Karlsruhe Institute of Technology

Topics in Water Chemistry and Water Technology

2016

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

ENGLER-BUNTE-INSTITUT

Dear colleagues and friends,

This year is marked by the loss of our longtime colleague Elly Karle (you can see her in the middle row, third person from right). Her passing in March left us all in sorrow and in disbelief that such a cheerful personality was taken away from us at such young age. Elly Karle was a highly respected person at the institute and we all still miss her being around.

Having this in mind, it is hard to report on the positive things which happened in 2016. I will thus mention only a few important events. Yes, we won new research projects and finished others. Three PhD students defended their theses and made a step into the next period of their lives. At the same time, we do have five new PhD students who started their projects.

Marius Majewsky left us and moved to Heidelberg University. I would like to thank him for the four years we could work together. We welcomed two postdoctoral researchers: Ewa Borowska joined our group to continue her work on organic micropollutants and Gisela Guthausen, the Head of the DFG Instrumental Facility Pro²NMR at KIT, has been associated to the Engler-Bunte-Institut.

This year, Gudrun Abbt-Braun was elected as President of the International Humic Substances Society (IHSS). I was elected as Dean of the Department of Chemical and Process Engineering.

The ABC (Advanced Biofilm Course) took place in Karlsruhe this year. Another course dealing with biofilms was given by Michael Wagner and myself in Frankfurt. The course is organized by the Water Chemistry Society (GDCh) every two years.

On the next pages, you will find a more detailed report on the projects and activities of our group in 2016.

I wish you all the best for 2017

Harald Horn

DVGW

Water Research Network Baden-Württemberg

Ulrike Scherer

Tanja Stahlberger

Since January 2016, three interdisciplinary joint projects (so called ‘researcher networks’) have been funded in the framework of the Water Research Network by the Land Baden-Württemberg (Ministerium für Wissenschaft, Forschung und Kunst (MWK), Ministry of Science, Research and the Arts):

The researcher network **CHARM** ‘Challenges of Reservoir Management’ of the universities Stuttgart, Konstanz and Freiburg investigates the deposition of sediments in reservoirs, the growth of algae, the generation of methane gas emissions as well as socio-economic and ecological aspects of reservoir operation at the Schwarzenbachtal dam. The universities Heidelberg, Tübingen and the KIT are involved in the researcher network **Eff-Net** ‘Effect Network in Water Research’. Eff-Net studies the impact of micropollutant emissions (pharmaceuticals, food additives and their metabolites) on the aquatic environment. In addition, the awareness of the society for micropollutant emissions will be monitored. The objective of the researcher network **DRIeR** ‘Drought Impacts, Processes and Resilience’ of the universities Freiburg, Heidelberg and Tübingen is to investigate the interactions of climate, land use, hydrology and water governance during droughts by using data from past drought events, field experiments and simulations.

In January 2016, the Kick-off Symposium of the Water Research Network took place in Stuttgart. 190 participants from universities, research institutions, authorities and industry attended the event. Following the opening of the symposium by Ministerialdirigent Michael Kleiner from the MWK, the funded projects as well as various other aspects of water research were presented. The key note lecture was held by Prof. James Kichner from ETH Zürich.

During the year 2016, seven universities of applied sciences in Baden-Württemberg joined the network. The advisory board, comprising all member institutions of the network, met for the first time in June 2016. The focus of the meeting was on the importance of humanities and social sciences for water research and the enhanced integration of these disciplines in future research projects.

Impressions of the Kick-off Symposium of the Water Research Network in Stuttgart on 14 January 2016 .

Funding:

Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg (MWK)

Partners:

University of Freiburg

Heidelberg University

University of Hohenheim

University of Konstanz

University of Stuttgart

University of Tübingen

Biberach University of Applied Sciences

Furtwangen, University

Karlsruhe University of Applied Sciences

Hochschule Konstanz University of Applied Sciences

Offenburg University of Applied Sciences

University of Applied Sciences Ravensburg-Weingarten

Stuttgart University of Applied Sciences

For further details see: www.wassernetzwerk-bw.de

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

Standardization activities to update 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', were continued in 2016. At present, about 10 projects on national level and 20 ISO projects under German lead are in progress in the technical committee DIN NA 119-01-03 AA 'Water examination'. For determination of sulfide by gas extraction, a method using electrochemical detection of H_2S in the gas phase has been developed; for the optional photometric detection, a more stable calibration substance than in the old version of the standard has been specified. The standard on determination of sewage sludge COD is being amended by a procedure for non-dried samples in order to better allow for the analysis of co-substrates with low softening points. The dilution and seeding method for BOD_n determination is also under revision. In the field of organic trace analysis, LC-MS/MS methods are being standardized for the determination of pharmaceutical ingredients, polyfluorinated alkyl substances (PFAS) or microcystins. As German mirror committee, the committee 'Water examination' dealt with about 70 further standardization items initialized by other members of ISO/TC 147 'Water quality' or CEN/TC 230 'Water analysis' by delegating experts to their meetings in Sydney and Delft and commenting on draft documents.

Birgit C. Gordalla

Funding:

Water Chemistry Society (Division of the Gesellschaft Deutscher Chemiker e. V., GDCh)

The Umweltbundesamt (UBA)

Instrumental Facility Pro^{2}NMR

The year 2016 brought diverse tasks and changes to the instrumental facility Pro^{2}NMR. From the scientific point of view, the main projects were the investigation of oil aging and dispersed systems, the *in situ* measurement and interpretation of filtration processes in hollow fiber membranes, the characterization of new contrast agents and polymer solutions as well as the detection of the influence of shear on polymer states. The activities were performed in close collaboration of several institutes at KIT, including all modalities of NMR (nuclear magnetic resonance), namely spectroscopy, imaging, diffusometry and relaxometry at different magnetic fields. Imaging of biofilms and their interaction with the local environment resulted in two publications and will be pursued in 2017.

Apart from the scientific efforts, which are also summarized in several master and bachelor theses, the highlight of the year was the installation of a new imaging hardware, which brings us up to date and opens the door to methodical developments concerning fast imaging and semi-solid state imaging. The 'MR in Food 2016' conference was held in Karlsruhe and led to new cooperations. In autumn, a µCT scanner (micro-computed tomography) was installed, which allows complementary studies to MRI (magnetic resonance imaging).

Gisela Guthausen

Funding:

German Research Foundation (DFG)

The Land Baden-Württemberg

Partners:

Several institutes at KIT, national and international research institutions

Assessing the Influence of Biofilm Surface Roughness on Mass Transfer by Combining OCT and 2D Modeling

Chunyan Li

Michael Wagner

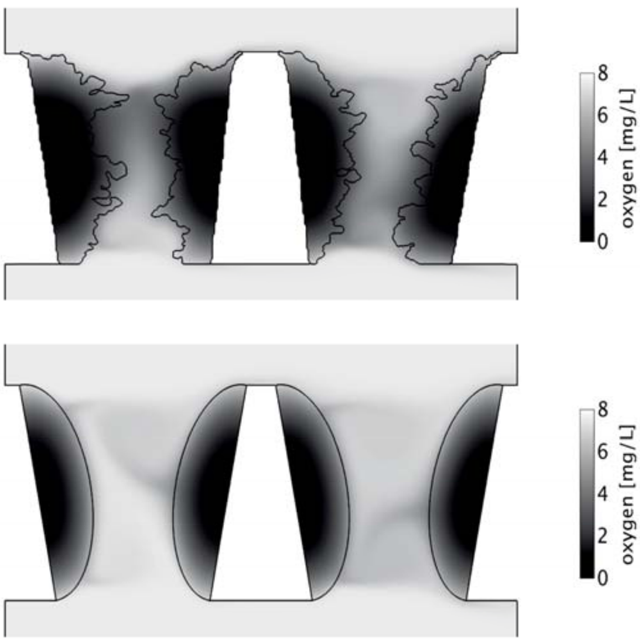
Among the various approaches in biofilm research, imaging and modeling are the two major ones to understand the physical and biochemical processes involved in biofilm development. Typically, biofilm imaging and mathematical modeling are used separately. We developed a methodology which combines both tools.

