Dear colleagues and friends,

With respect to water research and water management, 2018 was an exciting year. Especially the latter was exciting as we had nearly no rain but very high temperatures in Germany for more than six months. Water consumption went up due to heavy use of water for gardening. As a consequence, the water levels in reservoirs and rivers in most parts of Germany are still extremely low and people working in water works, agriculture and forestry are hoping for a wet winter time. Interestingly, a project of the University of Freiburg within the frame of the Water Research Network Baden-Württemberg (page 2) dealing with 'Drought impacts, processes and resilience: making the invisible visible – DRiTeR' has been running since 2016. When the project started 4 years ago, it was discussed whether droughts are really an issue in Germany with an average rainfall of around 800 mm a⁻¹. Obviously yes!

Another less exciting but highly important topic for people in research is the ongoing negotiation between German and Swedish universities and Elsevier about the access to Elsevier journals. Since July this year, we have not had any chance to download manuscripts from Elsevier journals for 2018. This conflict did not appear out of nowhere. People who have been working in research for more than a decade did see a dramatic change in how research results are traded worldwide. It seems it is a business like selling any other high-quality product with one goal: pleasing the shareholders of the publishing companies. One maybe minor consequence of the change can be seen at the end of this report: At the end of this year, we do have 'printed' versions of our manuscripts from the future, i.e., 2019.

If I had to highlight a specific topic at the Engler-Bunte-Institut in 2018, it would be the inauguration of the new Engler-Bunte-Institut lecture building (page 20). After four years, we can again give our lectures directly at the Engler-Bunte-Institut. Moreover, we can run the practical courses in chemistry for students in the first semester of Chemical and Bio Engineering in March and April 2019 in new laboratories. This is really a step forward for the reconstruction of the Engler-Bunte-Institut, which will be an ongoing process for the next 8 to 10 years.

As you can see from the report (page 18), we do have three new PhD students in Water Chemistry and Water Technology. Together with Johannes Gescher from the Institute of Applied Biology, we won two new projects where we will work with bioelectrochemical systems. Moreover, we had guest scientists from Australia (Prof. Yiwen Liu), China (Prof. Guihu Xu, France (M.Sc. Rana Hatoum) and Poland (Dr. Marta Gmurek), working on biological and chemical water treatment processes as well as the degradation of micropolutants. I hope you can find one or the other interesting topic on the following pages.

I wish you all the best for 2019

Harald Horn
Water Research Network Baden-Württemberg

In 2018, two conferences were organized by the Water Research Network Baden-Württemberg: The symposium 'Environmental risks in water research – Inter- and transdisciplinary evaluations and perspectives', held in Karlsruhe in January, dealt with approaches and methods from different disciplines to enhance integrative approaches in water research. Ortwin Renn (Director of the Institute for Advanced Sustainability Studies, Potsdam) gave a keynote on the challenges of systemic risks. Janet Hering (Director of Eawag, Dübendorf, Switzerland) commented on the role of science in the decision process to upgrade Swiss waste water treatment plants for micropollutant removal and Katrien Termeer (Professor of Public Administration and Policy, Universität Wageningen, the Netherlands) presented her research on governing climate change adaptation through a strategy of 'small wins'. Furthermore, speakers from universities in Baden-Württemberg took a closer look at risks from different disciplinary perspectives. One session focused on pollutants in the water cycle, while another dealt with risks arising from extreme events. About 130 participants attended the symposium. Particularly gratifying was the great interest of young scientists.

In October, the three joint projects, funded in the framework of the Water Research Network by the Ministerium für Wissenschaft, Forschung und Kunst (MWK), presented their interim results in Stuttgart for an audience from research and public authorities as well as engineering consultants.

Due to the positive response to the networking workshops held in 2017, this measure was continued in 2018. In summer 2018, a two-phase workshop on 'The triangle flow – biofilm – sediment' took place at the University of Stuttgart. The aim was to analyze the complex interactions between hydrodynamics, biofilms and sediments by means of the application of different measuring and imaging techniques. In October, an exchange of experience on careers of women in the water sector was organized for female PhD students and postdocs by the University of Tübingen.

Dr. Caroline Liepert (MWK), opening the colloquium in Stuttgart (left), audience at the symposium on 'Environmental risks' in Karlsruhe (upper right), workshop on careers of women in the water sector (bottom center) and Michael Wagner (EBI), conducting biofilm imaging at the University of Stuttgart (bottom right).

