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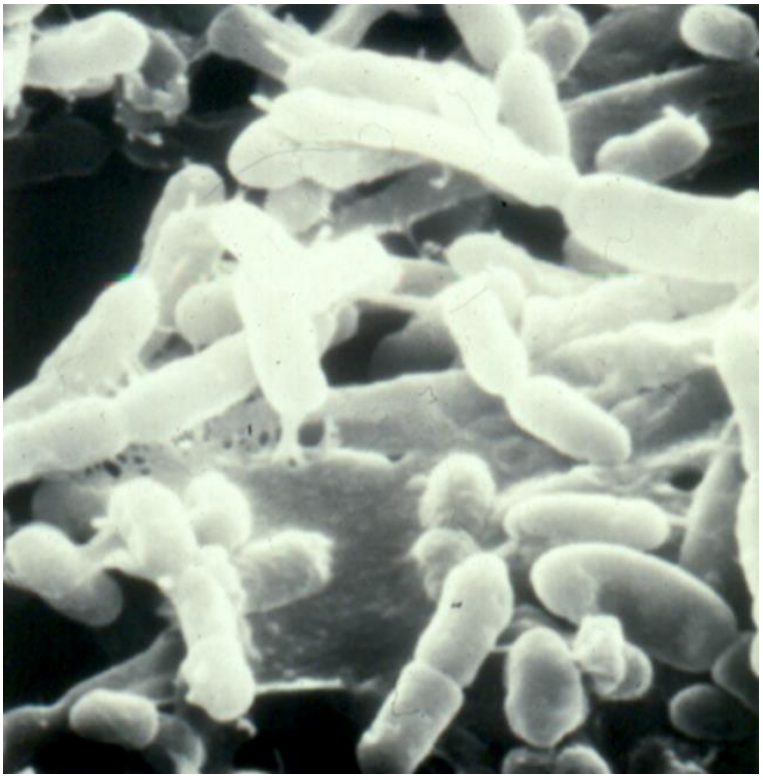
Biofilm Centre
Annual Report 2013



Aquatic Microbiology
Aquatic Biotechnology
Molecular Enzyme Technology & Biochemistry

Biofilm Centre

The Biofilm Centre is composed of three groups: Aquatic Microbiology, Aquatic Biotechnology and Molecular Enzyme Technology & Biochemistry. It is part of the Faculty of Chemistry and exists since 2001 at the University of Duisburg-Essen. By the end of 2010, the Biofilm Centre moved completely from three different locations at the campus Duisburg into one location at the campus Essen. That was a major effort, but now we have settled and make more and more use of the new, improved situation. An example is the project on "Stress response on biofilms of the thermoacidophilic archaeon *Sulfolobus acidocaldarius*", in which all three groups of the Biofilm Centre are involved. The success of the Biofilm Centre is also reflected in the total amount of funding of the projects running in 2013, which increased from 5.25 Mio € in 2012 to over 7 Mio € in 2013!

The Biofilm Centre comprised in 2013 a staff of 56, with three professors, one Research Group Leader, 3 laboratory heads, 1 technical coordinator and biocorrosion consultant, 4 postdocs, 29 Ph. D. students, 7 Technicians, 1 Secretary.

The focus of the **Aquatic Microbiology** lies in fundamental biofilm research and on the role of biofilms as a habitat for pathogens with Jost Wingender as leader of the "Research Group Pathogens in Biofilms". Hans-Curt Flemming is affiliated to the IWW Water Centre as a Member of the Board of Directors. During the last three years, a DFG founded project on the role of plankton as a habitat for hygienically relevant microorganisms has been almost completed. Furthermore, five large projects, funded by the Ministry of Research and Technology on biofilm management in drinking water installations, on the efficacy of silver nanoparticles in implants, and on the desiccation resistance of microorganisms under space conditions have been granted and are currently in process.

In 2012, a new research project was granted with the title „Safe Ruhr". It is dedicated to the occurrence of hygienically relevant microorganisms in River Ruhr water in terms of recreational use and of optimization of drinking water treatment. The project has a volume of 780.000 € and lasts until 2015

Also in 2012, Prof. Flemming was appointed as the chairman of the BioCluster, a joint specialist group of the International Water Association (IWA) and the International Society of Microbial Ecology. This cluster is an approach to bring water specialists in closer contact with the latest developments in microbial ecology. The BioCluster was successfully presented to the IWA council in *December 2013*. A BioCluster award was announced which was granted to Prof. Bruce Rittmann from Arizona State University and to Dr. Mari Winkler from TU Delft, a graduate of Water Science. The awards will be presented at the IWA Conference in Lisbon, September 22-24, 2014

In 2013, Prof. Flemming was appointed as a member of the Strategic Council of the IWA.

As Hans-Curt Flemming will leave office in September 2014, the procedure to find a successor was initiated in 2013. Among 22 candidates, 6 were invited for presenting their research and ideas; three out of them were shortlisted. The Rector of the University has announced the call to Candidate No 1 and promising negotiations are on their way. There is a good chance that he might begin his service in October 2014.

Aquatic Biotechnology mainly focuses on fundamentals and applications of biological leaching processes, biocorrosion and the prevention of acid mine drainage. The group deals with microbial surface processes such as the bioleaching of metal sulfides and the microbial influenced corrosion (MIC) of concrete and steel and its inhibition by biological measures. Consequently, our scientific expertise is the microbiology of the iron, manganese, nitrogen and sulfur cycles, which include moderate and extreme acidophilic iron and sulfur compound-oxidizing species (e. g. of the genera Acidithiobacillus and Leptospirillum) and manganese ion-oxidizing as well as sulfate-reducing bacteria. A worldwide cooperation in the fields of bioleaching and biocorrosion was established, including the Marie-Curie training network BIOCOR

In 2012, a project on “Urban Mining” was granted by the Ministery of Research and Technology. It is dedicated to the extraction of valuable elements from brown coal ashes by bioleaching. Furthermore, a cooperative research project with the Ruhr-Universität Bochum indicates that switching from planktonic lifestyle to Biofilm lifestyle causes in acidophilic bacteria the activation of about 100 genes and the inactivation of 50 genes. This was published in Proteomics in 2013, indicating that upon change from planktonic to biofilm lifestyle more than 120 proteins were induced or repressed within the first 12 hours after commencement in cells of *Acidithiobacillus ferrooxidans.*

Prof. Sand was appointed as a Guest Professor at the Central South University in Changsha, Hunan, China, from 2012 bis 2016. A scholarship was granted by the Shanghai Institute of Technology as an Outstanding Foreign Teacher for the academic year 2013 to 2014.

Molecular Enzyme Technology and Biochemistry concentrates on elucidating archaeal carbohydrate metabolism and stress response including transcription and transcription regulation. Beside classical biochemical, molecular biological and genetic approaches in collaboration with (inter)national partners state of the art high-throughput approaches (e.g. transcriptomics, proteomics, metabolomics and modeling) are used to gain a system level understanding (systems biology).

The general aim of the group of Prof. Siebers (MEB) is to unravel carbohydrate metabolism and its regulation, stress response as well as biofilm formation (e.g. synthesis of extracellular polymeric substances in archaeal model organisms (Siebers et al. 2012, Ulas et al. 2012, Esser at al. 2012). Based on fundamental research one major focus are biotechnological applications of extremozymes, extremophiles (metabolic engineering) as well as catalytic biofilms (Koerdt et al. 2012).

A new research project was granted in 2012 by the Ministery of Research and Technology (2013-2015): e:Bio – Innovationswettbewerb Systembiologie (Verbundprojekt Modul II, Transfer), „Applied **Sulfo**lobus **Sys**tems Biology; Exploiting the hot archaeal metabolic potential for **Biotec**hnology - SulfoSYS^{BIOTEC}.

Under the leadership of Dr. Siebers 10 partners, amongst others Sigma Aldrich, will be engaged to unravel the central carbohydrate metabolism of the thermoacidophilic Archaeon *Sulfolobus solfataricus* (78-80°C, pH 2-3). The aim is to provide new enzymes “extremozymes” for the production of fine chemicals and process optimization (e.g. cellulose degradation in the area of renewable resources). (Total budget 2.550.940 €, Siebers Group: 623.666 €)

With an overall funding of ~5.2 Mio € for projects currently in progress, it ranks among the top groups of our university. The funding is provided by the German Research Foundation, the Ministry for Research and Education, the Ministry for Environment, the German Environmental Foundation the Ministry for Economy, the European Union and from industry.

For the Biofilm Centre:

Bettina Siebers

Hans-Curt Flemming

Wolfgang Sand

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Agate Materla	Secretary

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Astrid Dannehl	Technician
Barbara Derricks	Ph. D. student
Zenyta Dwidjosiswojo	Ph. D. student
Jan Frösler	Ph. D. student
Marina Horstkott	Ph. D. student
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Witold Michalowski	Postdoc
Alexa Pillen	Ph. D. student
Conny Rosengarten	Technician
Miriam Tewes	Ph. D. student
Janine Wagner	Ph. D. student

Aquatic Biotechnology

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Claudia Janosch	Ph. D. student
Beate Krok	Ph. D. student
Andrzej Kuklinski	Ph. D. student
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Qian Li	Ph. D. student
Ruiyong Zhang	Ph. D. student
Nanni Noel	Ph. D. student
Christian Thyssen	Ph. D. student
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Katharina Kruse	Ph. D. student
Marcel Blum	Ph. D. student
Frank Schult	Ph. D. student
Claus-Rüdiger Wallis	Ph. D. student

Safe Ruhr – Bathing Water and Drinking Water for the Ruhr Area

Collaborative Project - Partners: IWW Water Centre, RWW Rheinisch-Westfälische Wasserwerksgesellschaft mbH, University of Duisburg-Essen (Aquatic Ecology; Institute for Communication Science; Institute for Sociology), Ruhrverband, University of Bonn, University of Bochum, RWTH Aachen, Karlsruhe Institute of Technology (KIT), aquatune GmbH, Xylem Water Solutions
Coordinator: Dr. Martin Strathmann (IWW)
Funded by: Federal Ministry of Education and Research (BMBF); Network: Risk Management of Emerging Compounds and Pathogens in the Water Cycle (RiSKWa)
Project period: 01.01.2012 until 31.12.2014
Homepage: www.sichere-ruhr.de

The River Ruhr is of important local value for recreational activities as well as source water for drinking water treatment. Despite current prohibition it is already used for bathing. During a wide sampling campaign data on distribution and concentration patterns of pathogenic microorganisms were obtained. This enables for detailed quantitative microbial risk assessment. Depending on findings novel concepts for a temporal use of the river are developed taking different stakeholder groups into account. Insights derived from this project can be transferred to rivers used for similar purposes.