A heterotrophic waste water biofilm cultivated on plastic carriers in a moving bed biofilm reactor was used as model biofilm. Biofilm imaging was conducted at the mesoscale by means of optical coherence tomography (OCT). The acquired imaging data served as structural template within a 2D biofilm model. Thereby, the impact of the biofilm structure on local mass transport and transfer was revealed. Additionally, a simplified smooth geometry was created to investigate the influence of the heterogeneity of the bulk-biofilm interface on substrate transport phenomena. Fluid flow, transport/transfer and biochemical conversion of substrates were incorporated into the model, simulating the hydrodynamics in the vicinity of biofilm surface as well as the substrate distribution. The results depict in detail the variation of hydrodynamics and mass transfer characteristics at the bulk-biofilm interface.

Comparison between the real, heterogeneous and the simplified but smooth biofilm structure revealed that the real structure allowed higher substrate fluxes (i) when diffusion was dominating as well as (ii) at elevated flow velocities along the biofilm surface.

Funding:

Helmholtz Atmosphere and Climate Program (ATMO)



Simulated dissolved oxygen concentration field for real (above) and simplified (below) biofilm geometry, respectively. The flow direction is from left to right, the inflow velocity equals 0.05 cm/s. Dissolved oxygen concentration in the inflow equals 8 mg/L. Subfigure cross-sections have a dimension of 2.8 × 1.4 mm^2.

Interaction of Biofilm Systems and Suspended Solids

Fabian Brunner

Chunyan Li

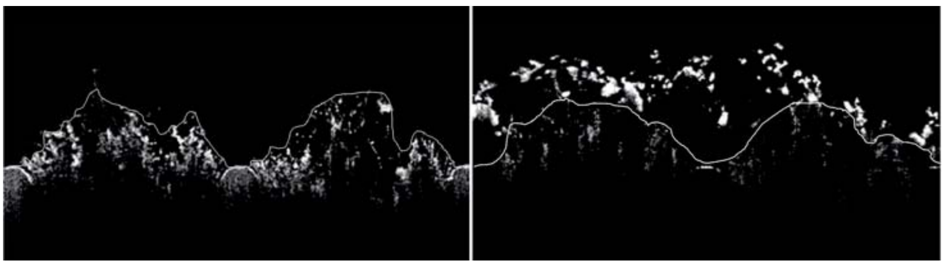
The chemical oxygen demand (COD) in municipal waste water is largely linked to particulate matter. In this research, the impact of the deposition of certain particle fractions onto biofilms was investigated. Two methods for biofilm characterization were combined: optical coherence tomography (OCT) and microsensor measurements.

Particles from waste water of a local treatment plant were separated into different size fractions, namely 28 – 45 µm, 45 – 100 µm, 100 – 250 µm and 250 – 500 µm. Heterotrophic biofilms growing on carriers were placed in a lab-scale flume, in which tap water with additional glucose (COD: 300 mg/L O_2) was recirculated. After 1 h of adaptation to the conditions in the flume, OCT images of the biofilm were taken and oxygen profiles over the biofilm depth were measured. Afterwards, the particles of the different size fractions were added into the flume. For each particle size fraction two experiments were conducted, one with OCT imaging and one with microsensor measurements.

As an example, the OCT images for the fraction of 100 – 250 µm are shown in the Figure (left side: before particle deposition, right side: after particle deposition). As can be seen from the images, the biofilm and the deposited particles can be clearly distinguished. Moreover, a slight compression of the biofilm was observed. In addition, the volume of particles and the mean particle layer thickness (between 250 – 350 µm) were extracted from the images.

Based on the microsensor measurements, a decrease of the oxygen flux (from the surface into the biofilm matrix) of around 20 %, caused by the layer of deposited particles, was observed.

In conclusion, this experiment showed that the deposition of particles on a biofilm can be visualized in detail by using OCT imaging and that the particle layer influences the biofilm surface structure. Additionally, the particle layer physically hampers oxygen transport to the biofilm and decreases the oxygen flux within the biofilm. Therefore, substrate conversion rates might be reduced and even the microbial community may be affected in the long run.



OCT images of the biofilm before (left) and after (right) particle deposition. The white outline illustrates the biofilm/liquid interface.

Funding:

German Research Foundation (DFG)

Autogenerative Two-Phase High Pressure Fermentation (AG-HiPreFer)

Marc Tuczinski

Florencia Saravia

High pressure fermentation would allow a direct feeding of the produced methane into the national gas grid. In order to achieve this goal, a fixed bed methane reactor should be operated under high pressures of up to 100 bar. The fixed bed reactor has to be fed with a highly concentrated influent, the so-called percolate, containing volatile fatty acids as the best substrate for the methanogenic bacteria. The percolate can be produced by a thermophilic hydrolysis stage (55 °C) fed with renewable energy crops like corn.

A well-known problem for such two stage biogas production systems is the liquid-solid separation after the hydrolysis step. Within the project, different aspects of the application of microfiltration to separate the particulate material from dissolved organic components are evaluated. Research activities include membrane process selection and investigations of fouling, membrane performance and long-term-behavior of the filtration process.

Series of studies were performed to select the suitable type of membrane material and design as well as the optimal mode of operation, either crossflow or submerged. Ceramic membranes proved to be the better option to treat the percolate due to their chemical and temperature resistance. The crossflow filtration ended up with a sustainable flux between 12 and 17 L/(m^2h), which could be kept stable over several weeks. Fouling could be controlled with the induced shear by operating the system under high crossflow velocities of more than 1 m/s. Critical flux measurements (see Figure) with submerged membranes showed that the highest sustainable flux is approx. 7 L/(m^2h), which is much lower than the flux of the crossflow system. Further analyses of the percolate and the permeate showed that the rejected total organic carbon (TOC) fraction and thereby the loss of organic carbon in the permeate does not significantly reduce the methane yield. Current investigation focuses on the optimization potential of the submerged membrane operation.