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
Stuttgart University of Applied Sciences

For further details see: www.wassernetzwerk-bw.de
Standard Methods for the Examination of Water, Waste Water and Sludge

The loose-leaf collection 'Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung' (DEV), jointly edited by the German Water Chemistry Society and the DIN Standards Committee 'Water Practice', comprises more than 300 methods in the format of DIN, DIN EN, DIN EN ISO or DIN ISO standards, their years of publication ranging from 1980 through 2018. Standards are checked for their up-to-dateness every five years. The experts of the technical committee DIN NA 119-01-03 Arbeitsausschuss 'Wasseruntersuchung' have to decide whether a standard should be confirmed, withdrawn or revised in order to match the current state of the art. Revision was necessary, e.g., for DIN EN ISO 10634 (DEV L 19), preparation of poorly water-soluble organic compounds for subsequent biodegradability testing, as several solubilizing agents specified in the 1995 edition had turned out to be easily biodegradable, banned, or no longer available on the market for other reasons. Another example for a recent revision is ISO 5815-1 (DEV H 51), the dilution and seeding method with allythiourea addition for BOD₅ determination. The 1998 edition has been amended by a list of validity criteria to be met and by protocols for control analysis and multitesting.

Besides amending existing methods and discarding outdated ones, technical committees deal with emerging approaches resulting from pre-normative research. Thus, CEN/TC 230 'Water analysis', in charge with development of the analytical repertoire for water monitoring according to European directives, revised its business plan to include genetic examination of water quality in addition to physical, chemical, biochemical, biological and microbiological methods. This was the result of some years of discussion and – as the ultimate kick-off – a joint meeting of CEN/TC 230/WG 2 'Biological methods' with scientists involved in the EU COST Action DNAqua-Net.

Funding:
Water Chemistry Society (Division of the Gesellschaft Deutscher Chemiker e.V., GDCh)
The Umweltbundesamt (UBA)

Molecular approaches for monitoring of aquatic ecosystems and biodiversity. Right: Members of CEN/TC 230/WG 2 'Biological methods' and researchers of the EU COST Action DNAqua-Net, initiating a standardization project on eDNA sampling in waters. Left: Reporting the outcome in the format of Recommendations. CEN/TC 230 meeting week in Brussels, 2018.

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Reactor Cascades for the Biotechnological Conversion of Waste into Platform Chemicals – RECICL

The aim of the project (RECICL) is to implement a combination of biotechnological processes for the conversion of biogenic residual and waste streams into platform chemicals. The first step consists of a stirred-tank hydrolysis reactor operating at acidic pH to convert waste to volatile organic acids (Figure a). Our research focuses on the optimization of the hydrolysis process to obtain a higher propionic acid production by integrating a propionic acid-producing bacterial culture. Types, feeding rate and configurations of the hydrolysis reactor have been investigated in the last months.

The results of the lab scale experiments and the hydrolysis reactor showed that a higher propionic acid production can be obtained at mesophilic temperature (30 °C) and by using a propionic acid producing culture, whereas more than 50 % of organic waste was mainly converted to volatile organic acids (Figure b). The maximum concentration of propionic acid was 5.7 g/L (76.5 mmol/L) during the time of operation. Furthermore, the gas analysis confirmed the lack of methanogenic activity inside the reactor as no methane was detected in any of the gas samples.

A microbial electrolysis cell (MEC) is applied as a second step in order to reduce the concentration of the non-relevant volatile organic acids (e.g., acetic and butyric acid) while propionic acid remains in the solution. Additionally, the MEC leads to hydrogen production, which can be used as an alternative energy source.

A two-chamber MEC was designed to characterize the biofilm structure and morphology on the anode side by means of optical coherence tomography (OCT) in order to correlate biofilm properties to the MEC performance. In the MEC, different filtering anode materials will be tested using anode respiring bacteria such as Geobacter sulfurreducens.

Funding:
Federal Ministry of Education and Research (BMBF)
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Partners:
Prof. Johannes Gescher, Institute of Applied Biology (IAB), KIT
Department of Microsystems Engineering (IMTEK), University of Freiburg, Germany
Fraunhofer Institute for Solar Energy Systems (ISE), Germany

(a) Photograph of the hydrolysis reactor. (b) Total organic acids production for acetic, propionic, and butyric acids, which was measured for a study period of 95 days.
Increasing Water Availability in Arid Areas: A Method to Assess the Impacts and Adaptability of Different Water Technologies for Sustainable Adaptation

Arid areas are challenging for water management: Natural low water availability forces decision makers to commonly work in overstressed water contexts with the subsequent social, environmental and economic conflicts, and overall difficulties to achieve a sustainable development. Sea water desalination and waste water reuse are two of the most common paths to increase water availability when focusing on water offer. Nevertheless, they present new challenges such as a) the distribution of costs and benefits of ‘new’ resources among different users, b) the environmental impacts of waste disposal, and c) the legal framework that will regulate the water use and administration. In this context, decision makers need to understand and manage the challenges for these technologies in order to be sustainable long-term solutions.