Contribution of the Biofilm Centre to the consortium:
Identification of influence factors on concentrations of pathogenic bacteria in the River Ruhr and their elimination during drinking water treatment. Implementation of rapid tests and online monitoring systems in cooperation with the IWW Water Centre.
Selected facultative (*Aeromonas spp, Pseudomonas aeruginosa*) and obligate pathogenic (*Campylobacter spp*., *Salmonella enterica*) organisms were detected in water samples and quantitative data was acquired using cultural methods. Generally the detection frequencies and concentrations of the facultative pathogens, that occur naturally in aquatic environments, were found to be higher compared to those of the obligate pathogens, especially regarding to *Aeromonas* spp.. Effects of point and non-point sources on the pathogen levels appear to be lower than expected. To evaluate the risks for human health, measured concentrations of the gastrointestinal pathogens *Campylobacter* spp. and *S. enterica* are used for quantitative microbial risk assessment (QMRA). Strain isolates obtained within the sampling campaign will be used for further geno- and phenotypical population analysis.
Furthermore rapid identification techniques are currently tested (e.g. the mbOnline-Coligard® device and quantitative PCR) for indicator organisms included in the EU bathing water directive; first observation show that the continuous operation has to be optimized.

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Recognition and countermeasures to transiently non-culturable pathogens in drinking water installations

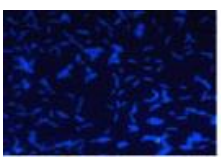
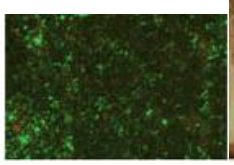
Collaborative Project, Project Partners: University of Duisburg-Essen, University of Bonn, Technical University of Hamburg-Harburg, DVGW, Technical University of Berlin, IWW Water Centre Mülheim

Coordination: Prof. Dr. Hans-Curt Flemming
Funded by: Federal Ministry of Education and Research
Project period: 01.09.2010 until 28.02.2014
Homepage: www.biofilm-management.de

Project of Biofilm Centre within the consortium: How do hygienically relevant microorganisms enter the VBNC state, how do they become culturable again and how virulent are they then?

Copper plumbing materials can be the source of copper ions in drinking water supplies. For the pathogens *P. aeruginosa* and *Legionella pneumophila*, inactivation of planktonic cells as well as of biofilms by copper ions has been topic of this research. Copper-mediated inactivation of bacteria was usually studied based on culture-based methods, but these do not necessarily verify cell death. It could be shown that copper-stressed planktonic cells of *P. aeruginosa* and *L. pneumophila*, although not detectable with the cultural methods designed for their detection, maintain essential viability markers (e. g. membrane integrity, 16S rRNA). Thus, they cannot be considered to be dead. Integrated in a biofilm, *P. aeruginosa* could even tolerate higher copper concentrations up to 100 mM before losing culturability, while maintaining viability. The minimal inhibitory concentration was found to be about 20 fold higher than that found for planktonic cells.

Under nutrient-limited conditions and with the addition of the copper chelator sodium diethyldithiocarbamate (DDTC), non-culturable copper-stressed *P. aeruginosa* return to a culturable state (resuscitation) within 14 d. This effect could be shown to occur faster with the addition of nutrients. Current experiments deal with the resuscitation of non-culturable cells of *L. pneumophila* upon co-culture with *Acanthamoeba castellanii* under nutrient-limited and nutrient-rich conditions. The hygienic importance of pathogens in the VBNC state is still unclear and presents a key issue of this study. To investigate the cytotoxicity and genotoxicity of *P. aeruginosa*, human bronchial epithelium cells (BEAS-2B) were exposed to *P. aeruginosa* in the VBNC state and after resuscitation. Determining the membrane integrity (trypan blue staining), mitochondrial activity (MTT assay), cell proliferation (exCELLigence) and chromosomal damage (micronucleus test), the vitality of BEAS-2B was analyzed. It could be shown that *P. aeruginosa* in the VBNC state were not harmful towards human bronchial epithelium cells (BEAS-2B), whereas resuscitated *P. aeruginosa* showed the same cytotoxic and genotoxic effect as the original strain. From a health perspective, the relevance of pathogens in the VBNC state may be underestimated, because it has to be taken into account that they can regain their virulence and are able to initiate infection when they revert to the culturable state. Thus, pathogens in the VBNC state represent an infectious potential when present in drinking water systems.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Nanosilver particles - mechanisms of action and study of possible interaction with tissue, cells and molecules. Definition of their relevant potential for intolerance.

Collaborative Project - Project Partners: aap Biomaterials GmbH, EXcorLab GmbH, Justus Liebig Universität Gießen, rent a scientist GmbH
Coordination: C. Sattig, aap Biomaterials GmbH
Funded by: Federal Ministry of Education and Research
Project period: 01.10.2010 until 31.03.2014
Homepage: www.nanosilver-project.info

The toxic effect of silver has been known for a long time. With increasing resistance of microorganisms against antibiotics, the use of silver has gained interest in medical applications. Nanomaterials such as nanosilver (AgNPs) may have new and different characteristics in contrast to the bulk material. Until today little is known about possible side effects of nanomaterials towards humans. Substances that are categorized as well-tolerated can exhibit toxic side effects, if they are present as nanomaterial. Aim of this study is to investigate possible concerns associated with AgNPs referring to long-term effects in humans as well as possible silver resistance of pathogens.

Project of Biofilm Centre within the consortium: Antimicrobial effect of AgNPs and possible generation of microbial resistance

Aim of this study is to obtain information about the efficiency of AgNPs against clinically relevant bacteria in order to evaluate application spectra and limitations.
It was found that AgNPs are much less effective towards the inhibition of biofilm formation and the inactivation of established biofilms than silver ions. Attached biofilm bacteria are significantly less sensitive towards silver (AgNP and $AgNO_3$) than planktonic bacteria.

Furthermore, one response of microorganisms to silver stress may be the transition into a viable-but-nonculturable- (VBNC-) state, from which microorganisms are able to resuscitate and therefore are able to regain infectivity. An indication for the VBNC-state in silver exposed *Pseudomonas aeruginosa* was investigated within this project. Non-culturable silver exposed *P. aeruginosa* showed clear signs of viability when investigated by culture-independent methods. They still displayed intact DNA and ribosomal RNA, maintained their membrane integrity and contained significant amounts of ATP. Therefore, it was concluded that silver ions and AgNPs in concentrations at which culturability was inhibited, P. aeruginosa still remained in the VBNC state. This applies to both planktonic and biofilm cells. From the view of in-vitro experiments, the employment of AgNp seems not to be promising for preventing or removing biofilms on implants.

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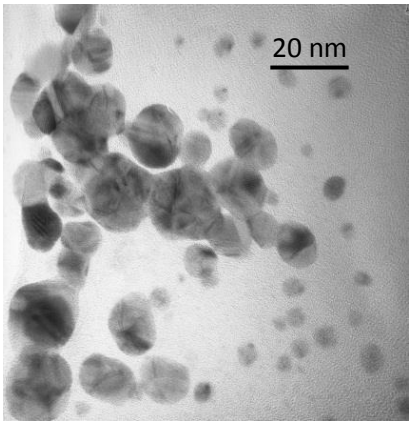

Transmission Electron Micrograph of Silver Nanoparticles

Microbial ochering in technical systems

Collaborative Project - Project Partners: TU Berlin, Friedrich Schiller University Jena, HTW Dresden, Berlin Centre of Competence for Water, Berliner Wasserbetriebe, Institute for Scientific Photography, Hammann GmbH, ARCADIS Deutschland GmbH, AUCOTEAM GmbH, KSB Aktiengesellschaft, RWE Power AG, VATTENFALL Europe Mining AG
Funded by: Federal Ministry of Education and Research
Project period: 01.02.2011 until 31.07.2014
Homepage: www.anti-ocker.de

In water-bearing technical systems, particularly in wells or pipe networks, microbial influence may cause the formation of clogging materials. These precipitates negatively affect the performance of wells by blocking the screens and the pump. The aim of the project is to gain a better understanding of the process of microbial clogging and to develop new techniques to prevent or dissolve the incrustations.

Contribution of Biofilm Centre to the consortium: Integration, persistence and control of hygienically relevant bacteria in iron oxide incrustations in wells

Ochering of wells leads to huge porous surfaces. Hygienically relevant microorganisms and pathogens can enter wells via contaminated groundwater or directly. Question of the study therefore was if those organisms can integrate in ochreous incrustations, if they can persist or even grow there and if they have the potential to contaminate the water in which they are in contact with.