Funding:

Federal Ministry of Education and Research (BMBF)

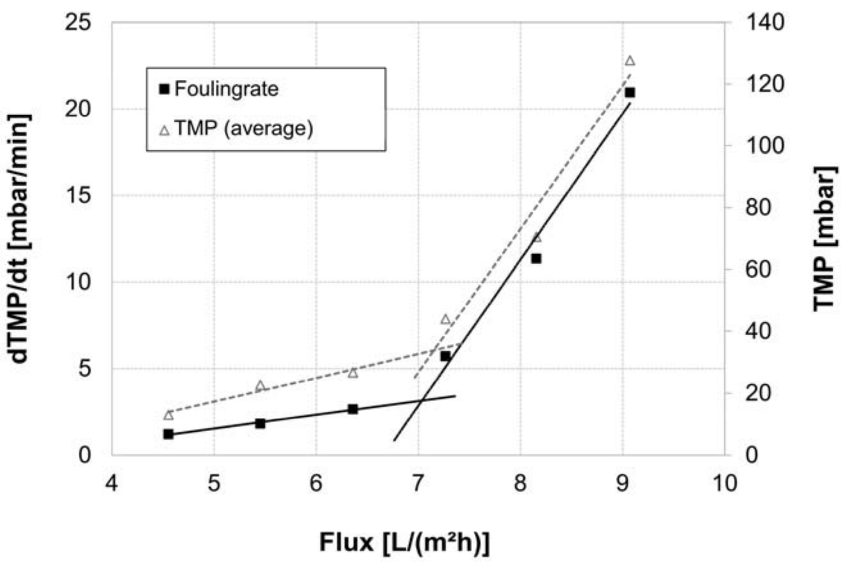
Partners:

University of Hohenheim, State Institute of Agricultural Engineering and Bioenergy

DVGW Research Laboratory at the EBI of KIT, Division Gas Technology

Johannes Gutenberg University Mainz, Institute of Microbiology and Wine Research

BIOFAR-EVA, Belvaux, Luxembourg



Critical flux measurements in the submerged membrane system (TMP is transmembrane pressure).

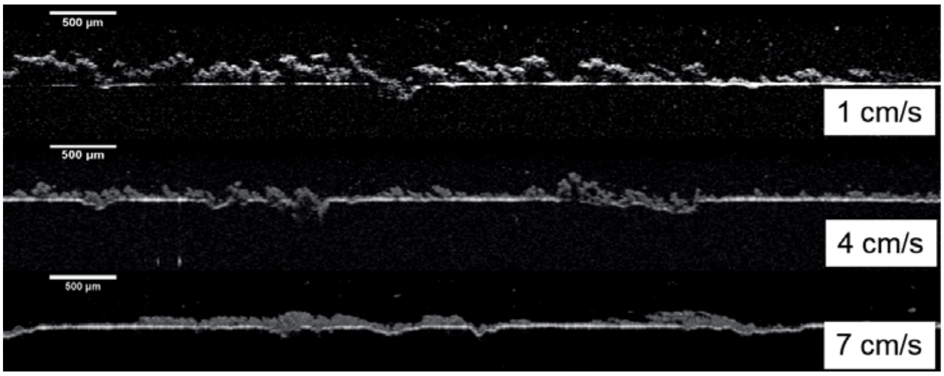
Influence of Flow Velocity on the Structure of Biofilm Growing in Microbial Fuel Cells Using Municipal Waste Water as Feed

Dominic Breitkopf

Michael Wagner

The structure of a biofilm is governed by the flow and nutritional conditions of the growth environment. In microbial fuel cells (MFC), the biofilm structure is linked to the electrical power production. Hence, MFCs were designed to obtain three-dimensional structural information about biofilms by means of optical coherence tomography (OCT). In an experiment, three MFCs were operated with municipal waste water from the waste water treatment plant Karlsruhe at mean flow velocities of 1 cm/s, 4 cm/s and 7 cm/s, respectively. The chemical oxygen demand (COD) of the waste water ranged between 50 mg/L and 190 mg/L. During the experiment, the electrical power of each MFC was measured. MFCs were operated at their individual maximum power point.

The experiment aimed at understanding the relation between flow velocity and biofilm structure. OCT images from day 7 of the biofilm growing on the anode of the MFC are shown in the Figure. The influence of the flow velocity on the mean biofilm thickness is obvious: The higher the flow velocity, the more compact and less heterogeneous the biofilm structure. As a measure of the biofilm structure, the mean biofilm thickness was calculated. It was 175 µm at 1 cm/s, 48 µm at 4 cm/s and 38 µm at 7 cm/s, respectively. Unexpectedly, the electrical power output was almost independent of the flow velocity.



Biofilm grown at different flow velocities on anodes of microbial fuel cells. Flow direction is from right to left.

Funding:

Federal Ministry of Education and Research (BMBF)

Partners:

Clausthal Institute of Environmental Technology (CUTEC), Germany

TU Braunschweig, Germany

Clausthal University of Technology, Germany

Eisenhuth GmbH & Co. KG, Osterode, Germany

EURAWASSER Betriebsführungsgesell-schaft mbH, Goslar, Germany

For further details see: www.bio-bz.de

Impact of Inorganic Particles on the Stability of Biofilms in Drip Irrigation Systems

Jueying Qian

Michael Wagner

In (semi-) arid regions, treated waste water (TWW) is extensively used in drip irrigation. Organic and inorganic particles in the TWW influence the biofilm development as well as the fouling potential of the drip irrigation system. The objective of this study was thus to investigate the impact of inorganic particles on the structure and stability of biofilm inside the drippers.

In this study, 3D printed microfluidic devices (MFD) mimicking the internal dripper geometry (see sketch inserted in Subfigure a) were operated to assess the biofilm formation in detail.

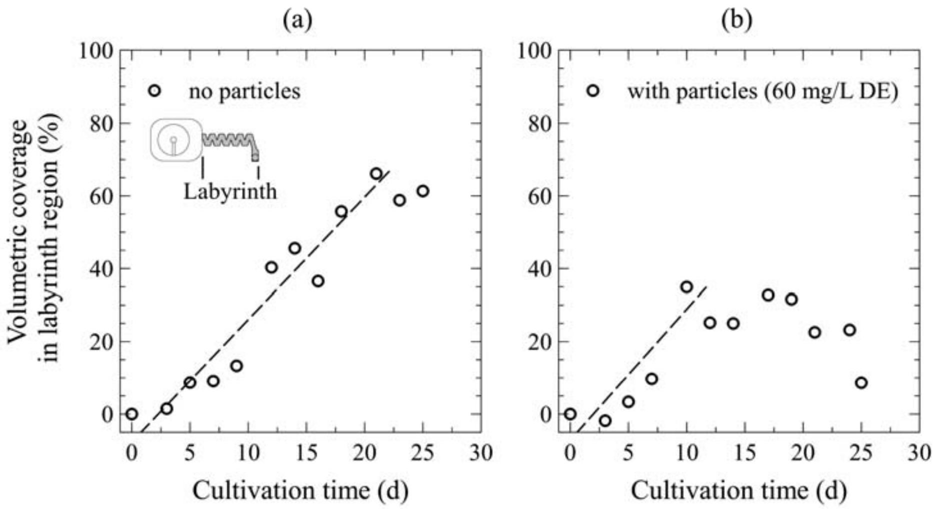
Biofilms were cultivated at ambient temperature using synthetic waste water as irrigation medium (chemical oxygen demand: 20 mg/L O_2). The volumetric flowrate during irrigation cycles was 1 L/h. Diatomaceous earth/diatomite particles (DE, dp_{50} = 36.5 µm) were suspended in the irrigation medium to simulate a total suspended solid (TSS) concentration of 60 mg/L. The biofilm formation inside the MFD was monitored over 25 days *in situ* and non-invasively by means of optical coherence tomography (OCT).

The fraction of biomass occupying the labyrinth compartment was quantified from OCT datasets. Subfigure (a) shows that a biofilm cultivated without particles accumulated steadily until day 24. With addition of 60 mg/L DE, the biofilm accumulated comparably until day 10. However, with ongoing cultivation, detachment events occurred, causing a decreasing volumetric coverage of the labyrinth compartment (see Subfigure b).

