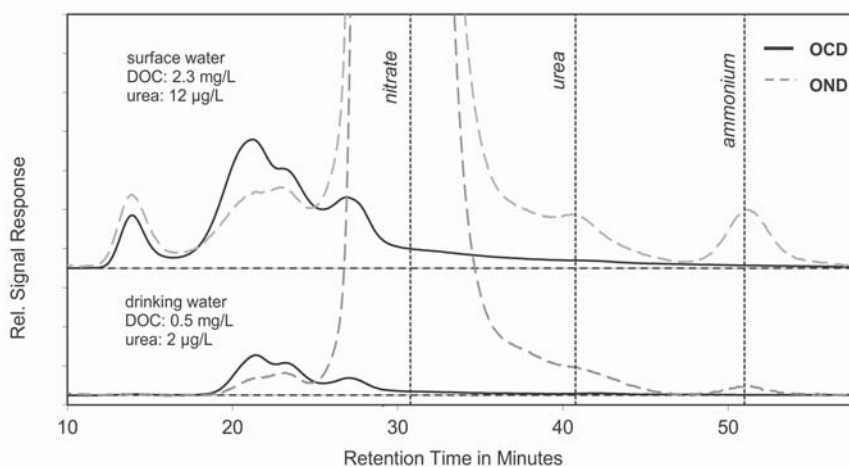
Dissolved organic nitrogen (DON) is an important constituent in the nitrogen and carbon pools in the environment. By means of size exclusion chromatography (SEC), coupled with the so-called DONOX-reactor, different N-species such as nitrate, ammonium and urea can be separated from other N-containing natural organic substances (e.g., humic substances).

Urea is an ubiquitous compound. It is excreted by mammals, can be found in many consumer products and is the most important synthetic fertilizer. Due to the non-ionic character and high water solubility, it cannot be removed by ion exchange resins or other sorption processes.

By using the described SEC technique, urea can be analyzed even in the low  $\mu\text{g}$ -range. A screening of urea in different raw water samples used for drinking water and during different treatment processes showed that the concentrations ranged from 0.5 to 59  $\mu\text{g/L}$ .

The Figure shows SEC chromatograms (organic carbon (OC)- and N-detection) of river water before and after treatment (slow sand filtration, ozonation, multi-layer filtration, activated carbon filtration and UV disinfection). The dissolved organic carbon (DOC) is reduced from 2.3 mg/L in the raw water to 0.5 mg/L in the resulting drinking water. It is obvious that the organic composition is changing during the treatment process. The high molecular fraction at  $t = 15$  min (which can be assigned to compounds like polysaccharides and proteins) is almost totally removed. In the chromatogram, the signal intensity of the organic nitrogen detection (OND) has been increased five times to point out the differences of the urea concentration in the two samples. The amount of urea is decreasing from 12  $\mu\text{g/L}$  in the raw water to 2  $\mu\text{g/L}$  in the drinking water. It was shown that urea is mainly reduced during the slow sand filtration, although the mechanisms are not yet investigated.

Further studies have to show whether urea could be used as a tracer to indicate the microbial stability of water during different treatment steps.



Size exclusion chromatograms with online organic carbon and nitrogen detection (OCD, OND) of raw water (surface water, above) and after treatment (drinking water, below).

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DOC-Labor Dr. Huber,  
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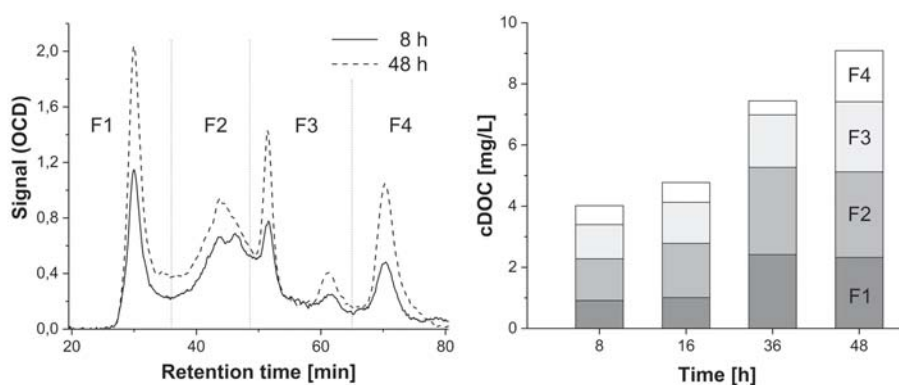
## Impact of Waste Water Particles on the Aerobic Treatment Process

Particles constitute over 50 % of the organic load in domestic waste water. In waste water treatment plants, the particles are partly removed mechanically during the pre-treatment steps. However, smaller particles remain in the waste water and reach the biological treatment steps where they are supposed to be degraded.