Survival of target organisms (*Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*) spiked in ochre and well-water was tested over time in microcosm experiments and under flow through conditions. Those experiments showed:

- No increase of target organisms in ochre or well water, but all target organisms still culturable after 14 days
- Culturablity of target organisms over time differ among one another and in suspensions of different ochres; mostly a decrease in culturability was observed which however was more or less pronounced
- A slighter decrease of FISH(Fluorescence in situ hybridization)-positive cells than of culturability of target organisms spiked into well water gives a hint to the occurrence of viable but nonculturable (VBNC) bacteria
- Under flow through conditions target organisms attached to ochre showed a similar persistence over time despite of their different ecology: In a week nearly no decrease of culturability was observed; after two weeks a decrease of at most one order of magnitude. Target organisms originating from the ochre were detected in the water phase over the whole period of 14 days

Conclusion: Target organisms survive under low nutrient conditions and low temperatures attached to ochre. Ochre seems to be a habitat for hygienically relevant bacteria from which the water can be contaminated over a longer period of time.

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Test of survival of target organisms in ochre over time under flow through conditions

Dynaklim ("Dynamic adaptation of regional planning and developmental processes to the impact of climate change in the Emscher-Lippe region (Ruhrgebiet)"

Joint project, partners: IWW Water Centre
Funded by the Federal Ministry of Education and Research
Homepage : www.dynaklim.de
Contribution of Biofilm Centre within the project:
The task of the Biofilm Centre is to perform research on **the influence of temperature on hygienically relevant microorganisms in drinking water biofilms** in close collaboration with the IWW Water Centre (Mülheim/Ruhr).

Field Experiments
Variations in temperature of about 10 °C at the sampling sites could be detected in summer. These variations were climatope-dependent and more intense in highly sealed areas (e.g. city center). For the tested drinking water no temperature effect on the detection of hygienically relevant microorganisms could be observed. Compared to the drinking water, more hygienically relevant bacteria could be detected in drinking water biofilms. Moreover, the frequency of detection of cultural coliform bacteria was temperature dependent (summer> autumn> winter). By means of Fluorescence in situ hybridization (FISH) the target organisms could be detected at all sampling sites at least for one sampling time point. This might be an indication for bacteria being present in a viable but non culturable state. However, further research is required to elucidate the influence of water temperature on the presence of bacteria in the VBNC state. The frequency of detection of *Escherichia coli* using FISH increased with elevating temperatures, while it decreased for *Pseudomonas aeruginosa*. Biofilm formation on EDM and PE was enhanced by higher temperatures posing a risk of contamination of the drinking water in summer.

Laboratory Experiments
Under oligotrophic conditions the incorporation (number of cultural cells of the target organisms 1 d after inoculation) of *E. coli* decreased at temperatures above 21 °C on PE. The same effect was observed for *Klebsiella pneumoniae*. The incorporation of *P. aeruginosa* increased at temperatures above 21 °C on PE and EPDM. On both materials the incorporation of *Legionella pneumophila* was similar at temperatures above 8 °C. No effect of nutrient addition was observed on the incorporation of *E. coli* and *K. pneumonia*. However, the incorporation of *L. pneumophila* decreased at temperatures above 21 °C under nutrient rich conditions on both materials. Under oligotrophic conditions the persistence (period of cultural detection) of the target organisms was not influenced by temperature except for *L. pneumophila*, whose persistence increased at temperatures above 21 °C. Nutrient addition significantly enhanced the period of culturally detectable *E. coli* and *K. pneumonia*, whereas the duration of culturally detectable *L. pneumophila* decreased. Regardless of water temperature and nutrient situation, *P. aeruginosa* was always culturally detectable. With FISH *E. coli* and *K. pneumonia* were detectable over 28 d under all conditions indicating the presence of these bacteria in a VBNC state. In 100 mL Volume of the reactor effluents the target organisms could be mostly detected at least for the same time as they were detected in the biofilms indicating a contamination of the water phase by detachment of the hygienically relevant bacteria from the biofilm.

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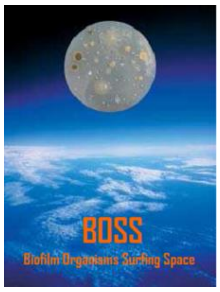
Annular reactor for investigation of the influence of water temperature on incorporation, persistence and survival of hygienically relevant bacteria in drinking water biofilms.

BOSS – Biofilm Organisms Surfing Space



Collaborative Project - Project Partners: DLR Cologne (Germany), CEPSAR (UK), University of Rome (Italy), University of Salzburg (Austria)
Coordinator: Dr. Petra Rettberg, DLR Cologne
Funded by: Federal Ministry of Economics and Technology
Project period: 01.05.2011 - 31.10.2014

When living as a biofilm, embedded in a matrix of self-developed extracellular polymeric substances, microorganisms are generally more tolerant towards environmental stressors than planktonic cells of the same species. It is the hypothesis of BOSS, that due to this phenomenon the biofilm form of microbial life is able to survive under the detrimental effects of space and Martian environments.
In the course of this project, various test organisms such as extremophile bacteria will be exposed to simulated space conditions (e.g. desiccation, UV irradiation) at the German Aerospace Centre DLR in Cologne. Besides these simulation experiments, a space mission with a launch scheduled for summer 2014 is planned. Mounted within the EXPOSE R-2 facility attached to the International Space Station, the BOSS samples will be exposed to a real space environment.

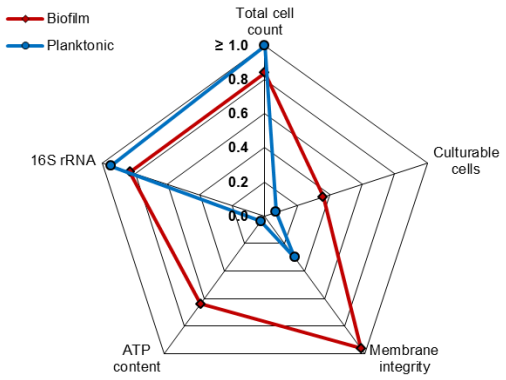


Fig. 1. Relative viabilty of biofilms and planktonic cells after 1 month of desiccation (values are in reference to fresh cultures).

The test organism investigated at the Biofilm Centre is *Deinococcus geothermalis*, a highly desiccation- and radiation resistant bacterium isolated from hot springs.

Long-term (2 months) desiccation experiments showed that, both biofilms as wells as planktonic cells survived in the dried state.
However, biofilms cells were in fact able to maintain important viability functions (intracellular ATP content, membrane integrity, culturability) at a higher level than planktonic cells (cf. Fig. 1).

The effects of various space-relevant parameters on dried biofilms and planktonic cells of *D. geothermalis* were assessed using cultural methods and fluorescence microscopy (Live/Dead differential staining). Whilst high doses (1-10 kJ/m^2) of monochromatic UV irradiation decreased the culturability of the samples by 1 to 2 orders of magnitude, other stressors such as vacuum or freeze-thaw cycles did not seem to affect viability. So far, the results indicate that *D. geothermalis* is a well-suited candidate for the foreseen space mission.

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Composition, dynamics and function of extracellular polymeric substances in drinking-water biofilms

Project description

Biofilms are ubiquitous on surfaces in drinking-water distribution systems, where they can act as a reservoir for pathogenic microorganisms, posing a potential threat to human health. Extracellular polymeric substances (EPS) are major components of microbial biofilms. They form the extracellular matrix of biofilms, mediate the mechanical stability of biofilms and afford protection for biofilm organisms against disinfectants like chlorine. For prevention and control of biofilm formation, knowledge of the composition and function of the EPS components is essential, since EPS are important targets for measures aimed at the inactivation and removal of biofilm organisms.

The aim of this study was the characterization of drinking-water biofilms and their EPS on microbiological, molecularbiological and biochemical level. The biofilms were grown on an EPDM substratum which is known to promote biofilm growth in a biofilm reactor which was continuously perfused with drinking water. A miniaturized method based on the cation exchange resin Dowex was established for the isolation of EPS from drinking-water biofilms. The composition of the EPS was determined by analyzing for carbohydrates, proteins and DNA as characteristic components of microbial EPS. Proteins were further studied by gel electrophoretical means. Of particular interest was the function of EPS proteins as hydrolytic enzymes, which provides information on the metabolic potential of a biofilm as reaction to environmental conditions such as nutrient availability. Enzyme activity of eight groups of enzymes commonly found in the EPS of environmental and pure culture biofilms was tested.

This study demonstrated the dynamics of EPS components in the course of biofilm formation, indicating continuous changes to the EPS matrix induced by the constituent organisms. Drinking-water biofilms were shown to be another type of biofilms, in which proteins represent the main EPS component, followed by polysaccharides and DNA. EPS proteins in drinking-water biofilms exhibited metabolic, transport and regulatory functions. The pattern of expression of extracellular enzymes could be interpreted as graduated response to the nutrient supply. The initial stages of biofilm formation were influenced by the leaching of organic components from the EPDM material (e.g. plasticizers), while later stages were characterized by the modification of the EPS matrix or its utilization as nutrients. Thus the EPS matrix functions as an external digestive system of biofilm organisms, which can adapt to the nutrient conditions, and furthermore, it serves as a nutrient reservoir.

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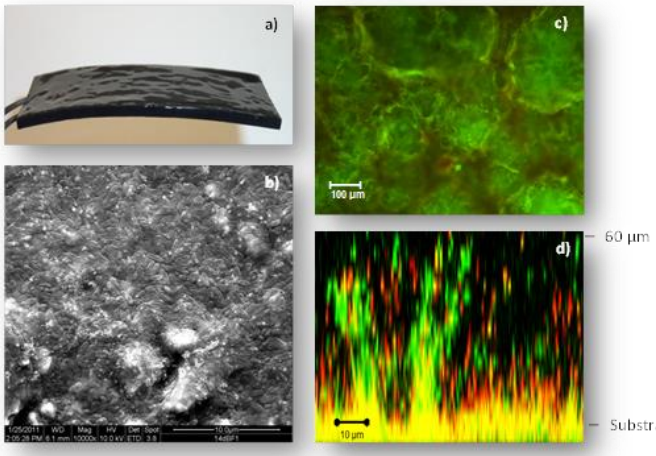

Images of 14 d-old drinking-water biofilms grown on EPDM showing a) a macroscopical view of a biofilm, b) an SEM top view of a dried biofilm at 10,000x magnification, c) an epifluorescence microscopic top view of a live/dead stained biofilm at 100x magnification, and d) a CLSM side-view (z-stack) image of a live/dead stained biofilm at 1,000x magnification.