This project aims to contribute to the sustainable development of arid areas through the selection of technological solutions that will allow to increase the water volumes available with minimum negative impacts. For this, a decision support method based on the Integrated Concept of Sustainability (ICoS) will be developed, consisting of a series of indicators that will be applied in three scenarios for the city of Arica (Chile), projecting different socio-economic conditions for 2030.

With the purpose of reducing the pressure on the scarce existing water resources, this project assesses the sustainability performance of waste water reuse and sea water desalination through reverse osmosis, as the most used and developed technology, and membrane distillation, which presents itself as an interesting option due to the high solar energy potential.

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**Flow chart of sustainability assessment based on context-specific indicators.**

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Monitoring and Quantification of Fouling Development in Membrane Distillation by means of Optical Coherence Tomography

Membrane distillation (MD) is a promising thermally driven technology for desalination applications and waste water treatment. A temperature difference between feed and condensate side induces a water vapor gradient along the microporous hydrophobic membrane and allows a separation performance of theoretically 100%.

Fouling formation in membrane distillation limits process stability and causes, besides wetting, a rapid flux decrease, making the process inefficient. Process performance is conventionally assessed by flux monitoring without online proof. Analysis of the fouling layer is to date mainly performed ex situ at distinct intervals or at the end of process operation.

Recently, optical coherence tomography (OCT) has been introduced for the in situ monitoring of biofouling development in several studies. However, inorganic fouling layers were not considered. We thus developed an innovative methodology for 3D OCT dataset analysis allowing for the detection of scaling dominated fouling on the membrane surface in a fully operated direct contact membrane distillation system (DCMD).

Defined fouling parameters permit a correlation between process performance indicators, especially flux decrease and quantified fouling layer coverage. Additionally, structural information about the fouling layer was derived from the fouling parameters calculated from 3D OCT datasets across the feed channel within the flat sheet membrane unit.

The developed approach links scalable fouling parameters with macroscopic process parameters. Hence, this approach allows for making performance monitoring possible while enabling an adapted process control with a view to membrane cleaning strategies.

Topographic visualization of the membrane fouling layer – bulk interface and the corresponding histogram for hot spring water. (A) was acquired at time t = 0 directly after starting the experiment. (B) and (C) show the fouling layer – bulk interface after 10 and 30 days, respectively. The calibration bar corresponds to the thickness of the fouling layer above the membrane in mm. The histogram represents the thickness distribution.
Measuring the Effect of Organic Fouling on Concentration Polarization in Reverse Osmosis and Nanofiltration

Reverse osmosis (RO) and nanofiltration (NF) continue to increase in popularity for total or partial de-ionization of natural waters of any kind. The main drawback in continuous RO/NF operation is the occurrence of fouling, which leads to reduced process efficiency, increased energy requirements and ultimately to replacement of the fouled membrane modules. Organic fouling, biological fouling and scaling (inorganic) are the main fouling types. Most research focusses exclusively on one type and uses macroscopic performance indicators and retrospective analysis as main research techniques.

To gain a fundamental understanding of occurring fouling mechanisms, we investigate the application of Raman microspectroscopy (RM) for in situ measurement of concentration polarization (CP) in a flat-sheet crossflow membrane unit equipped with a NF membrane. This technique allows for locally resolved measurement of CP, which is a gradient of solutes towards the membrane surface. CP is the precursor of scaling as it may cause local super saturation. CP and cake layer formation also lead to organic fouling. In practice, both fouling phenomena are usually present simultaneously and affect each other. With RM, we can study how existing organic or biological fouling layers on NF membranes affect the CP layer of sulfate, a common scaling factor.

Firstly, an organic fouling layer is established and characterized in situ using optical coherence tomography (OCT) to determine the dimensions, i.e., thickness, of the fouling layer. Biofilm, agar-agar and humic substances were used as natural and model foulants, respectively. Secondly, the fouled membrane was operated in the flat-sheet membrane unit with a pure MgSO₄ solution and linked to the RM to measure CP profiles. The concentration profiles can be directly compared to those from clean membranes. Thus, we are able to investigate the influence of various organic fouling phenomena on a major mechanism of scaling.

(a) Visualization of fouling layer using OCT. (b) Influence of an agar-agar fouling layer on CP. (c) Dismounted membrane fouled with humic substances.