The investigation aims at determining the impact of waste water particles on the aerobic and anaerobic treatment process. Typical water quality parameters such as soluble chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total suspended solids (TSS) are not sufficient to comprehend the particles hydrolysis, which is the limiting step in the process. A better understanding of the effect of the particle fraction on the treatment can be obtained by the characterization of the dissolved organic carbon (DOC) by means of size exclusion chromatography coupled with an online dissolved organic carbon detection (SEC-OCD). By using this method, the composition changes of the soluble hydrolyzed fractions of the organic matter can be monitored during the different processes.

Laboratory experiments with particle concentrations of 1 g/L TSS (particle size: 25 to 250  $\mu\text{m}$ ) showed accumulation of soluble COD and DOC between 8 and 48 h, suggesting a production of slowly or non-biodegradable material.

The left Figure shows SEC chromatograms obtained after 8 and 48 h. They can be divided into four fractions (F1 to F4) related to decreasing molecular size; the DOC distribution of the four fractions is given on the right. It is obvious that major changes occur after 36 h where there is a strong DOC increase. The relative DOC distribution in the different fractions is changing within the time investigated; it is quite similar until 16 h, while F4 is decreasing after 36 h and increasing again after 48 h. Further studies, including experiments under anaerobic conditions, will be performed to provide more information about the significance of different particle size fractions for the character of the hydrolyzed organic matter during the different treatment steps.



Left: Size exclusion chromatograms of hydrolyzed chromatographable DOC (cDOC) after 8 and 48 h. Right: cDOC in the four fractions during the aerobic treatment of municipal waste water particles after 8, 16, 36 and 48 h (corresponding cDOC, mg/L: 4.0, 4.7, 7.4 and 9.1).



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Stephanie West

Gudrun Abbt-Braun

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## Productive Biofilms



Laure Cuny



Andrea Hille-Reichel

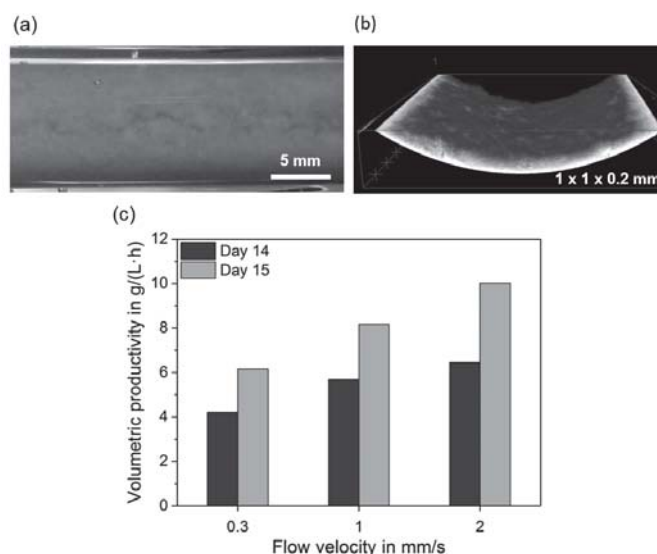
The application of biofilm technology for the production of both low-cost and valuable compounds allows the white biotechnology to benefit from the advantages of biofilms compared to suspended cultures. In this study, we focus on the production of optically pure lactic acid. Recently, the interest in lactic acid has greatly increased, especially due to the synthesis of polylactic acid, a biodegradable and biocompatible polymer. However, the high costs of lactic acid are responsible for its low competitiveness to petroleum-derived plastics.