Freshwater plankton as a habitat for hygienically relevant microorganisms

Funded by: DFG (German Research Foundation)

Project period: 01/2009 until 12/2012

Collaboration: NRF-funded project: “Plankton as a vector for hygienically relevant bacteria in eutrophic waters”; Prof. Dr. T. E. Cloete, University of Stellenbosch, South Africa

In aqueous environments bacteria can occur planktonically in the water phase, or associated in biofilms attached to solid surfaces or other phase boundaries. During high proliferation periods in summer, plankton organisms in surface waters provide external surfaces which can be colonized by biofilms. Zooplankton surface can add up to an overall area of more than 3.000 km^2 in a lake. Possible associations of potentially pathogenic bacteria with phyto- and zooplankton were observed in a field study in a freshwater environment (Lake Baldeney, Essen, Germany) and in laboratory experiments.

Summary of field study

As a result of the field study, it can be concluded that overall surfaces of plankton can be very large. All organisms of hygienic relevance considered in this study were found in water, plankton and *Elodea* samples at 4–6 orders of magnitude higher concentrations than in water. Plankton and *Elodea* both provide a microhabitat for these pathogens. *Legionella* spp. was only detected by molecular methods. The presence of 16S rRNA indicates presence of ribosomes and, thus, may be considered as viability sign. This is supporting (but not proving) the hypothesis that these organisms may be in a VBNC state.

Summary of interaction study

Co-cultivation experiments showed both colonization on the carapace and ingestion of bacteria into the gut of the daphnids. As the FISH numbers were consistently higher than those obtained by cultivation, the assumption was supported that the bacteria may have entered a VBNC state. This state might have been induced by association with *D. magna*. From such a state, in which the cells are not dead but only no more found with the standardized method for their detection, it has to be taken into account that resuscitation is possible. Ingested microorganisms such as *E. faecalis* can replicate in the plankton organism gut and can be released either unharmed, or in VBNC, or digested (dead).

The results of this study indicate that it is of concern to consider plankton organisms acting as habitat and vector for human pathogens. This is of relevance considering human health in drinking water production and use of recreational waters.

The project has been finished and Miriam Tewes got her Ph.D. awarded in 2013.

Person in charge: Dr. Miriam Tewes, MSc

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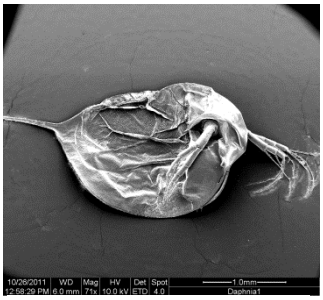

SEM micrograph *D. magna*

Advanced Technologies for Water Resource Management (ATWARM)

Marie-Curie project (http://www.atwarm.com)

Project consortium: QUESTOR centre (UK), ATWARM, University of Duisburg-Essen, Queen's University Belfast (UK), Cranfield University(UK), IWW Water Centre, Industrial partners: NI Water (UK), T.E. Laboratories Ltd (Ireland).

Coordinator: Prof. Mike Larkin, Queens University, Belfast

Contribution of Biofilm Centre and Institute of Analytical Chemistry within the project:

Priority substances in activated sludge: Incidence, accumulation, source tracking emitter identification & prevention strategies

This project is aimed at finding innovative applications of microbial wastewater biofilms. Due to their ability to interact with many organic and inorganic pollutants, the wastewater microbial biofilms potentially represent a useful tool in order to trace pollution processes in wastewater piping systems and to prevent pollution events. Therefore, it is important to develop a novel analytical method for detecting organic pollutants absorbed by sewer biofilms. In this regard, fluorescence spectroscopy was employed in order to detect PAHs (polycyclic aromatic hydrocarbons) inside sewer biofilm samples using portable devices for on-field measurements and performing faster analyses than the traditional methods.
The fluorescence lifetime was measured analyzing 4 different portions of the same biofilm matrix, that was taken from the sewers of the urban waste water treatment plant in Bielefeld, Germany For the analyses each sample was placed in QS glass cuvettes (1 mm pathlenght, chamber volume 350µL). A USB-Spectrograph OceanOptics 65000 was used for the acquisition of the fluorescent signal from each sample with a fiber optic device in front-face mode. A Deuterium lamp linked to a second fiber optic device was used to irradiate the biofilm sample. The fluorescent spectrum of each sample was collected before and after the exposure to a deionized water solution of phenanthrene (PAHs) at 1.5 ppm for a period of 24 hours. For all samples, a higher fluorescence lifetime in the biofilm was related to the presence of phenanthrene (Table 1). Parallel to biofilms as sorbents, other hydrogels have been included in the study with promising results, indicating sorption of target substances and easier to handle than microbial biofilms. Such substances can be exposed in suitable devices and exposed for monitoring. In cases of detection of contaminants in systems without monitoring devices, biofilms are the best choice to detect the occurrence of contaminants even after they cannot be found any more in the water phase.

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Table:Total fluorescence lifetime of each sample tested during the experiments. Samples exposed to the phenanthrene solution show highest. "–"= not significant value. Values obtained after exposure to phenanthrene are indicated as "phe. +", while the samples which were not exposed to phenanthrene are indicated as "phe. –". I, II, III, IV are the roman numeral indicating the order of the samples

Sample	Fluorescence lifetime (hours)
I phe.+	16.6
I phe.-	4.7
II phe.+	4.3
II phe.-	-
III phe.+	10.00
III phe.-	-
IV phe.+	7.45
IV phe.-	3.95

Membrane biofouling: hydraulic resistance of biofilms

Funded by: Wetsus, centre of excellence for sustainable water technology

Project period: 01.04.2009 until 30.06.2013

Supervisors: Prof. Dr. Hans-Curt Flemming and Dr. Hans Vrouwenvelder

Homepage: http://www.wetsus.nl/research/research-themes/biofouling

High quality water can be produced with membrane filtration processes such as nanofiltration and reverse osmosis. A serious limitation in the application of these membrane processes for water treatment is biofouling. Biofouling occurs when the growth of biofilms negatively impacts the membrane performance parameters feed-channel pressure drop and transmembrane pressure drop, both reducing the water production (permeate flux).

The main objective of this thesis was to determine the hydraulic resistance of pure biofilms and how it was affected by (operational) parameters such as: permeate flux, crossflow velocity, biodegradable substrate content, and feed spacer presence.
A monitor was developed to assess the (i) hydraulic biofilm resistance and (ii) performance parameters feed-channel pressure drop and transmembrane pressure drop. By using a microfiltration membrane (pore size 0.05 µm) in the monitor salt concentration polarization was avoided, allowing operation at low pressures enabling accurate measurement of the hydraulic biofilm resistance. Extensive validation tests showed that the small-sized monitor is a suitable tool to study the hydraulic biofilm resistance under controlled conditions.
With that system, studies were performed at two fluxes (20 and 100 L m^{-2} h^{-1}) and constant crossflow velocity (0.1 m s^{-1}). The intrinsic biofilm resistance reached values up to 50 × 10^{12} m^{-1}. The resistance was not caused by biofilm cells but by the EPS matrix. The hydraulic biofilm resistance was higher when the permeate flux was increased. The biofilm resistance was high compared to the resistance of the employed microfiltration membrane but low compared to the resistance of nanofiltration (20%) and reverse osmosis (2%) membranes.
Biofilm accumulation, transmembrane (biofilm) resistance and feed-channel pressure drop were studied as a function of the crossflow velocity (0.05 and 0.20 m s^{-1}) and feed spacer presence in the monitors operated at a permeate flux of 20 L m^{-2} h^{-1}. Acetate was dosed to the feed water to enhance biofilm accumulation in the monitors. Biofilm formation in membrane systems increased both the feed-channel and transmembrane pressure drop. The hydraulic biofilm resistance was increased by a high biodegradable substrate load, high cross flow velocity, and feed spacer presence. Current membrane practice (high crossflow velocity and feed spacer) increased the impact of biofouling on membrane performance.
Based on new insights obtained by the studies described in this thesis potentially effective biofouling control strategies and recommendations for future research were given (Chapter 5). Biofilm development can be delayed by reducing the substrate concentration in the water by pre-treatment. The impact of biofilm accumulation on membrane performance can most likely be reduced by a (i) low permeate flux, (ii) low crossflow velocity and (iii) use of thicker/modified feed spacers. Concentration polarization may be increased by a lower crossflow velocity but reduced by a lower flux. The impact of modified operation and spacers on membrane performance, cleanability and concentration polarization are not clear and should be part of future biofouling research. (Dreszer et al., 2013).