Results show a correlation between the TSS concentration in the irrigation medium and biofilm stability: The higher the TSS concentration, the lower the coverage of the dripper geometry. Results further indicate that biofilms cultivated at a certain concentration of inorganic particles seem to be unstable and susceptible to detachment compared to those formed without additional particles in the irrigation medium. Additional experiments with clay particles (TSS) support these initial findings (data not shown).

Funding:
Federal Ministry of Education and Research (BMBF)

Partners:
The Hebrew University of Jerusalem, Rehovot, Israel
Lagotec GmbH, Magdeburg, Germany
Netafim, Hanegev, Israel



Volumetric coverage of biofilms in the labyrinth region of a dripper. (a) Without addition of particles: biofilm accumulation rate = 3.4 %/d. (b) With addition of 60 mg/L DE: biofilm accumulation rate = 3.6 %/d.

Rejection of Trihalomethanes (THM) by NF Membranes

Di Peng

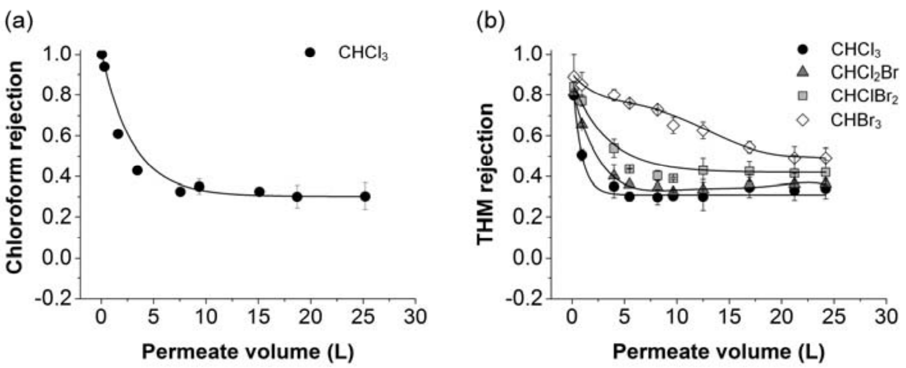
Florencia Saravia

Gudrun Abbt-Braun

Nanofiltration (NF) can be considered as an interesting alternative to remove unwanted disinfection by-products, especially trihalomethanes (THM). However, literature regarding the THM rejection by NF has been contradictory. In this work, the rejection of four THM by three commercial NF membranes of different materials was investigated, considering the effect of adsorption and organic fouling on the rejection performance.

Results indicated that, in general, NF membranes actually showed a limited rejection of THM, even when the molecular weight of the THM molecules was larger than the molecular weight cut-off (MWCO) of the membrane. MWCO alone does not seem to be an appropriate property to describe membrane rejection. NF90, the tightest investigated NF membrane, showed a steady-state rejection of 33 %, 36 %, 42 % and 49 % for chloroform, bromodichloromethane, dibromochloromethane and bromoform, respectively (see Figure).

The other two membranes, NTR-7470pHT and SB90, presented no THM rejection at steady-state. Adsorption has a significant influence on rejection, facilitating the mass transport of THM through NF membranes. The membrane material plays a substantial role in the intrinsic adsorption capacity and consequently influences rejection. The cellulose acetate membrane SB90 showed little adsorption capacity of THM but at the same time, THM could pass cellulose acetate very quickly. It can be assumed that the occupation extent of the available adsorption capacity in the membrane plays a significant role in how the adsorption facilitates the transport of molecules through the membrane and thus decreases membrane rejection. Natural organic matter in the feed solution and in the organic fouling layer had little effect on THM rejection. Organic fouling lowered the adsorption of less adsorptive THM due to blocking of the membrane surface.



Rejection of (a) chloroform and (b) four THM by the membrane NF90.

Funding:

German Environmental Foundation (DBU)

German Technical and Scientific Association for Gas and Water (DVGW)

Partner:

W.E.T. GmbH, Kasendorf, Germany

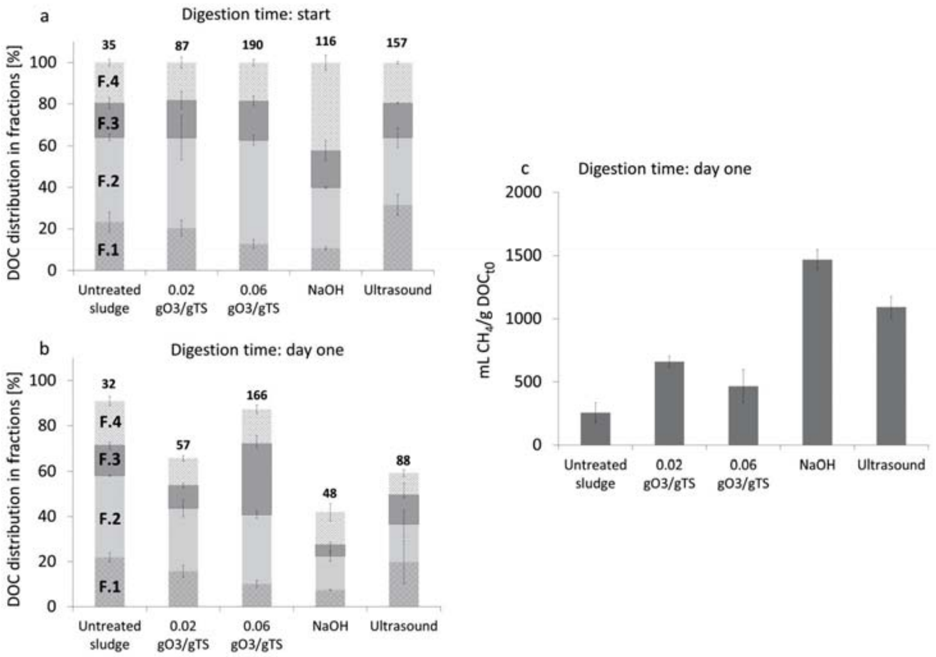
Comparing Different Sludge Disintegration Techniques and their Impacts on the Molecular Weight Distribution of Dissolved Organic Carbon (DOC)

Elham Fatoorehchi

Stephanie West

Hydrolysis of organic matter is the rate limiting step in anaerobic sludge digestion. To solubilize organic matter and enhance methane production, sludge flocs can be disintegrated before digestion. In this study, ozone, sodium hydroxide and ultrasound were selected for sludge disintegration. The performance of each method on methane production was studied in batch anaerobic digestion experiments. Sludge disintegration with sodium hydroxide and ultrasound increased methane production twice as much as with ozone. The changes in the molecular size of organic carbon during anaerobic digestion and their impacts on methane production were studied by size exclusion chromatography with online organic carbon detection (SEC-OCD).

Distribution of DOC in four fractions at the start of the anaerobic digestion and after one day is presented in Figures (a) and (b). In the reactor fed with the supernatant of the high ozonated sludge (0.06 g O_3/g TS), there is a transformation of intermediate molecules in F.2 to smaller molecules in F.3. This indicates that the type of organic carbon released during high sludge ozonation is not easily degradable and could explain the lower methane production compared to low ozonated sludge (0.02 g O_3/g TS). Sludge disintegration with sodium hydroxide highly improved methane production (Figure c). This could be attributed to the significant degradation of very small molecules in F.4 produced during treatment (Figures a and b). On the other hand, in the reactor fed with the supernatant of sonicated sludge, large and intermediate molecules in F.1 and F.2 were also highly degraded and significantly enhanced methane production. This indicates that organic carbon molecules produced by ultrasound are easily broken down and hydrolyzed by anaerobic bacteria.