Funding:
Federal Ministry of Education and Research (BMBF)
Helmholtz Association, Atmosphere and Climate Program (ATMO)
The Raman microscope is provided by Prof. Thomas Leisner from the Institute of Meteorology and Climate Research (IMK-AAF), KIT.

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Determination of Particle Intake in Packed Beds of Aerobic Granules Visualized by means of Magnetic Resonance Imaging

Aerobic granules in sequencing batch reactors show a high metabolic rate per volume. In contrast to commonly applied reactors, waste water enters from below and flows through the sedimented packed bed of aerobic granules under laminar flow conditions. Around 33% of the carbon sources of waste water are available as particles in the micrometer range. Hence, they are an important fraction of waste water. As these particles are affected by the local flow field, the aim of this project is to study their fate and bioavailability in the packed beds.

A model setup, using a column, was realized applying constant flow rates. Reference particles consisted of microcrystalline cellulose that have been saturated with iron. Different magnetic resonance imaging (MRI) methods have been chosen to study the development of preferential flow paths and the spatially resolved distribution of microparticles inside the packed bed: Flow maps have been acquired to characterize the local flow while 2D and 3D imaging characterizes the distribution of particles and granules. \( T_2 \)-weighted images allow to determine the structure of the packed bed in order to identify pore spaces and redistribution of the aerobic granules. \( T_2 \)-weighted images indicate particle intake and distribution inside the packed bed.

According to the preliminary results, microparticles are filtered by the granules and lead to a blockage of the pore space. As a consequence, preferential flow paths develop. Further particle accumulation occurs next to these areas, leading to an irregular distribution of the micro particles in the packed bed.

![Intake of particles in a packed bed of aerobic granules under constant flow.](image)

Funding:
Karlsruhe Institute of Technology (KIT)

Partners:
Department Process Engineering, Eawag, Dübendorf, Switzerland

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
The Effect of Sludge Retention Time, Hydraulic Retention Time and Biomass Concentration on Elimination of Micropollutants with Activated Sludge

Due to the design and current operation modes of waste water treatment plants (WWTPs), a complete elimination of many persistent anthropogenic micropollutants (MPs) present in waste water cannot be achieved. On the other hand, according to some studies, in real conditions, the biomass responsible for MPs degradation is in an excess (total suspended solids, TSS ~ 3 g L⁻¹), and therefore, its concentration is expected not to play a role in MPs removal. The question arises which operational parameters could be modified to improve waste water treatment efficiency. The aim of this study was to investigate the relation between biomass concentration of activated sludge, the operational parameters, sludge retention time (SRT) and hydraulic retention time (HRT) of biological treatment, and removal efficiency of selected MPs.

The results showed that SRT and HRT can influence the MPs removal efficiency to some extent, which depends mainly on their physico-chemical properties. For highly biodegradable compounds (e.g., caffeine, CAF, the effect of SRT and HRT is difficult to capture, whereas, in the case of moderately biodegradable compounds such as sulfamethoxazole (SMX) or benzotriazole (BZT), an increase of these two parameters improves the removal efficiency. For persistent compounds (carbamazepine, CBZ; diclofenac, DCF, the positive effect of prolongation of SRT could be observed only for very high sludge age, which is unrealistic in existing WWTPs. Interestingly, our results also indicated that the increase of biomass concentration from 3 to 5 gₜₚS L⁻¹ increased the values of the pseudo-first order rate constant of the highly and moderately degradable compounds (CAF; SMX; BZT; erythromycin, ERY; roxithromycin, ROX, whereas a further increase to 8 gₜₚS L⁻¹ had no effect (see Figure). This suggests that aforementioned assumption of neglectable effect of biomass concentration is valid only for relatively high TSS values, here above 5 gₜₚS L⁻¹.

As a next step, a more elaborate model to describe the relation between rate constant and biomass concentration will be investigated.

Funding:
German Technical and Scientific Association for Gas and Water (DVGW)
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Partners:
Prof. Olivier Potier,
Reactions and Chemical Engineering Laboratory (LRGP), Nancy, France
Prof. Joumana Toufaily,
Laboratory of Materials, Catalysis, Environment and Analytical Methods, Beirut, Lebanon

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Photolytic Degradation of Antibiotics of Last Resort under Simulated Environmental Conditions

Antibiotics of Last Resort (AoLR) are pharmaceuticals used to treat infections caused by multiresistant bacteria. These substances and their metabolites excreted with urine and faeces of patients reach waste water treatment plants (WWTPs). Due to the limited efficiency of existing treatment methods, the residuals of these substances leave WWTPs with the effluent and end up in surface water.