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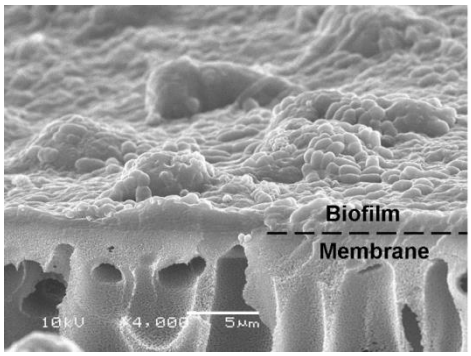

Manipulation of biofilm formation by mesophilic leaching bacteria – effect of physiological stress factors on biotechnological processes

Originaltitel: Manipulation der Biofilmbildung mesophiler Laugungsbakterien – Effekt physiologischer Stressfaktoren auf biotechnologische Prozesse

Collaborative project - Project Partners: Prof. Dr. Wolfgang Sand, Universität Duisburg-Essen (UDE), Biofilm Centre, Aquatische Biotechnologie; Prof. Dr. Galina Iutynska, Zabolotny Institut für Mikrobiologie und Virologie (IMV), Kiew

Persons in charge: Sören Bellenberg, MSc; Dr. Mariia Boretska, Prof. Dr. W. Sand
Project Funding: BMBF
Project period: 01.08.2013 until 31.07.2015

Colonization of metal sulfides is a prerequisite of efficient bioleaching. The aim of this project is to identify factors that influence the attachment of leaching bacteria to metal sulfides. Physiological stress factors have been shown to affect and sometimes induce surface colonization in other bacterial or archaeal systems. Therefore testing media supplementations or modified cultivation conditions regarding attachment to and dissolution of metal sulfides likely provides novel insights into the relevant bioleaching and biofilm formation mechanisms. Among the stress factors within the scope of this project are heavy metal ions, non-optimal cultivation temperature and pH values, availability of nitrogen and phosphate, ionic strength as well as oxidative stress. Oxidative stress is to be managed by all forms of aerobic life, but in microorganisms that grow in acidophilic habitats on metal sulfides, such as pyrite, reactive oxygen species (ROS) are of fundamental importance. ROS play an important role in leaching habitats since metal sulfides are releasing ROS due to chemical reactions of oxic waters with pyrite. Hence, ROS and the biofilm formation process by *Acidithiobacillus* spiecies might be linked. The aim is to manipulate bioleaching processes by altering microbe-mineral interactions.

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Attachment to and dissolution of metal sulfides by moderately thermophilic archaeon *Ferroplasma*

Topic: Biofilm formation and extracellular polymeric substances analysis of acidophilic archaea during bioleaching

Funded by: China Scholarship Council/Universität Duisburg-Essen

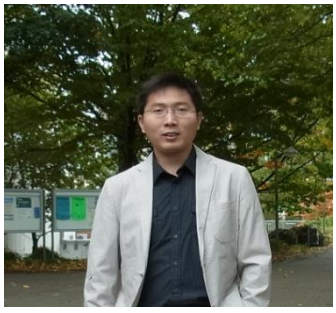
Running time: 2011-2014

The attachment of microorganisms to mineral surfaces is of great importance for the mineral dissolution process. Extracellular polymeric substances (EPS) mediate the connection between cells and the mineral sulfide and play a crucial role in interfacial interactions. Acidophilic archaea such as *Acidianus* spp., *Sulfolobus* spp. and *Ferroplasma* spp. play important roles in bioleaching and AMD systems, and have attracted significant attention for both, fundamental research and commercial application.

Contribution of Biofilm Centre within the project: Investigations on the process of attachment to and biofilm formation on surfaces during bioleaching, extraction and analysis of EPS involved in attachment and biofilm formation by acidophilic archaea. Our study aims to get an improved understanding of interfacial interactions between archaeal and surfaces in the bioleaching process.

Work in Progress: (1) Lectin screening tests (in collaboration with Dr. T. R. Neu, UFZ-Magdeburg) offer us a panel of useful lectins for visualization of glycoconjugates of EPS in biofilms of acidophilic archaea. (2) Cells of *Ferroplasma* grew heterogeneously on polycarbonate filters over time and seem to adjust their biofilms according to the substrate (e,g, glucose supplementation). EPS were evident in *Ferroplasma* biofilms on pyrite or polycarbonate filters. A low coverage of the pyrite surface by cells seems to be correlated with a low leaching ability. Bioleaching conducted by *Ferroplasma* occurs possibly via combined contact and non-contact mechanisms. (3) Advanced microscopy techniques (SEM, CLSM, AFM in combination with EFM) were applied to examine spatial distribution of cells and biofilms, as well as EPS excretion on pyrite and elemental sulfur. Cells of *Acidianus* RZ1 colonized these surfaces heterogeneously over time. Moreover, biofilm formation is dependent on energy substrates and surface properties. (4) Fluorescent lectin binding analysis (FLBA) as well as biochemical analysis indicate carbohydrates are a major part of archaeal EPS. Monosaccharides like glucose, mannose and fucose were detected in these archaeal EPS.

Person in charge: Ruiyong Zhang (M.Sc.), Prof. W. Sand



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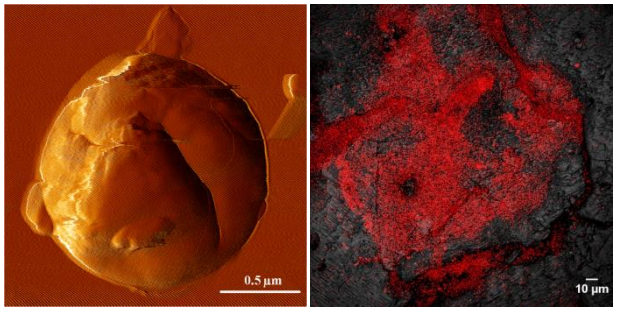

Microscopic images of cells of *Acidianus* RZ1. Left: a planktonic cell visualized by AFM. Right: attached cells (biofilms) on sulfur stained by SyproRed and visualized using CLSM

Urban mining – Recovery of metals from lignite ashes

Originaltitel: r^3 - Strategische Metalle , Verbundvorhaben: Kraftwerksasche - Chemisch-biotechnologische Gewinnung von Wertstoffen aus Braunkohlenkraftwerksasche. TP 3 - Mikrobielle Laugung mit thermophilen Schwefeloxidanten und Nitrifikanten

Collaborative project - Project Partners: − G.E.O.S. Ingenieurgesellschaft mbH; Prof. Dr. A. Schippers, BGR (Bundesanstalt für Geowissenschaften und Rohstoffe); Prof. Dr. W. Sand, Universität Duisburg – Essen; Prof. M. Bertau und Prof. G. Heide TU Bergakademie Freiberg; Prof. H. Wotruba RWTH Aachen; Prof. Dr.-Ing. Horst-Michael Ludwig, Bauhaus-Universität Weimar; Nickelhütte Aue GmbH; Loser Chemie GmbH; Vattenfall Europe Mining AG; Sunicon GmbH

Persons in charge: Sören Bellenberg, MSc; Dr. Tilman Gehrke, Prof. Dr. W. Sand
Project Funding: BMBF
Project period: 01.011.2012 until 31.10.2015

Raw material supply is a mayor issue for ensuring success of the European industry, economy and society. Critical metal resources are essential for almost all high technology electronic products. Due to their metal contents, lignite ashes are considered as a potential novel resource. Lignite fired power plants are a backbone of the energy production in Germany and tremendous amounts of ashes were landfilled in the past.

Growth of acidophilic sulfur oxidizing bacteria and archaea in presence of 25 % (wt/v) lignite ashes was demonstrated. Metal solubilization concerning aluminum and iron from the ashes was observed to be more efficient at a pulp density below 20 % (wt/v). Using raw ashes from landfill sites without chemical or physical pretreatment in biological experiments allowed for solubilization of up to 50% of the iron and 30% of the aluminum content. Growth of nitrifying bacteria was also successfully demonstrated, even though ash supplementation and metal solubilization are lower than in experiments with acidophilic sulfur oxidizers. Improved metal recovery is expected to be achieved after physical pretreatment of the raw material. This may enable for the separation from not leachable silicates prior leaching. Separation of materials may allow for winning of a product that may be worth for use in construction material. Hence, a selection of materials which are more accessible by bioleaching, which is a combined action of acid attack, complex formation and biological catalysis of redox chemistry in biofilms on mineral surfaces, will enable us to increase the metal recovery.

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Projektträger Jülich
Forschungszentrum Jülich
Bundesministerium
für Bildung
und Forschung

Microcalorimetry – a Measuring Technique for the Detection of Biomining

Funded by: Federal Institute for Geosciences and Natural Resources
Project period: 01.10.2010 until 31.12.2014
Project Partners: Federal Institute for Geosciences and Natural Resources & Universität Duisburg-Essen, Biofilm Centre, Aquatic Biotechnology

The demand (price) of metals, especially copper is increasing since many years. The mining techniques for the recovery of metals are cost intensive and environmentally harmful. High grade ore deposits are running off and low grade ores as well as recycling of metals have to be considered as valuable resources.

Biomining is an environmentally friendly, cheap and simple mining technique. Some microorganisms are able to solubilize minerals from their ores. Since more than 40 years biomining is used for the recovery of metals from low grade ores to win metals like copper, nickel and uranium.

Contribution of Biofilm Centre to the consortium:

Development of a microcalorimetric measuring technique for biomining

Aim of this study is the development of a measuring technique for the quantification of biomining of three different copper sulfides (Chalcopyrite, Chalcocite, Covellite).

Microcalorimetry is a non-invasive, non-destructive analytical technique, which provides information about the metabolic activity of microorganisms. In combination with analytical techniques, which can track the chemical products produced during the bioleaching process, the bioleaching efficiency may be quantified and predicted.

The copper sulfides will be leached with microorganisms in different temperature ranges. Microcalorimetric measurements will be performed to investigate the microbial activity and Atomabsorptionspectroscopy, High-Pressure Liquid Chromatography, Ion Chromatography and UV/VIS spectrophotometry will be performed to analyze the reaction products copper, sulfur, sulfate & thiosulfate and iron.

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The results show that bioleaching activity can be tracked by Microcalorimetry in the early stage of bioleaching. Additionally, the copper recovery correlates with the heat emission detected by Microcalorimetry.
However, later on iron, sulfur and copper species formed during the leaching process hinder the copper sulfide dissolution significantly.