For the production of lactic acid, we cultivate a monoseptic biofilm consisting of bacteria with a strong preference for planktonic growth (provided by BASF SE). Biofilm growth is achieved in a glass tube reactor (Figure a). The biofilm system can be cultivated in a continuous mode and kept monoseptically for at least 3 weeks. It can be shown that the higher cell densities lead to a significantly increased space-time yield compared to the planktonic culture. Thus, the use of biofilms proves to be a promising method to increase the productivity and thereby reduce production costs of lactic acid.

For a better process understanding and optimization, biofilm formation and structure (e.g., biofilm thickness and wall coverage) are investigated by optical coherence tomography (OCT), a non-invasive imaging tool that can be applied *in situ* during reactor operation without disturbing the system or process (Figure b).

The analysis of biofilm performance at different flow conditions revealed that productivity increases with the flow velocity (Figure c). This can be explained by the decreased retention time at higher flow velocities and, thus, a minor pH drop caused by the released lactic acid. At low flow velocity, the pH drops to a value where growth and production are significantly inhibited. The increase in production from day 14 to day 15 results from the growth of the biofilm.

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(a) Biofilm in tube reactor. (b) OCT image of biofilm. (c) Volumetric productivity for different flow velocities on two different cultivation days.

## Application of a Membrane Biofilm Reactor for Biological Methanation

In this joint project, we investigate the applicability of membrane biofilm reactors (MBfR) to anaerobic biological methanation. The idea is to grow a biofilm of hydrogenotrophic methanogenic archaea on a membrane submerged into a streaming liquid phase, while delivering the gaseous substrates  $H_2$  and  $CO_2$  via the membrane. Thus, we try to overcome the limitations of low mass transfer rates of hydrogen into the liquid phase, since hydrogen is directly transferred to the biofilm (bubble-free). We expect to be able to increase turnover efficiency significantly due to the reduction of losses of substrate gases. A second promising feature of the MBfR is that the slowly growing hydrogenotrophic methanogens are immobilized in the film. As has been shown for other biofilm reactor systems, this does not only increase cell density in the system, but is also predicted to enhance resilience and durability of the process.

In technical MBfRs, normally hollow fiber membranes (outer diameter  $d < 1$  mm) are used because of their high specific surface area. Instead, we constructed a reactor which contains a bundle of tubular membranes ( $d = 9$  mm; Figure a) in order to be able to determine the behavior and growth of the biofilm by means of optical coherence tomography (OCT).

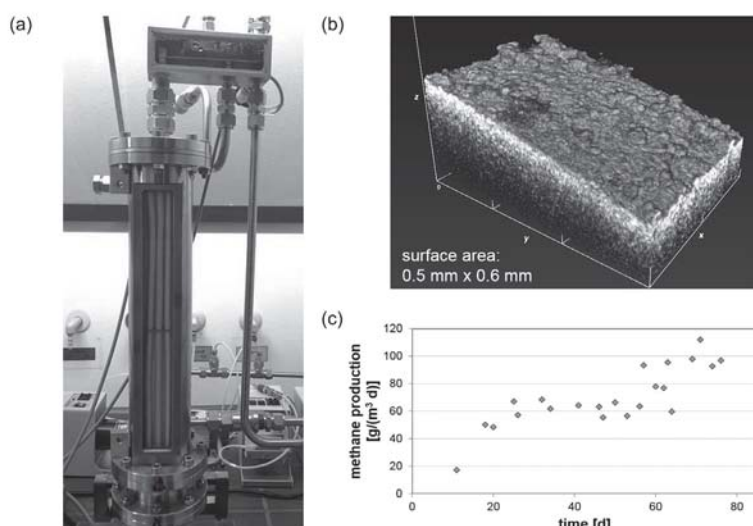
In a first run, the reactor was operated similarly to a biogas plant and fed with an artificial medium containing glucose, yeast extract and peptone as additional (organic) carbon sources. We could prove biofilm development (Figure b) and methanation activity (Figure c), yet the methane was at least partly produced by the microorganisms suspended in the bulk phase, probably mainly by acetoclastic archaea. Currently, the process is investigated with regard to the selection of hydrogenotrophic autotrophs, which implies that the carbon source is limited to  $CO_2$ .



Isa Heiler

Stephanie West

Andrea Hille-Reichel



(a) Photograph of the membrane module with gas trap (on top). (b) OCT image of biofilm on a tubular membrane. (c) Methane production rate per reactor volume during a course of 77 d operation.