DOC distribution in the four fractions during the anaerobic digestion of the supernatants of untreated and disintegrated sludge, (a) at the beginning and (b) after one day of anaerobic digestion experiments. Total DOC concentrations in mg/L are written above the columns. (c) Methane production during anaerobic digestion after one day.

Funding:

Federal Ministry of Education and Research (BMBF)

German Academic Exchange Service (DAAD)

Partners:

Hugo Vogelsang Maschinenbau GmbH, Essen/Oldendorf, Germany

Galilee Society Institute of Applied Research, Shefa-'Amr, Israel

Mekorot, Tel Aviv, Israel

Sustainable Management of Available Water Resources with Innovative Technologies (SMART-MOVE) – A Handbook on Brackish Water Usage

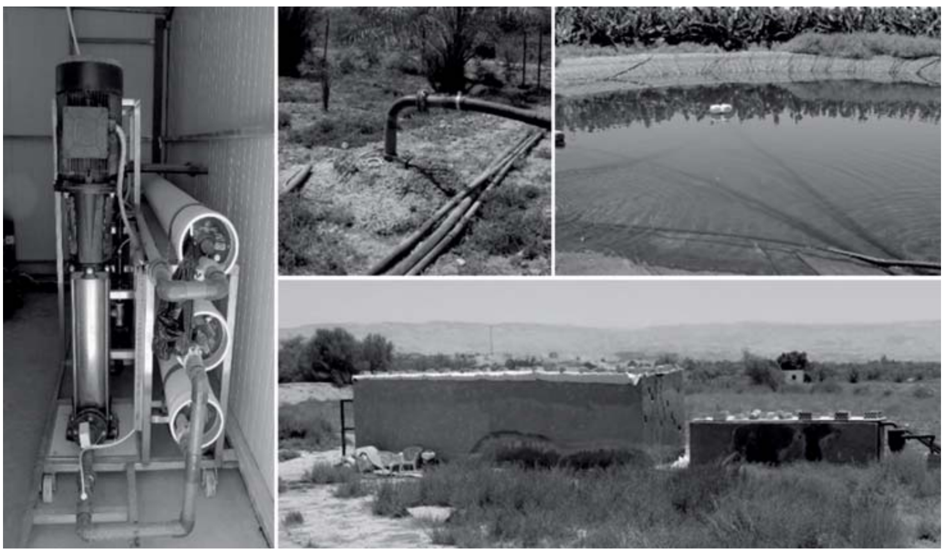
Oliver Jung

Florencia Saravia

Gudrun Abbt-Braun

In the framework of the BMBF funded SMART project, a handbook on brackish water usage in the lower Jordan rift valley (LJRV) is developed. The use of brackish ground water is necessary as the available quality of the water source is insufficient for current agricultural irrigation practices. The handbook is thought to provide standards for brackish water desalination and aims specifically at the possibilities present in the LJRV. A number of suppliers and operators of reverse osmosis (RO) units were visited in the region, from governmentally run drinking water supply stations to privately run farm stations. Water samples have been collected and analyzed in the lab to evaluate system performance and water quality. The data helps extracting key issues concerning operation and weigh the multiple challenges faced in the region.

The handbook focuses on small scale farm units as they have the biggest impact environmentally as well as economically. Most farmers have a similar demand of about 30 to 40 m³/d of treated water to supplement their irrigation water source. The total number of such RO systems in the area exceeds 50. The typical investment of such a unit is comparatively low (about 50,000 €) with a very basic design, minimal pre-treatment steps and minimal stages. These units are not designed to maximize product output and quality but rather to be functional with minimal operating efforts and minimal capital costs. The downside of such an approach is a smaller efficiency, smaller lifetime of membranes and system parts and thus higher operating costs and less product quality security. As a consequence, the analysis of the data shows that most systems do not run well either because of high salt concentration in the permeate or due to mechanical problems and leakages. However, any strategy for improvement has to take into account that these units are not run by qualified workers. The handbook will fill the gap and help operators to improve decisions on their RO systems.



From left to right: RO unit on a farm, well on site, typical product storing pond, container for RO unit on a farm.

Funding:

Federal Ministry of Education and Research (BMBF)

Partners:

Göttingen University, Germany

Helmholtz Centre for Environmental Research, Leipzig-Halle, Germany

KIT, Institute of Applied Geosciences, Germany

Mekorot Water Company Ltd., Tel Aviv, Israel

Tel Aviv University, Israel

Environmental and Water Resources Engineering Ltd., Haifa, Israel

Ministry of Water and Irrigation, Amman, Jordan

The University of Jordan, Amman, Jordan

Al-Balqa' Applied University, Amman, Jordan

Palestine Water Authority, Ramallah, Palestinian Territories

Ministry of Agriculture, Al-Bireh, Palestinian Territories

Al-Quds University, Jerusalem, Palestinian Territories

Fate of Pharmaceuticals in the Aquatic Environment

Alexander Timm

Ewa Borowska

Marius Majewsky

Funding:

German Research Foundation (DFG, D-A-CH)

Partners:

FHNW Basel, Switzerland

EAWAG, Switzerland

Betalactam antibiotics belong to the most used antibiotics in human and veterinary medicine. Thus, high amounts of these substances end up in the aquatic environment and undergo different degradation pathways. The present project focuses on the photolytic degradation of the four betalactam antibiotics amoxicillin, ampicillin, penicillin V and piperacillin. For the analysis of these substances and their transformation products (TPs), LC-MS/MS (liquid chromatography - tandem mass spectrometry) is used. In previous method development, limits of detection between 1 and 5 ng/L were achieved for water samples with direct injection. By using online solid phase extraction, the sensitivity could be improved up to 0.2 ng/L. Photolysis experiments with UV light (254 nm) showed that all analytes are photolytically degradable. For each compound, several transformation products were observed. The elucidation of their molecular structures is planned using QToF-MS (quadrupole time-of-flight mass spectrometry) measurements. Further research will focus on the investigation of the photolytical and biological transformation of the two antibiotics of last resort linezolid and tedizolid as well as on the elucidation and characterization of their metabolites.

Another task will concern the application of photocatalysis with modified ZnO nanoparticles induced by solar light for removal of selected pharmaceuticals from water. The aim of the study is to verify if this type of heterogenous photocatalysis may give higher removal efficiencies in comparison to the direct photolysis and if it induces formation of similar TPs.

Dissemination and Spread of Clinically Relevant Antibiotic Resistance Genes

Philip Brown

Funding:

German Technical and Scientific Association for Gas and Water (DVGW)

Partner:

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

Due to their extensive use, antibiotics are continuously introduced into the aquatic environment. 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 and are therefore subject to modern environmental microbiology. However, the question of how bacterial communities evolve and respond to antibiotic stress and selection pressure in river ecosystems has rarely been addressed. For this reason, the analysis of resistance genes and the impact of antibiotics on microbial populations are of fundamental environmental relevance and in the focus of this research. Particular attention will be paid to the quantification of mobile genetic elements.

The project aims at studying the absolute and relative abundance of clinically relevant antibiotic resistance genes and their carrier, how these genes are spread and in which genetic context they are found. Antibiotic resistance genes and mobile genetic elements will be quantified by using quantitative PCR (polymerase chain reaction) and digital PCR. Environmental sediment samples originating from river ecosystems before and after discharge of waste water will be analyzed and compared. Moreover, with the help of lab-scale sediment reactor experiments, the impact of antibiotics on natural bacterial populations will be characterized and genetic events occurring in natural biofilms under selection pressure will be promoted and investigated.