This study focuses on the environmental fate of two AoLR – linezolid and tedizolid – in presence of sunlight. Both substances were degradable by simulated sunlight (1 kW m$^{-2}$, equal to irradiance in Middle Europe at high noon in summer), with half-lives of 32 h for linezolid and 93 h for tedizolid in ultrapure water. The Figure shows the degradation rate constants of both antibiotics with and without exposure to simulated light (bright and dark samples, respectively) in different types of water: pure solutions, Rhine river water and Alb river water (a tributary of the river Rhine). To compare the degradation kinetics, each type of water was spiked with 1 μg L$^{-1}$ of both antibiotics and their decay was monitored over time.

Determined photodegradation rate constants of linezolid were in a comparable range in all tested types of water. Very low degradation rate constants, achieved without exposition to light, suggested a neglectable effect of hydrolysis and/or biodegradation. Tedizolid was less susceptible for photodegradation than linezolid. Interestingly, in the natural water, the degradation rate constants had comparable values for both, with and without light exposure. This might result from microbial degradation or adsorption on particles and dissolved organic matter. Structure elucidation by liquid chromatography coupled to high-resolution mass spectrometry provided information about 7 and 5 phototransformation products for linezolid and tedizolid, respectively. The morpholine moiety of linezolid was the main target site for photolytic transformation. For tedizolid, the main transformation occurred on the oxazolidinone ring.

Degradation of linezolid and tedizolid in ultrapure and surface water.
Transport of Antibiotic Resistances from Waste Water Discharges into the Sediment of the Receiving River

It is well established that the emission of antibiotic resistance genes (ARGs) leads to an increase of ARGs in receiving rivers. However, also the role of sub-inhibitory antibiotic concentrations is being discussed. Results obtained in this study suggest that, at environmental concentrations, antibiotics do not have an effect on the selection of ARGs. Instead, we emphasize the significance of ARG transport, i.e., waste water particles that carry high loads of microorganisms (Brown et al., 2019). We demonstrated that ARGs (ermB, blaTEM, tetM, qnrS) as well as facultative pathogenic bacteria (FPB) (enterococci, Pseudomonas aeruginosa, Acinetobacter baumannii) inside the particulate fraction of waste water treatment plant (WWTP) effluents are very likely to remain in the riverbed of the receiving water due to sedimentation (see Figure).

The sedimentation potentials out of 1 m² were 7.6 g of TSS, 10 mg of DNA, and between 10⁶ and 10¹² gene copies (GC) of FPB and ARGs. Additionally, ARG and FPB numbers measured in the settleable fraction strongly correlated with the delta ARGs and FPB numbers measured in the receiving river sediment (downstream compared to upstream) (R² = 0.93, p < 0.05) (data not shown). Apparently, the sheer amount of settleable ARGs and FPB from the WWTP effluent is sufficient to increase abundances in the receiving riverbed by 0.5 to 2 log units. Finally, there is a strong indication that the increased ARG abundances in downstream sediments are due to a continuous import of ARGs from effluents. We hypothesize that if the import is removed, ARG numbers will decrease to an initial state again. This issue is addressed in an on-going lab-scale experiment with batch reactors in which real river sediments are exposed to waste water particles. For details, see Brown et al. (2019).

<table>
<thead>
<tr>
<th>parameter</th>
<th>concentration in effluent</th>
<th>amount in fraction [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>13.6 ± 1.9 mg/L</td>
<td>0 10 20 30 40 50 60 70 80 90 100</td>
</tr>
<tr>
<td>DNA</td>
<td>33.4 ± 5.0 µg/L</td>
<td></td>
</tr>
<tr>
<td>enterococci</td>
<td>3.3 ± 0.5 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.8 ± 0.1 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>A. baumannii</td>
<td>1.7 ± 0.5 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>ermB</td>
<td>6.3 ± 0.4 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>blaTEM</td>
<td>5.7 ± 0.4 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>tetM</td>
<td>4.2 ± 0.4 log GC/mL</td>
<td></td>
</tr>
<tr>
<td>qnrS</td>
<td>3.3 ± 0.5 log GC/mL</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations of total suspended solids (TSS) and DNA, FPB, and ARGs measured in WWTP effluent (left), and their amount measured in settleable and suspended fractions (right). GC = gene copy number. Mean values and standard errors of the means are shown (n = 3). According to Brown et al. (2019).