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Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam-Bornim, Germany

## Microbial Electrosynthesis for Production of Bioplastics from Flue Gas – BioElectroPlast



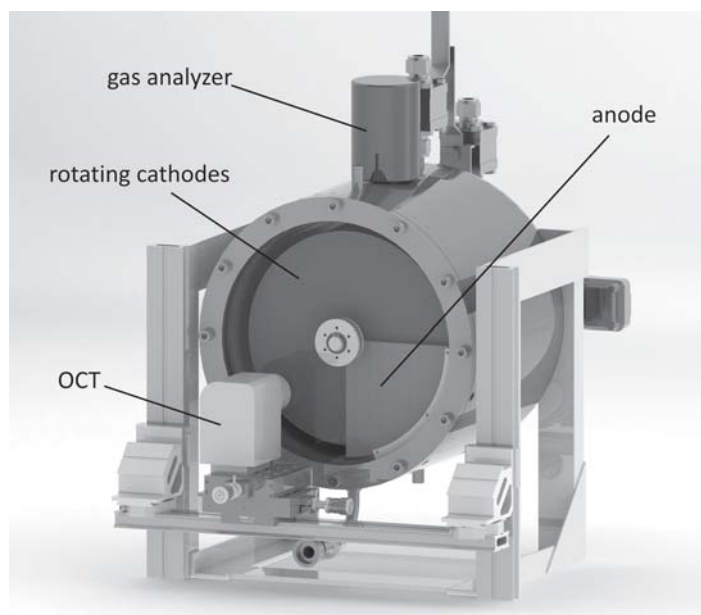
Max Hackbarth

Andrea Hille-Reichel

Michael Wagner

The aim of the project BioElectroPlast is the use of carbon dioxide as feedstock for the production of the biopolymer polyhydroxybutyrate (PHB). Therefore, electrolithoautotrophic microorganisms, which directly use electricity as electron source, are grown on cathodes in so-called microbial electrosynthesis cells (MECs). These MECs are usually designed as small-scale H-Cells or as batch reactors with undefined flow conditions. A challenge of this type of process is to provide the biofilm with the gaseous substrates. To overcome these limitations, the idea of this project is the construction of a gas-liquid contactor for the technical realization of this biotechnological production process.

Below, a 3D rendering of the gas-liquid contactor is shown. The biofilm is growing on rotating cathode discs made of graphite. The reactor is filled half with a mineral solution and half with a mixture of the gaseous substrates carbon dioxide and oxygen. As uptake and conversion of substrates and electrons are strongly dependent on the structure of the biofilm, three-dimensional visualization will be performed with optical coherence tomography (OCT) to correlate the biofilm structure with the biopolymer production. Hence, the shape of the anodes is designed as a quadrant, so the biofilm can be visualized in the gas and the liquid phase through the front window. To realize the harvest of the PHB-loaded biomass from the cathodes, the gas-liquid contactor can be equipped with a contrivance for mechanical abrasion.



3D rendering of the gas-liquid contactor (volume 10 L). OCT = optical coherence tomography.

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## Development of a Multi-Physics Biofilm Model Incorporating Biofilm Mechanical and Structural Characteristics from 3D Imaging Datasets

To understand the mechanical behavior of biofilms (e.g., deformation at certain shear stress levels), it is necessary to get more insights into their material and mechanical properties. Studies showed that, for example, different iron concentrations and shear stress levels can have diverse effects on the biofilm structure and, thus, their mechanical properties. To date, mechanical characterization of biofilms is performed invasively. Moreover, reliable estimations of parameters are challenging.

Thus, the standardization of methods and analyses as well as the application of multi-dimensional modeling for parametrization and interpretation of obtained data are in focus of the project. Numerical simulations are performed by our project partner at the Technical University of Munich, whereas biofilm experiments are conducted in our labs. In detail, different  $\text{Fe}^{2+}$  concentrations and shear stress levels will be applied to investigate the development, fluid-structure interactions and detachment of *Bacillus subtilis* biofilms.