Advanced Characterization of Refractory Organic Matter in Raw Waters

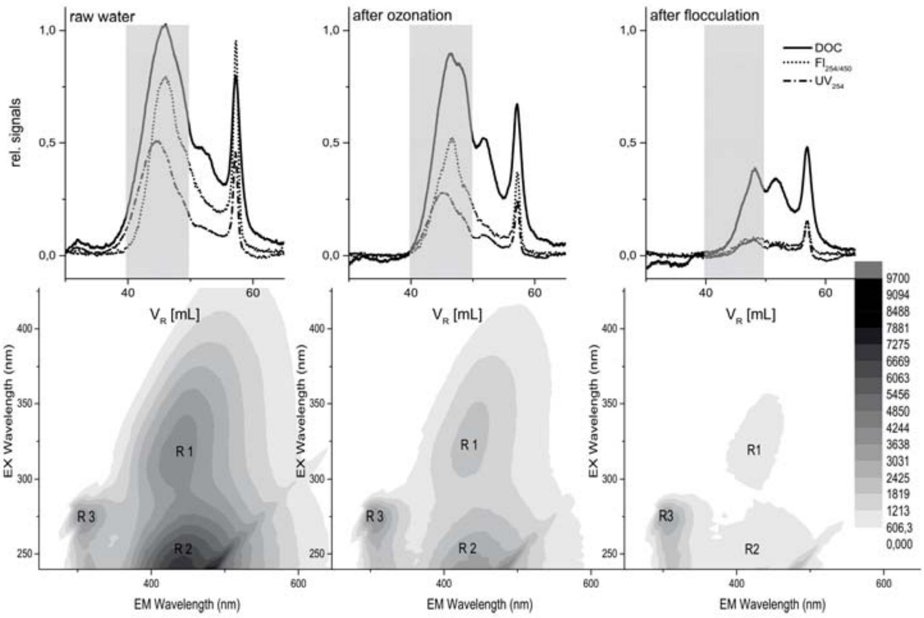
The GROM-project aimed for the advanced characterization of different kinds of raw waters used for drinking water production (ground water, river water, reservoir water) and for the study of changes in the DOC quality during the treatment steps. The analysis was performed by means of size exclusion chromatography (SEC) coupled with UV (254 nm), fluorescence (FLU) (λ(excitation, EX) = 254 nm, λ(emission, EM) = 450 nm) and organic carbon (OC) detection and by excitation-emission (3D-EEM) fluorescence spectroscopy.

The Figure shows the 3D-EEMs for raw water from a reservoir (1.2 mg/L DOC), after ozonation (1.0 mg/L DOC) and after flocculation ($FeCl_3$, 0.4 mg/L DOC). There are three dominant peaks observed: humic and fulvic organic matter (R1: EX(330 to 350 nm), EM(420 to 500 nm); R2: EX(250 to 260 nm), EM(380 to 480 nm) and protein- and algal-derived organic matter (R3: EX(250 to 280 nm), EM(280 to 350 nm)).

During ozonation, the fluorescence chromophores related to humic substances are decreasing, whereas the chromophores of protein- and algal-derived substances are not influenced (3D-EEM). The SEC shows that the molecular size of the DOC is slightly decreasing in the oxidation step and that there is a rise of smaller molecular weight organic acids. After flocculation, the high molecular size and UV(254 nm)-absorbing compounds related to R1 and R2 (humic substances) were almost eliminated. In contrast, the protein- and algal-derived substances cannot be removed by the flocculation step. The remaining DOC is low in molecular size and shows fluorescence chromophores for protein- and algal-derived organic matter.

Gudrun Abbt-Braun

Fritz H. Frimmel



3D-EEM of reservoir water, after ozonation and after flocculation (below) and the corresponding SEC chromatograms (OC, UV(254 nm) and FLU(254 nm/450 nm) detection (above)).

Funding:

German Technical and Scientific Association for Gas and Water (DVGW)

Partner:

DVGW Water Technology Center (TZW), Dresden Branch, Germany

Identifying the Poisson's Ratio of Biofilms by means of Optical Coherence Tomography (OCT)

Florian Blauert
Michael Wagner

Biofilm modeling can be used, for instance, to predict biofilm deformation. However, to perform such simulations correctly, experimental studies have to be conducted to access the mechanical properties of biofilms. A technique capable of visualizing biofilm structures of several mm³ at a high resolution of a few µm thus becomes necessary. These requirements are fulfilled by optical coherence tomography (OCT). OCT has several advantages over other imaging techniques used with biofilms: No staining is required, it is non-invasive and is applicable *in situ*. Moreover, OCT acquires images really fast. Two-dimensional cross-sections through biofilm structures are obtained within milliseconds and whole volumetric representations within seconds.

OCT was thus used in time-lapsed deformation experiments to determine mechanical properties of biofilms, namely the shear modulus G as well as the Young's modulus E. These parameters measure the rigidity and elasticity of the biofilm structure. Another important mechanical parameter used in biofilm modeling is the Poisson's ratio ν. It is the fraction of the longitudinal over the transverse elongation of biofilms under deformation. Valid estimates of this parameter for biofilms are lacking. For biofilm, the Poisson's ratio is assumed to be in the range of 0.3 – 0.5. Fortunately, the Poisson's ratio relates the shear and Young's modulus. By plotting E over G, the Poisson's ratio is derived from the slope of the linear fit as shown in the Figure. In this study, deformation experiments with several heterotrophic waste water biofilms (cultivated with acetate, glucose or phenylalanine as substrate) were performed to calculate G and E as well as to determine the Poisson's ratio. The Poisson's ratio of these biofilms was estimated to be ν = - 0.31. This finding may cause a change in paradigm as for biofilms, a Poisson's ratio ν > 0 has been assumed for decades.

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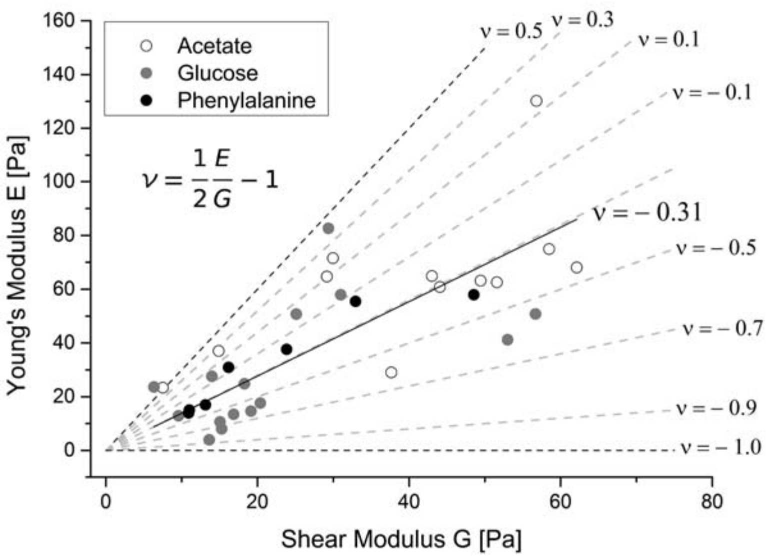
For further details see:
https://blogit.itu.dk/evoblissproject/



The slope of the linear fit (solid line) gives the Poisson's ratio ν of - 0.31 for the heterotrophic waste water biofilms (cultivated with three different substrates) being investigated. In theory, the Poisson's ratio can take values between - 1 and 0.5 (dashed lines).