For an efficient bioleaching, the attachment of microorganisms to the ore is required. The attachment is mediated through the extracellular polymeric substances (EPS) layer produced by microorganisms. The EPS layer plays a crucial role as reaction space for the bioleaching process. To investigate the microbe-mineral interactions more in detail, Atomic Force (AFM) and Epifluorescence Microscopy (EFM) will be used. With the AFM, force distance curves will measured, while with EFM the sugar moieties of the EPS will be analysed by the use of fluorescently labled lectins.

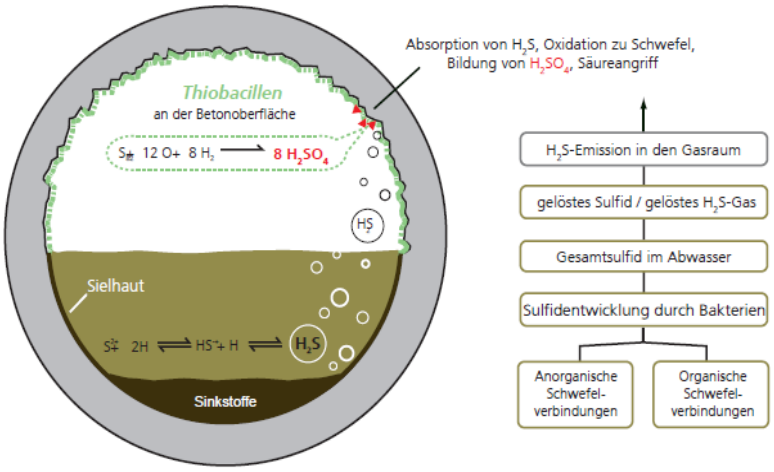
Biogenic sulfuric acid corrosion of different materials

Project Partner: Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT in Oberhausen

Biogenic sulfuric acid corrosion is a chemical attack to surfaces of different materials such as concrete, iron and polymers. It is caused by sulfuric acid producing bacteria. Gaseous sulfuric compounds are released and accumulate in the head space. Chemical oxidation of H_2S to elemental sulfur and the following biological oxidation via thiosulfate and other polythionates cause a decrease of pH (<7). The reduced sulfur compounds are oxidized to sulfuric acid, resulting in suitable conditions for the growth of Thiobacilli (*T. neapolitanus, T. intermedia*) which further decrease the pH. Below pH 5.5 *A. thiooxidans* colonize the surface. Between pH 2.0-3.0 this organism find its optimal growth conditions. Sulfuric acid is produced as a metabolite of these organisms causing a chemical attack on different materials. The aim of this study is the optimization and further development of different materials against biogenic sulfuric acid corrosion

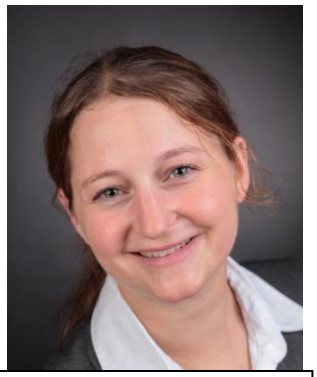
Contribution of Biofilm Centre to the consortium: Microbiological and analytical investigation of materials which were stored in the pilot plant at Fraunhofer Institute

In cooperation with Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT in Oberhausen a pilot plant was developed and the effect of biogenic sulfuric acid corrosion on different materials was tested. All samples were investigated using analytical as well as microbiological techniques. Microbiological analysis included the search for like acidophilic and neutrophilic iron- and sulfur oxidizers, sulfate reducing bacteria, nitrite oxidizers, fungi and chemoorganotrophic bacteria.



Nach: Bock, E.; Sand, W.; Pohl, A.: Bedeutung der Mikroorganismen bei der Korrosion von Abwasserkanälen, TIS Tiefbau- Ingenieurbau- Straßenwesen, Sonderdruck zum 4. Statusseminar "Bauforschung- Technik", 1983, S. 47-49

Person in charge: Nanni Noël



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Bio-derived corrosion protection for metallic materials by analogues of microbial exopolymeric substances from renewable resources

AIF collaborative project together with: DFI – DECHEMA Research Institute
Funded by: Programme for Industrial Collective Research (IGF) of the German Federal Ministry of Economy and Technology under the auspices of AIF, Project ZN04204-10
Period: 01.02.2011 – 31.12.2013

Microbial biofilms and their surrounding extracellular polymeric substances (EPS) can both proliferate and inhibit corrosion. The first is denoted as microbiologically influenced corrosion (MIC), the latter as microbiologically influenced corrosion inhibition (MICI). Both effects are influenced by the interactions between the EPS matrix and the constructional material itself, with the EPS' chemical composition determining its detrimental or beneficial nature. Comparable to classical interfacial corrosion inhibitors, specific functional groups of the EPS are crucial for EPS-surface interactions and thus facilitation of cell adhesion. This project aims to investigate EPS-analogue substances to protect metals against (a)biotic corrosion and formation of detrimental biofilms on their surface. The blocking of electrochemically "active sites" should suffice to prevent bacterial chemotaxis to (iron) ions and thus to reduce attachment of detrimental microbes to these sites.

Contribution of the Biofilm Centre: Extraction and analysis of microbial EPS, MIC simulation with SRB + manganese oxidizers and visualization of the metal-coating-bacteria interfaces
The Aquatic Biotechnology working group is constantly growing biofilms of detrimental and beneficial microorganisms to extract and analyze their EPS. In conformity with relevant literature the main components are carbohydrates, proteins, lipids and eDNA. Our MIC-simulations with sulfate-reducing bacteria showed a reduction in corrosion rates of mild steel coated with EPS analogues by up to 90% compared to an uncoated references over a period of 3 month. Pitting corrosion of highly alloyed steel was successfully shown for manganese oxidizing microorganisms. A potential impact of the coatings on pitting corrosion of highly alloyed steel by Mn-oxidising bacteria is currently under investigation. Biofilm formation of both groups of organisms was only slightly reduced, but the coatings effectively prevented contact of the microorganisms to the material surface. The biodegradability of the substances by pure cultures of SRB as well as enrichment cultures from soil ("worst case") in short-term was negligible, long-term studies over a period of 3 month showed no degradation of pure cultures of the respective organism. Improved coating procedure and crosslinking of monomers enhanced the coating stability up to 3 month. Currently, the influence of surfaces, bacteria and coatings on each other is studied using state-of-the art high-resolution visualization techniques such as combined epifluorescence with both Kelvin probe force microscopy (EFM-KPFM). Additionally, a novel electrochemical AFM-cell will be used to combine online electrochemical corrosion measurement and the high spatial resolution of the AFM.

In summary we successfully applied EPS analogue substances as a coating to significantly reduce MIC caused by SRB.

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Elucidation of the biofilm lifestyle of acidophilic leaching bacteria

Mario Vera, Sören Bellenberg, Claudia Janosch, Nanni Nöel, & Wolfgang Sand

Biomining uses acidophilic microorganisms for the recovery of metals from sulphide ores in tanks, heaps and dumps. Bioleaching of secondary copper minerals such as chalcopyrite ($CuFeS_2$; the largest copper resource in the word) is done in engineered heaps and accounts for approximately 15% of the present world copper production.
Bacterial cells can effect this metal sulfide dissolution via iron(II) ion and/or sulfur compound oxidation. In most cases, the biofilm forming cells are efficient in terms of bioleaching kinetics. In biofilms, bacteria are embedded in extracellular polymeric substances (EPS), which complex iron(III) ions (Fig. 1). EPS are responsible for an electrostatic mediation of cell attachment to metal sulfides, while their second function is the oxidative dissolution of the metal sulfide[1]. Biofilm formation is a genetically regulated process where the microorganisms involved undergo a series of molecular adaptations to their sessile lifestyle. One of the main phenotypic changes in an intense EPS production[2]. In this work we have studied the molecular adaptations to the biofilm lifestyle in model leaching bacterium *At. ferrooxidans* by high throughput proteomics, in collaboration with Dr. Ansgar Poetsch´s lab, from the Ruhr Universität Bochum.

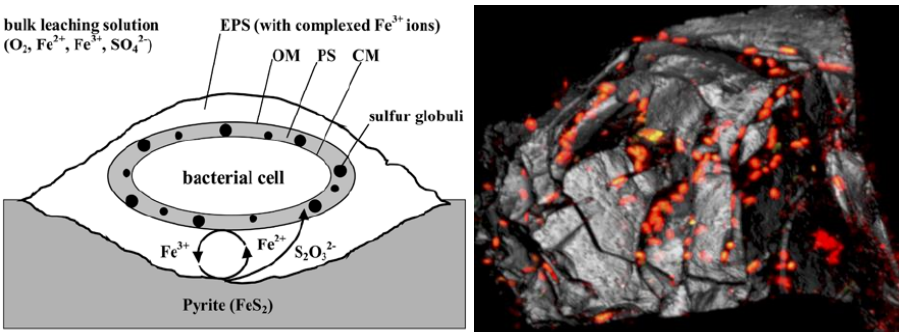

Fig. 1. *At. ferrooxidans cells embedded and their EPS attached to pyrite.* Left:CM, cytoplasmic membrane; PS, periplasmic space; and OM, outer membrane. *Right:* Confocal laser microscopy image showing a 3D projection of a pyrite grain (50-100 µm) after 7 days of biofilm development. *At. ferrooxidans* colonisation pattern correlates with surface imperfections.

Our results show that the early steps of *At. ferrooxidans* biofilm formation consist of a set of metabolic adaptations where extracellular polymeric substances biosynthesis seem to be pivotal. This first high-throughput proteomic study will also contribute to the annotation of several unknown *At. ferrooxidans* proteins found[3]. Recently we have applied a similar high throughput proteomic approach to study the sulfur oxidizing *Acidithiobacillus caldus* by comparing sulfur and thiosulfate grown cells (manuscript in preparation).