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

Partners:
Prof. Thomas Schwartz, Institute of Functional Interfaces (IFG), KIT

Publication:

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Trace Analysis of Urea in Surface, Ground and Drinking Water with Gel Chromatography and Organic Carbon and Nitrogen Detection

Urea, being an important nutrient in the nitrogen cycle, is by far the most important fertilizer worldwide. It is considered to be the primary source of nitrogen for plants and algae and is usually transformed by urease and microorganisms into accessible nitrogen sources. Besides the manifold usage of urea in agriculture and being the main N-containing compound of the excretion of mammals, there are almost no data available about the urea concentration in ground waters. By using the improved Gel Chromatography and Organic Carbon and Nitrogen Detection (LC-OCD-OND) technique, several advantages are obvious for the analysis of urea in aqueous samples: analysis time of only 70 min, a low limit of detection (1 ± 0.1 μg/L urea), and the peaks of urea, nitrate and ammonium are well separated.

For the case study, ground water, surface water influenced by anthropogenic activities, surface water classified as oligotrophic, and drinking water, which was gained from treated ground water, have been analyzed. Almost all of the 100 water samples investigated contain urea. Surface waters showed the highest concentration of urea, ranging from 4.0 to 59.3 μg/L, whereas ground water gives lower values (< 0.5 to 24.0 μg/L). For drinking water, the concentration was lowest, namely 0.5 to 12 μg/L.

As expected, the urea concentrations are highest in surface water, which is influenced by anthropogenic activities. This can be explained by the run-off from agricultural areas and/or by the discharge of waste water effluents. In addition, the studies show that even in oligotrophic water, the amount of urea is relatively high, although the total nitrogen content is low. In addition, it is evident that even in ground water, urea is present. During the treatment process, urea is mainly removed during the sand filtration step, although it cannot be removed totally.

Based on these results, it will be investigated if urea could be used as an indicator for the microbial stability of water during different treatment steps.

![Box-Whisker Plot](image)

(a) Urea and (b) total nitrogen (TN) of different types of water, presented as box-whisker-plot (IQR: interquartile range).

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

Partner:
DOC-Labor Dr. Huber, Karlsruhe, Germany
Hydrolysis of Particulate Organic Matter from Municipal Waste Water under Aerobic and Anaerobic Conditions

Around 50% of the organic load in municipal waste water is in the form of particulate matter. The primary clarifier of a municipal waste water plant removes 50 – 70% of the total suspended solids (TSS). However, it is not completely understood to which extent and how fast solids are degraded by microorganisms in the biological stage. The particulate organic matter needs to undergo a hydrolysis process in order to be consumed by microorganisms. Hydrolysis is known to be the limiting step in biological processes.

In this work, particles (size: 25 – 250 µm, concentration: 1 g/L TSS) originated from municipal waste water were treated under a) aerobic and b) anaerobic conditions. The challenge was to observe both, hydrolysis of particles and degradation of the released organic carbon. By using size exclusion chromatography coupled with online detection of dissolved organic carbon (SEC-DOC), the soluble hydrolyzed products of the particulate organic matter were monitored.

The Figure shows chromatograms obtained on day 0, 3 and 13 for the aerobic treatment (a) and on day 0, 5 and 10 for the anaerobic treatment (b). The chromatograms can be divided into four fractions (F1 to F4), related to decreasing molecular size.

The results showed a maximum accumulation of DOC on the third day for the aerobic process (Figure a) and on the fifth day for the anaerobic process (Figure b). This suggests a production of slowly biodegradable matter. After 13 days of aerobic treatment, almost all of the slowly biodegradable organic matter, represented by fraction F1, was hydrolyzed (Figure a), while after 10 days of anaerobic treatment, only half of F1 was degraded (Figure b). Further anaerobic experiments will be conducted in order to identify to which extent the slowly biodegradable matter can be degraded.

Size exclusion chromatograms of hydrolyzed chromatographable DOC on day 0, 3 and 13 under aerobic conditions (a) and on day 0, 5 and 10 under anaerobic conditions (b).

Funding:
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German Academic Exchange Service (DAAD)
German Technical and Scientific Association for Gas and Water (DVGW)

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Simulation of Productive Biofilms for Lactic Acid Production

The interest of white biotechnology in productive biofilms is continuously increasing as biotechnological processes benefit from the advantages of biofilms as compared to planktonic cells used in conventional processes. In the current project, we focus on the production of lactic acid (LA) as a model compound to evaluate the potential of productive biofilms.

A pure-culture biofilm of *Lactobacillus* bacteria was cultivated in a horizontal tubular biofilm reactor (TBR) made of glass (L = 400 mm, ID = 10 mm). At the end of the cultivation period of 3.5 weeks, the biofilm was visualized by nuclear magnetic resonance (NMR) (Figure a). A mean biofilm thickness of approx. 850 μm was achieved in the middle of the reactor length; however, the biofilm thickness was subject to huge variations over the cross-sectional area as well as over the reactor length.