Productive Biofilms

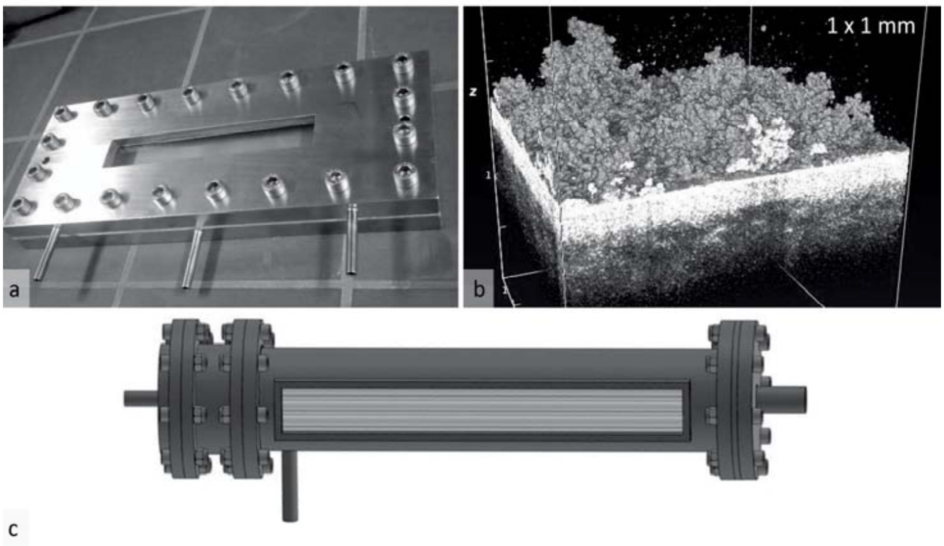
The production of valuable compounds in white biotechnology is usually accomplished with suspended cultures. Recently, many approaches have been made to use biofilm processes to benefit from the following advantages: biomass retention within the production system, resulting in higher space-time yield in continuous culture and lower susceptibility to unfavorable growth conditions. The potential of productive biofilms is investigated in two projects: The first project compares the productivities of monocultures cultivated as planktonic cells in batch mode on one hand and as biofilms in continuous mode on the other. We are mainly interested in product yield and product inhibition and their correlation with the biofilm structure (density, thickness). The impact of different process conditions and the type of substratum on biofilm formation are investigated by means of optical coherence tomography (OCT).

The second project focuses on the biotic production of methane from H_2 and CO_2 by digested sludge microorganisms in anaerobic membrane biofilm reactors (MBfR). The biofilm is grown on the membrane surface, in which it is exposed to a liquid phase containing mineral nutrients. Via the membrane, the cells are provided with gaseous CO_2 and H_2. Two different types of reactors have been constructed: a flow cell with a flat sheet membrane (Figures a and b) and, at a larger scale, a reactor with tubular membranes (Figure c). Results show that biofilm can easily be cultivated on the membrane, whereas it is not trivial to select for autotrophic methanogens. To favor these organisms over, e.g., autotrophic acetogens or sulfidogens, cultivation conditions have to be carefully adapted.

Laure Cuny

Isa Remdt

Andrea Hille-Reichel



(a) Flow cell with flat sheet membrane. (b) OCT image of biofilm on the flat sheet membrane. (c) Reactor with tubular membranes.

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Determination of Microplastics in Waste Water Samples Using Thermal Analysis

Marius Majewsky

Funding:
German Technical and Scientific Association for Gas and Water (DVGW)

Partner:
Elisabeth Eiche, KIT, Institute for Applied Geosciences (AGW), Germany

Microplastics – mostly defined as particles < 5 mm – are increasingly detected in the aquatic environment. To identify the polymer types, the most widely used method is Fourier-transform infrared spectroscopy (FT-IR). However, it requires particles to be identified one by one. In this study, a cost-effective and straightforward method was developed to determine the mass concentrations of polymer types. Characteristic endothermic phase transition temperatures were determined for seven plastic polymer types using thermogravimetry coupled to differential scanning calorimetry (TGA-DSC). By means of this method, extracts from waste water samples were analyzed.

Results showed that among the studied polymers, only polyethylene (PE) and polypropylene (PP) could be clearly identified, while the phase transition signals of the other polymers largely overlap each other. Subsequently, calibration curves were run for PE and PP for qualitative measurements. 240 and 1540 mg/m³ of solid material (12 µm to 1 mm) were extracted from two waste water effluent samples of a municipal waste water treatment plant of which 34 % (81 mg/m³) and 17 % (257 mg/m³) could be assigned to PE, while PP was not detected in any of the samples. The presented application of TGA-DSC provides a complementary or alternative method to FT-IR analyses for the determination of PE and PP in the environment.

Characterization of Dissolved Organic Carbon (DOC) and Organic Nitrogen in Water by means of Size Exclusion Chromatography (SEC)

Nico Seeleib

Alondra Alvarado
Gudrun Abbt-Braun

Funding:
German Technical and Scientific Association for Gas and Water (DVGW)
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Partner:
DOC-Labor, Dr. Huber, Karlsruhe, Germany

About 80 to 90 % of nitrogenous matter excreted by mammals is urea. It is by far the most important synthetic fertilizer and also an ingredient of many consumer products, including soaps and skin care products. Urea is not considered as harmful.

There are well-known approaches to detect urea in water, by photometry or enzymatic reaction. A new online-analyzer for urea has been developed by the DOC-Labor Dr. Huber. The urea in aqueous solutions is separated from other organic and inorganic compounds by size exclusion chromatography (SEC). Due to the non-ionic and hydrophilic character of urea, it can be separated from other nitrogen compounds like nitrate and ammonium. After oxidation of urea to nitrate at 185 nm within a special reactor (the so-called DONOX-reactor), it can be analyzed by a UV detector at 220 nm in the low µg-range.

The objective of this research is thus to study the dynamics of urea in natural environmental compartments as well as in the water treatment process. Furthermore, the enzymatic degradation of urea with urease will be studied in the µg-range with a close look at the kinetics.

Furthermore, SEC with online organic carbon and nitrogen detection can also be used to conduct a comprehensive study of the molecular size fractions of organic carbon during waste water treatment in both anaerobic and activated sludge systems.

Development of a Multi-Physics Biofilm Model Incorporating Biofilm Mechanical and Structural Characteristics

The EVOBLISS project revealed the influence of the growth conditions (e.g., substrate, hydrodynamics) on the mechanical characteristics of biofilms (i.e., Poisson's ratio). The objective of this recently funded project is thus to investigate in detail the influence of hydrodynamic and nutritional conditions on the mesoscale behavior of biofilms. By combining experimental and computational strengths, a comprehensive biofilm model will be developed that enables the reliable prediction of biofilm growth, architecture, interaction with the surrounding fluid and detachment for a wide range of different conditions. Visualization data acquired by means of optical coherence tomography will be transferred to the computational model and serve as basis for the determination of biofilm material properties using an inverse analysis approach. The multi-physics model will be utilized to gain more insights into mesoscale biofilm dynamics for a wide range of different conditions not necessarily realized experimentally.