1. **Vera M., Schippers A., Sand W** (2013) Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Mini Review Appl Microbiol Biotechnol 97:7529-41.
2. **Bellenberg S., Leon-Morales C. F., Sand W. & Vera M.** (2012) Visualization of Capsular Polysaccharide production induction in *Acidithiobacillus ferrooxidans*. *Hydrometallurgy* 129-130: 82-89.
3. **Vera M, Krok B, Bellenberg S, Sand W, Poetsch A** (2013) Shotgun proteomics study of early biofilm formation process of *Acidithiobacillus ferrooxidans* on pyrite. Proteomics 13: 133–1144.

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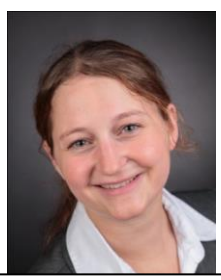

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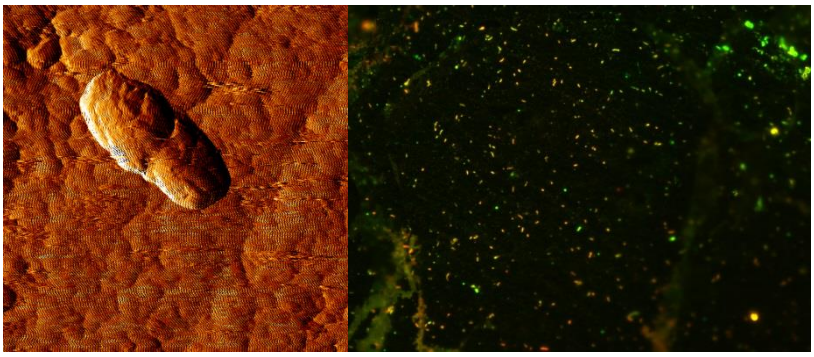
Interactions between pyrite and cells of Gram-positive leaching bacteria

Funded by: China Scholarship Council/University of Duisburg-Essen

Person in charge: Qian Li (M.Sc.)

Running time: 2013-2016

Over years, it has been confirmed that the bioleaching process is strongly influenced by the ability of bacteria to attach the minerals as it can improve the metal extraction rate and this behavior is mediated by extracellular polymeric substances (EPS). Therefore, the EPS involved bacterial attachment behavior has to be studied. In this study, we focus on *Sulfobacillus thermosulfidooxidans* which is Gram-positive leaching bacteria. Firstly, EPS analyzing will be done to figure out the main components affecting adhesion. Secondly, the adhesion force of *S. thermosulfidooxidans* to pyrite will be measured directly by atomic force microscopy (AFM). By doing this measurement, a serial factors that would affect adhesion will be investigated such as pH and ion intensity. We also study the effect of energy source on adhesion force. Additionally, AFM combined with epi-fluorescence microscopy (EFM) will be used to visualize the bacterial colonization on pyrite coupon surface during leaching. The aim of this study is to investigate the relationships between adhesion force, attachment behavior and bioleaching efficiency, thereby getting better understanding of the interfacial mechanism between leaching bacteria and minerals.



S. thermosulfidooxidans grow on pyrite visualized by AFM and EFM

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Sulfolobus acidocaldarius: a new chassis for metabolic engineering

Collaborative Project - Partners: Dr. Sonja-Verena Albers, Max-Planck-Institute for terrestrial Microbiology, Marburg, Germany
Funded by: University Duisburg-Essen, Max-Planck-Institute
Project period: 01.06.2013 until 31.05.2016
Homepage: https://www.uni-due.de/biofilm-centre/

The depletion of fossil resources leads to a high demand of alternative, **renewable energy sources**. One of these alternative energy sources is the production of biofuels by metabolically engineered microbial strains. However, the production of so called 1st generation biofuels is based on corn, sugarcane, rapeseed etc. and thus competes with human feedstocks and their cultivation areas. The 2nd generation biofuels have the potential to overcome these problems by the use of lignocellulosic biomass like straw, grasses, nutshells, banana waste or the daily newspaper, that we read. These biomass provides enough energy for metabolically engineered microorganisms to produce bioalcohols or other chemical compounds of industrial relevance without competition with human feedstocks.
In such biotechnological application and processes archaeal organisms are so far greatly neglected although their often extremophilic lifestyle could be of great advantage. The genetically tractable crenarchaeon ***Sulfolobus acidocaldarius*** is one of the best studied archaeal model organisms and its thermoacidophilic life style makes it very suitable for industrial applications since it persists the harch conditions used e.g. in the pre-treatment of lignocellulosic biomass .

Contribution of Biofilm Centre to the consortium
The aim is to establish and optimize *S. acidocaldurius* as host strain for the production of biofuels and other valuable chemical compounds. For the effective expression of enzymes involved in cellulose and hemicellulose degradation as well as in glycolytic flux optimization temperature inducible promoters are currently under investigation. Furthermore, suitable (novel) enzymes, particularly alcohol and lactate dehydrogenases, for the enhanced biofuel production (e.g. bioethanol, biobutanol) as well as for the synthesis of other chemical compounds (e.g. lactate) have been identified, the encoding genes were cloned and are currently transferred in the *S. acidocaldarius* host strain and the resulting production strains will be characzterized for their biotransformation efficiencies.

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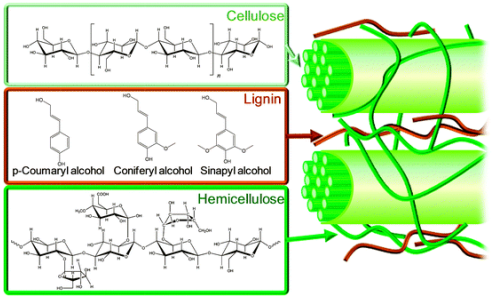
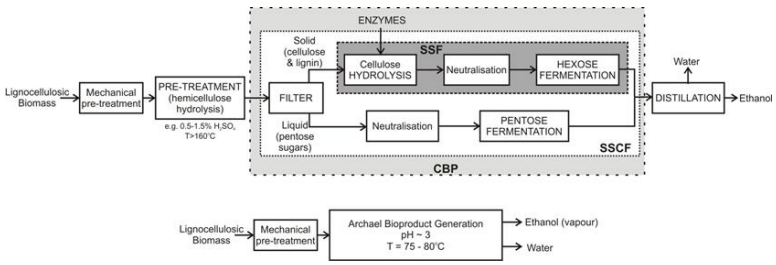
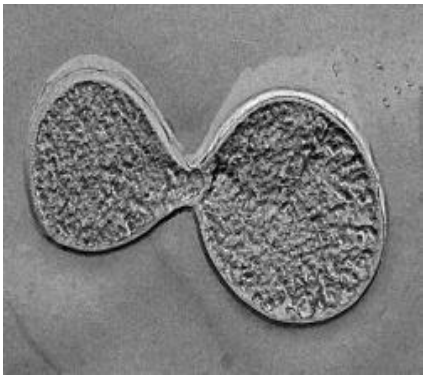




SulfoSYS^{BIOTEC}" Applied **Sulfo**lobus **Sys**tems Biology: Exploiting the hot archaeal metabolic potential for **Biotec**hnology

Funded by: Federals Ministry of Education and Research (BMBF)
Project period: 01.01.2013 until 31.12.2015
Collaborative Project - Project Partners: Dietmar Schomburg (TU Braunschweig), Jörg Schaber (University of Magedeburg), Alex Goesmann (University Gießen), Jörn Kalinowski (CeBiTec Bielefeld), Philipp C. Wright (University of Sheffield), Ida Schomburg (Enzymeta GmbH), Roland Wohlgemuth (Sigma Aldrich), Sonja-Verena Albers (MPI Marburg), Hans V. Westerhoff (University of Amsterdam), Jacky L. Snoep (Stellenbosch University)
Homepage: http://www.sulfosys.com

The SulfoSYS^{BIOTECH} project aims to unravel the complexity and regulation of the carbon metabolic network of the thermoacidophilic archaeon *Sulfolobus solfataricus* (optimal growth at 80°C and pH 3) in order to provide new catalysts `extremozymes´ for utilization in White Biotechnology. The growth of *S. solfataricus* is analyzed on ´alternative´ carbon sources (e.g. polymers, pentoses, sugar acids, alcohols, aldehydes) to elucidate the respective degradation pathways and to identify new enzymes involved. For these approaches the project will combine state of the art methods (genomics, transcriptomics, proteomics, metabolomics), bioinformatics, modelling, data management and classical microbiology, biochemistry as well as genetic/molecular biology techniques.



Electron microscopy picture of *S.solfataricus* (S.-V. Albers)

Contribution of Biofilm Centre to the consortium:
Metabolic pathway reconstruction
To investigate the metabolic capabilities and to identify the respective metabolic pathways the growth of *S. solfataricus* on different substrates like alcohols and different sugars is investigated with a special focus on the dehydratase and dehydrogenase enzymes involved. Currently, different alcohol dehydrogenases, acyl-CoA syntethases, sugar dehydratases and sugar dehydrogenase are investigated with respect to substrate and cosubstrate specificity as well as stability towards temperature, pH and organic solvents.

Person in charge: Claus-Rüdiger Wallis, M.Sc., and Katharina Kruse, Dipl.-Biol.