The analysis of biofilm performance under different flow conditions revealed that productivity increases with flow velocity. For a better understanding of the underlying processes in LA production, the knowledge of chemical gradients inside the biofilm is crucial. As not all components are accessible experimentally, a model was developed. It allows the representation of substrate and LA concentrations (cS and cP), as well as pH values across the biofilm depth for different flow velocities (Figure b). It can be seen that LA accumulates close to the substratum and creates pH values below 4.5, a range where pH inhibition occurs and LA production is significantly decelerated. Production is, thus, inhibited by mass transport limitations. Increasing the flow velocity improves the mass transfer into and out of the biofilm and results in different concentrations and pH values in the bulk phase and at the biofilm surface. This has an impact on the concentrations and pH values inside the biofilm: The magnitude of the pH limitation is decreased and consequently, LA productivity is improved. A control of biofilm thickness might overcome this inhibition and further increase the productivity.

**Funding:**
Karlsruhe Institute of Technology (KIT)
German Technical and Scientific Association for Gas and Water (DVGW)

**(a)** Visualization of the biofilm by MRI. The dotted line represents the mean biofilm thickness. **(b)** Substrate and LA concentrations (cS and cP) and pH values within the biofilm as calculated by the model for a biofilm thickness of 850 μm. The dotted line represents the biofilm thickness.
BioElectroPlast – Microbial Electrosynthesis for Production of Bioplastics from Flue Gas in Pressurized Flow-Cells

The aim of the project BioElectroPlast is the production of the biodegradable biopolymer polyhydroxybutyrate (PHB) in custom-made microbial electrosynthesis (MES) flow-cells. Therefore, the thermophilic, acidophilic and electrothoautotrophic microorganism *Kyrpida spormannii* was cultivated on the graphite cathodes poised with a constant potential of -531 mV against the standard hydrogen electrode. The flow-cells and the peripheral equipment are fully autoclavable, pressure-resistant and operated at a low pH value of 3.5. Medium temperature was maintained at 60 °C (double jacket boiler).

As inoculum, a heterotrophically grown pre-culture of *Kyrpida spormannii* was added to the flow-cells (optical density OD\textsubscript{600} = 0.01) after washing twice with cultivation medium. In order to supply the microorganisms with the gaseous substrates, the headspace of the boiler was pressurized to 1.5 bar with a mixture of 95 % CO\textsubscript{2} (v/v) and 5 % O\textsubscript{2} (v/v) while the liquid phase was continuously stirred. The medium enriched with the dissolved gases was recirculated through the flow-cells at a volumetric flow rate of 100 ml min\textsuperscript{-1}. The glass lid of the flow-cell allows for the in situ visualization of the cathodic biofilm by means of optical coherence tomography (OCT).

After 10 days of incubation, the cathode was covered by a biofilm-like structure with a mean thickness of 140 μm. Fluorescence microscopic analysis of the developed structures showed rod-shaped cells imbedded in a matrix of basically inorganic compounds. Additionally, staining with Nile Red revealed distinct PHB inclusions within the cells. Future investigations will aim at avoiding the accumulation of inorganic compounds as well as quantification of biofilm development and structure in order to derive accurate mass balances for the conversion of substrates into biomass and products.

3D rendering of the experimental setup.

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Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
Effect of Iron on the Structural Characteristics of *Bacillus subtilis* Biofilms Cultivated in Flow Cells

Structure and material properties of a biofilm play an important role in its development and maturation as well as in its resistance to external changes. To analyze biofilm characteristics, a fully-automated imaging setup for flow cells including optical coherence tomography (OCT) was developed.

Using 2D and 3D OCT images, first insights into Fe$^{2+}$ dependencies of *Bacillus subtilis* biofilms were gained performing (i) growth studies and (ii) dynamical mechanical analysis (DMA) — a method which determines the Young’s modulus $E$ by analyzing the deformation behavior of biofilms under hydrodynamic load.

In general, biofilms cultivated with a high Fe$^{2+}$ concentration of 5 mg/L showed a higher mean biofilm thickness and surface coverage after 7 days of growth compared to those grown at a low Fe$^{2+}$ concentration of 0.5 mg/L.

Additionally, the bulk-biofilm interface was more heterogeneous for the high Fe$^{2+}$ concentration.

In contrast, DMA revealed a higher loss of integrity for biofilms grown with 5 mg/L Fe$^{2+}$ compared to the 0.5 mg/L Fe$^{2+}$ condition, shown by a 2.5 fold higher reduction of the mean biofilm thickness after deformation experiments. Furthermore, surface roughness was doubled due to adhesive and cohesive failure. Results lead to an interpretation where γ-FeO(OH) particles incorporate into the biofilm matrix, leaving insufficient space for stabilizing polymeric substances.