Michael Wagner

Funding:

German Research Foundation (DFG)

Partner:

Technical University of Munich, Institute for Computational Mechanics, Germany

Microbial Electrosynthesis for Production of Bioplastics from Flue Gas (BioElectroPlast)

The use of the waste material CO_2 as feedstock for biotechnological production processes is highly interesting. Biotically catalyzed use of CO_2 usually requires organisms which convert solar energy or energy-rich molecules. Recently, it has been shown that some microorganisms are able to directly use electricity as energy and electron source when cultivated on electrodes. Exemplified by the production of the biopolymer polyhydroxybutyrate, this project aims at the technical realization of this so-called microbial electrosynthesis. The final goal is the construction and continuous operation of a pilot reactor fed with flue gas. As uptake and conversion of substrates and electrons are strongly dependent on the structure of the biofilm, three-dimensional visualization will be performed to correlate biofilm structure and biopolymer production.

Max Hackbarth

Andrea Hille-Reichel

Michael Wagner

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KIT (IAB, IFG), University of Freiburg, EnBW, Germany

Treatment and Valorization of Concentrates from Waste Water Recycling Processes (HighCon)

The goal of the HighCon project, which started in September 2016, is the development of innovative and selective processes to maximize water recovery, while at the same time, producing a solid product suitable for reuse in the industry. Well known processes (such as flocculation, membrane bioreactor, nanofiltration, reverse osmosis) and upcoming water treatment technologies (e.g., membrane distillation, monoselective electrodialysis, low temperature distillation-crystallisation) will be further developed. Following the development and adaptation of the treatment technologies, full scale experiments will be carried out at three locations. The main tasks of our group include: the characterization of the waste water sources, the application of different membrane processes for the waste water treatment, the investigation of scaling and fouling formation and the monitoring of the full scale application.

Annika Bauer

Florencia Saravia

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Apl. Prof. Dr. Gisela Guthausen	NMR/MRI
Dipl.-Ing. Dunja Haak	IT, network administration
Dr. Andrea Hille-Reichel	Biofilm systems
Dr. Marius Majewsky	Organic micropollutants
Dr.-Ing. Florencia Saravia	Membrane technologies
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Dr. Michael Wagner	Imaging
Dipl.-Ing. (FH) Stephanie West	Biofouling

PhD Students

M.Sc. Alondra Alvarado	Characterization of organic carbon in anaerobic and aerobic waste water treatment
M.Sc. Annika Bauer	Membrane filtration, waste water recycling
M.Sc. Florian Blauert	Mechanical properties of biofilms
M.Sc. Dominic Breitkopf	Microbial fuel cells in waste water treatment
M.Sc. Philip Brown	Dissemination and spread of antibiotic resistance genes in the environment
Dipl.-Ing. Fabian Brunner	Aerobic granules for waste water treatment
Dipl.-Ing. Laure Cuny	Productive biofilms
M.Sc. Elham Fatoorehchi	Sludge disintegration and anaerobic treatment (Dr.-Ing. since September 2016)
Dipl.-Ing. Norman Hack	Refractory organic substances in capillary fringes (Dr.-Ing. since October 2016)
M.Sc. Max Hackbarth	Bioplastics
Dipl.-Ing. Oliver Jung	Brackish water desalination
M.Sc. Oliver Kehl	Modeling of oxygen transfer in activated sludge systems
M.Sc. Chunyan Li	Influence of biofilm structure on reactor performance (Dr.-Ing. since December 2015)
M.Sc. Di Peng	Nanofiltration, pool water (Dr.-Ing. since November 2016)
M.Sc. Jueying Qian	Detection of biofouling in irrigation systems
M.Sc. Isa Remdt	Productive biofilms
Dipl.-Ing. Johannes Ruppert	Corrosion of piling wall steel (BAW, Karlsruhe)
Dipl.-Chem. Nico Seeleib	Organic nitrogen detection
M.Sc. Alexander Timm	Degradation of antibiotics
Dipl.-Ing. Marc Tuczinski	Microfiltration, solid-liquid separation in biogas production

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From left to right:
(front row) Gudrun Abbt-Braun, Laure Cuny, Sylvia Heck, Ulrike Scherer, Antje Decker, Elham Fatoorehchi,
(second row) Birgit Gordalla, Pari Arvind (guest student), Florian Blauert, Marc Tuczinski, Jueying Qian, Di Peng,
(third row) Ursula Schäfer, Dominic Breitkopf, Tanja Stahlberger, Isa Remdt, Fabian Brunner,
(last row) Florencia Saravia, Rafael Peschke, Harald Horn, Axel Heidt, Matthias Weber, Dunja Haak, Reinhard Sembritzki, Andrea Hille-Reichel

RECENT PUBLICATIONS

Peer-Reviewed Journal Publications

Delay, M., Schwegmann, H., Frimmel, F.H., 2015. Nanoparticles and refractory organic matter: Interactions and consequences. Journal of Environmental Chemical Engineering 3 (4), 2997–3004.

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Zemann, M., Majewsky, M., Wolf, L., 2016. Accumulation of pharmaceuticals in groundwater under arid climate conditions – Results from unsaturated column experiments. Chemosphere 154, 463–471.

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Herzog, B., Lemmer, H., Horn, H., Müller, E. (2016). Characterization of pure cultures isolated from sulfamethoxazole-acclimated activated sludge with respect to taxonomic identification and sulfamethoxazole biodegradation potential (Chapter 6). In: Sanchez, O. (Ed.), Environmental Engineering and Activated Sludge Processes: Models, Methodologies, and Applications. Apple Academic Press (Oakville, Canada), 137–158, ISBN 9781771883887 - CAT# N11670.

Publication Series of the Institute

Schriftenreihe des Lehrstuhls für Wasserchemie und der DVGW-Forschungsstelle am EBI:

Volume 66: Li, C., 2016: Using optical coherence tomography to quantify biofilm structure and mass transfer in combination with mathematical modeling.

Volume 67: Herrling, M.P., 2016: Nanoparticles in biofilm systems – assessment of their interactions by magnetic susceptibility balance and magnetic resonance imaging.

Volume 68: Fatoorehchi, E., 2016: Sludge disintegration techniques – assessment of their impacts on solubilization of organic carbon and methane production.

Conferences (Selection)

Majewsky, M., Glauner, T., Horn, H., 2016. Systematic suspect screening and identification of sulfa drug transformation products. Setac, 22 – 26 March, Nantes, France.

Timm, A., Majewsky, M., Peschke, R., Horn, H., 2016. Analysis of four β-lactam antibiotics by LC-MS/MS. LC-MS in der Umweltanalytik, 6 – 7 June, Leipzig, Germany.

Blauert, F., Pavissich, J.P., Wagner, M., Horn, H., Picioreanu, C., 2016. Evaluation of biofilm mechanical properties using optical coherence tomography (OCT) and fluid-structure interaction simulations. Biofilms 7, 26 – 28 June, Porto, Portugal.

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Cuny, L., Remdt, I.D., Hackbarth, M., Hille-Reichel, A., Horn, H., 2016. Reactor systems for investigation of productive biofilms. Biofilms 7, 26 – 28 June, Porto, Portugal.

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Qian, J., Sultan, Z., Wagner, M., Horn, H., 2016. Influence of inorganic particles on biofilm development in microfluidic devices monitored non-invasively by optical coherence tomography. MEWE and Biofilms IWA Specialist Conference, 4 – 7 Sept., Copenhagen, Denmark.

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