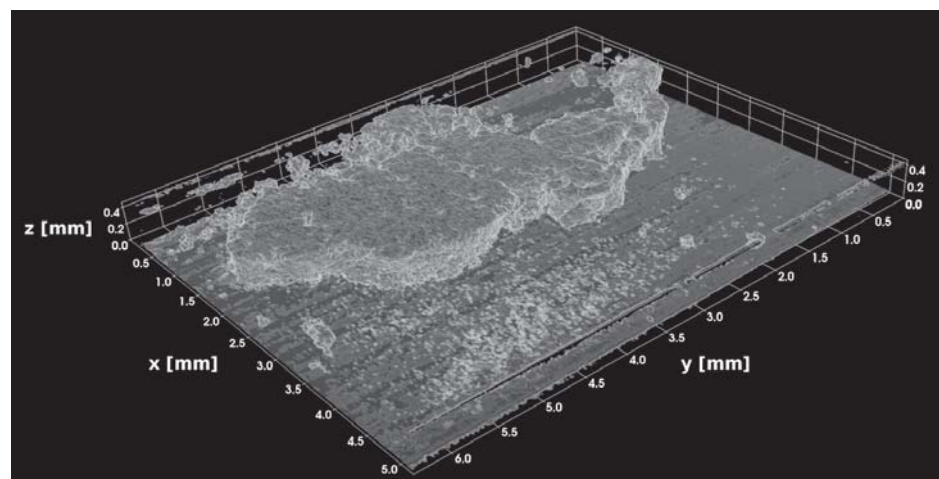
Initial experiments were used to optimize the experimental and imaging setup as well as substrate and nutritional conditions. First results revealed that flow cells are suitable to grow *B. subtilis* biofilms, which was denied in the past. A 3D rendering of a biofilm grown in a flow cell is shown in the Figure below.

Further experiments will include deformation measurements to determine material properties from 3D imaging datasets acquired by means of optical coherence tomography (OCT) before, during and after the deformation. Thereby, biofilms are characterized *in situ* and non-invasively with respect to their structural and mechanical features.



Luisa Gierl

Michael Wagner



3D rendering of a *Bacillus subtilis* biofilm grown in a flow channel of  $50 \times 5 \times 0.45$  mm ( $L \times W \times H$ ) for 8 days. Visualization was performed *in situ* and non-invasively using optical coherence tomography (OCT). The biofilm spreads along the direction of flow (left to right) and fills the space between substratum and top of the flow channel. Grayscale intensity is not related to any feature of the biofilm. Raw imaging data has been post-processed for elucidation purposes.

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Partner:  
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Characterization of organic carbon in anaerobic and aerobic waste water treatment  
Decision support for the implementation of water technologies in arid areas  
Membrane filtration, waste water recycling  
Mechanical properties of biofilms (Dr.-Ing. since March 2017)  
Dissemination and spread of antibiotic resistance genes in the environment  
Aerobic granules for waste water treatment  
Productive biofilms  
Mechanical and material properties of biofilms  
Microbial electrosynthesis  
Productive biofilms  
Brackish water desalination  
Modeling of oxygen transfer in activated sludge systems  
Membrane filtration  
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Visualization of water and biofilms in porous media (starting 2018)  
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Reinhard Sembritzki, Max Hackbarth,  
Florencia Saravia  
(last row) Yiwen Liu (Guest Researcher),  
Matthias Weber, Axel Heidt, Rafael Peschke,  
Daniel Reinig, Oliver Jung, Nico Seeleib



## RECENT PUBLICATIONS

### Peer-Reviewed Journal Publications

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## Publication Series of the Institute

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Volume 69: Hack, N., 2016: Refraktäre organische Substanzen im Kapillarsaum: ihre Dynamik, Gradienten und Reaktionen.

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Volume 71: Qian, J., 2017: Investigation of the fouling driving factors in drip irrigation systems.

Volume 72: Blauert, F., 2017: Investigating biofilm deformation using optical coherence tomography and fluid-structure interaction simulation.

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Cuny, L., Hille-Reichel, A., Ödman, P., Horn, H., 2017. Long term cultivation and modeling of productive biofilms. DECHEMA Himmelfahrtstagung 2017 'Models for Developing and Optimising Biotech Production', 22 – 24 May, Neu-Ulm, Germany.

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## Others

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