This is also reflected by an estimation of the biofilms $E$ moduli to be 0.71 Pa for the high and 11.6 Pa for the low Fe$^{2+}$ concentration. The $E$ modulus is a measure for elasticity, whereas high $E$ moduli indicate low biofilm displacement and thereby rigidity to higher flow velocities.

The obtained data will support the parametrization of 2D and 3D biofilm models. These models will help predicting data and analyzing strategies either supporting (e.g., in waste water treatment plants) or inhibiting (e.g., in industrial settings) biofilm growth.

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\tau_s = 0.08 \text{ Pa}
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\begin{array}{c}
\text{Fe}^{2+} = 5.0 \text{ mg/L} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Fe}^{2+} = 0.5 \text{ mg/L} \\
\end{array}
\]

\[
\tau_s = 2.0 \text{ Pa}
\]

\[
\begin{array}{c}
\text{Fe}^{2+} = 5.0 \text{ mg/L} \\
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\[
\begin{array}{c}
\text{Fe}^{2+} = 0.5 \text{ mg/L} \\
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\]

Biofilms grown at high and low Fe2+ concentration under growth conditions of $\tau_w = 0.08 \text{ Pa (a + b)}$ and a raised shear of $\tau_w = 2 \text{ Pa (c + d)}$. The biofilm grown with the high Fe2+ concentration shows less stability and tends to failure (c). The white outline illustrates the bulk-biofilm interface. Flow is from left to right. The scale bar equals 250 $\mu$m.
Development of a Novel Fixation Method of Microplastics from Sea Water

Since the start of the mass production of plastic in the 1950s, high amounts of plastic have been released into the marine environment due to incorrect disposal (e.g., plastic waste). Plastic accumulates in the water phase because of its low degradability. Over time, plastic is finally fragmented into countless small particles. Plastic particles smaller than 5 mm are classified as microplastics. Contamination with microplastics has been detected in all parts of the aquatic environment, which makes it ubiquitous. As numerous negative effects on marine organisms and ecosystems have been proven, they are considered as pollutants.

The aim of this project is to avoid microplastic contamination in processes using sea water. Currently, there are no techniques available to remove microplastics efficiently and cheap from sea water. Thus, a novel method based on the use of organosilanes, which attach to microplastic particles and link them by forming larger agglomerates, is developed. As these agglomerates float on the water surface, they can easily be removed. The organosilanes are tested on the lab scale and adapted to different polymer types and environmental conditions. Subsequently, the adapted method is optimized on the pilot-plant scale for an efficient application. The first practical application is planned for sea salt production. In order to avoid the contamination of sea salt with microplastics, it will be removed from the water flowing into the salt works.

Micro-Organisms and Turbulence: Towards a Numerical Laboratory for Water Quality Prediction – MOAT

The quality of surface water typically depends upon a complex interplay between physical, chemical and biological factors, which are far from being understood sufficiently. There still exist large uncertainties related to quality predictions based on state-of-the-art mathematical models of surface water bodies. A computer model (KIT, Institute for Hydrodynamics) is applied to determine the fate and transport of fecal indicator bacteria (such as *E. coli*) after a combined sewer overflow event into a streaming water body (such as a river or a canal) where the bacteria are initially present both in freely-suspended form as well as attached to the surfaces of the particles. Experimental data gained from lab-scale flume experiments are used to validate the model. Using flow cytometry, the transport of bacteria (domestic waste water) inside the water body of the flume is determined. Additionally, the transport of bacteria attached to model particles (agar-agar, diameter = 500 μm) is investigated.

These results will provide important insight into the mechanisms through which the spatio-temporal heterogeneities of the complex flow affect the self-purification capability of a contaminated water body, which will in turn pave the way for a general improvement of water quality assessment methods.

Chair of Water Chemistry and Water Technology and DVGW Research Center at the Engler-Bunte-Institut
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- Freundeskreis des Engler-Bunte-Instituts

**Miscellaneous**

On November 14, 2018, the official handover of the two new buildings (40.50 and 40.51) for the Engler-Bunte-Institut to KIT took place, attended by the Baden-Württemberg Finance Minister Edith Sitzmann, the Head of the Baden-Württemberg Ministry of Science, Research, and the Arts Ulrich Steinbach and the President of KIT Professor Holger Hanselka.

The new buildings in the South border on Richard-Willstätter-Allee (Photo: A. Cordts, KIT).