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Protein phosphorylation in Archaea: Central carbohydrate metabolism in *Sulfolobus* species

Funded by: DFG/ University of Duisburg-Essen
Project period: 01.07.2011 – 01.07.2014
Collaborative Project - Project Partners: Dr. Phillip C. Wright, University of Sheffield, UK; Dr. Sonja-Verena Albers, Max-Plank-Institute for Terrestial Microbiology, Marburg, Germany

Posttranslational protein modifications like phosporylation have a major impact on the activity and regulation of proteins and regulatory networks. In Bacteria commonly two-component systems including histidine kinases are involved in signal transduction processes whereas in Eukaryotes serine/threonine and tyrosine kinases play a major role in such regulatory networks by transducing signals and amplifying them. In the third domain of life, the Archaea, the common bacterial two component systems are largely absent in Crenarchaeota, but all archaea contain a set of eukaryotic type like protein kinases (ePKs) and protein phosphatases. However, only very few of these proteins have been studied and virtually no signal cascade has been unraveled in Archaea.
Model organisms of this study are the thermoacidophilic Crenarchaeota *Sulfolobus solfataricus* and *S. acidocaldarius*.

Contribution of Biofilm Centre to the consortium:
Investigation of the phospho-proteome, protein kinases, protein phosphatases and predicted target enzymes of the central carbohydrate metabolism and stress reponse
Previously, the phosphoproteome of *S. acidocaldarius* was investigated and 801 unique phosphoproteins were identified [Reimann et al., 2013]. The classification of the identified phosphoproteins revealed that 33.9% of them are involved in the central carbohydrate metabolism. To elucidate the impact of the protein phosphorylation on the regulation of the CCM, identified phosphoproteins, which are involved in glycolysis, gluconeogenesis and tricarboxylic acid cycle, where chosen for detailed biochemical characterization (glucose-1-dehydrogenase, aldehyde dehydrogenase 1-3, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, fructose-1,6-bisphosphate aldolase/phosphatase, malate dehydrogenase, 2-keto-3-deoxy-(6-phospho)-gluconate/galactonate aldolase, 2-keto-3-deoxy-gluconate/galactonate kinase). These target proteins were cloned using the pET vector system, heterologously overexpressed in *Escherichia coli* and purified to apparent homogeneity. In addition, four putative protein kinases were cloned, overexpressed and purified and first activity measurements were performed with histone H1, α-casein and myelin basic protein as artificial substrates and γ-[^{32}P]ATP as cosubstrate to verify the assumed phosphoryl transferring activity. Furthermore, protein-protein interaction studies will be performed with the selected target proteins and the so far over expressed protein kinases to unravel the mechanism of the phosphorylation.

Person in charge: Dominik Esser, Dr. rer. nat.

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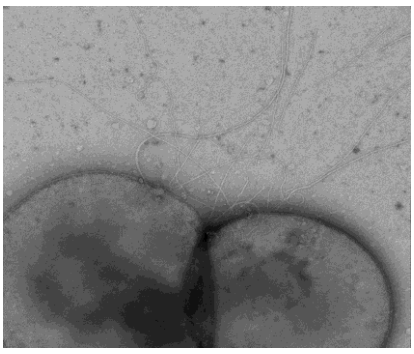

Electron microscopic image of *Sulfolobus acidocaldarius* cells

Construction and validation of a mathematical model on the gluconeogenic pathway in the thermacidophile *Sulfolobus solfataricus*

Collaborative Project - Partners: Prof. Jacky Snoep, Stellenbosch University, University of Manchester, Vrije Universiteit Amsterdam and Prof. Hans V Westerhoff, University of Amsterdam, Vrije Universiteit Amsterdam, University of Manchester
Funded by: Federal Ministry of Education and Research (BMBF); Biotechnology and Biological Sciences Research Council (BBSRC), South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa
Project period: 01.01.2013 until now
Homepage: www.sulfosys.com, http://jjj.biochem.sun.ac.za/

In thermophilic organisms (optimal growth 60 to 80°C) metabolic processes and specific biological functions have been adapted to these extreme conditions. In the past, focus was mainly on the structural stability of macro-molecules like proteins and membrane components; however, life at high temperatures also requires adaptations of the whole metabolism to cope with the influence of heat on metabolites and cofactors. Thermal instability of metabolites can lead to loss of free energy and carbon or to the accumulation of dead-end compounds. Within this study we aimed to quantify the extent of carbon loss in gluconeogenesis of the thermoacidophilic crenarchaeon *Sulfolobus solfataricus*. We established a minimal system that contains three thermolabile intermediates (glyceraldehyde 3-phosphate, 1,3-bisphosphoglycerate and dihydroxyacetone phosphate) as well as the interconverting enzymes of the gluconeogenic pathway *in vitro* and constructed and validated a detailed kinetic model which considered the temperature sensitivity. The mathematical model predictions are in very good agreement with the experimental results. Our study represents the first proof that thermal degradation of intermediates can significantly influence pathway efficiency and might represent a major challenge for life at high temperature.

Contribution of Biofilm Centre to the consortium:
Cloning, purification and kinetic characterization of the enzymes of *Sulfolobus solfataricus* involved in the conversion of the heat-instable intermediates in gluconeogenesis as well as the determination of half-life times of intermediates. Experimental validation of the mathematical model by reconstitution of the gluconeogenic pathway *in vitro* and determination of flux distributions over time.

Person in charge: Theresa Kouril, Dr. rer. nat

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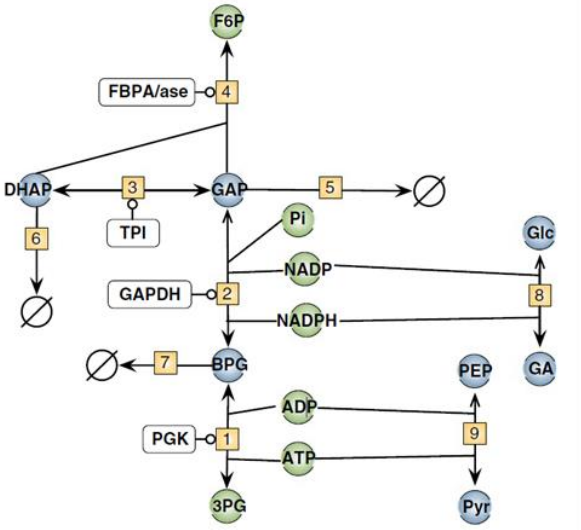


The model is accessible via JWS online.

Functional analysis of multiple general transcription factors in the crenarchaeal model organism *Sulfolobus acidocaldarius*

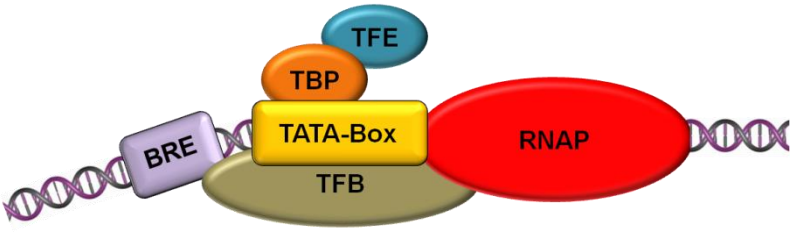
Funded by: Deutsche Forschungsgemeinschaft
Project period: 02.11.2009 until 30.09.2015
Collaborative Project - Partners: GRK1341 (Transcription, Chromatin Structure and DNA Repair in Development and Differentiation
Homepage: www.uni-due.de/zmb/studycourses/gk/gk_home.shtml

Beside unique features, archaea combine typical characteristics of bacteria and eukaryotes. Archaea belong to the prokaryotes since they lack a nucleus. Like bacteria, they contain a small circular chromosome and genes are organized in operon structures. In contrast, promoter structure and genetic information processing such as replication, transcription and translation, closely resemble that of their eukaryotic counterparts.

The archaeal transcription machinery consists of a multi-subunit RNA-Polymerase (RNAP) and the general transcription factors (GTF) representing homologues of the eukaryotic TATA-binding protein (TBP) and the transcription factor TFIIB (TFB). The actual understanding of the transcription initiation implies that TBP binds to the TATA-Box. Subsequently, TFB binds to the TBP-DNA-complex and forms sequence specific interactions with a purine-rich TFB-responsive element (BRE). The N-terminal region of TFB recruits the RNAP to build the ternary pre-initiation complex. RNAP, TBP and TFB are sufficient to initiate the transcription of archaeal promoters *in vitro*. Generally, the archaeal transcription machinery is considered as a simplified model of the more complex processes which are known from eukaryotes.

Contribution of Biofilm Centre to the consortium:
The thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*, which grows optimally at 80°C and a pH of 2-3, possesses three TFBs (TFB1, TFB2, TFB3) and one TBP. TFB1 seems to be the most commonly expressed TFIIB homologue under standard growth conditions. Although it has been reported that TFB3 is upregulated following UV-exposure and that it acts as a co-activator in the presence of TFB1, the role of multiple GTFs like TFB1-3 in crenarchaeota is still unclear, although a function similar to bacterial sigma factors has been suggested. The aim of this project is to study the functions of the TFB homologues, especially TFB2, and to investigate their roles in stress response. Overexpression of recombinant GTFs was performed successfully. Purification and denaturation/renaturation protocols are currently established and optimized. The purified proteins will be used for *in vitro* transcription and protein-DNA binding assays (EMSA). Furthermore, promoter activities of the different GTFs are currently tested in reporter gene assays.

Person in charge: Frank Schult, Dipl.-Biol.



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Stress response of biofilms from the thermoacidophilic Archaeon *Sulfolobus acidocaldarius*

Funded by: Mercator Research Center Ruhr (MERCUR)
Project period: 01.03.2012 - 28.02.2015
Collaborative Project - Project Partners: Prof. Dr. Andreas Schmid, Dr. Katja Bühler, Laboratory of Chemical Biotechnology, TU Dortmund
Prof. Dr. Hans-Curt Flemming, Aquatic Microbiology, University of Duisburg-Essen
Dr. Jost Wingender, Aquatic Microbiology, University of Duisburg-Essen

Biofilms are microbial aggregates which usually accumulate at solid-liquid interfaces. The cells are embedded in a self-produced matrix of extracellular polymeric substances (EPS). As biofilms in general often have higher resistances against toxic compounds and especially etremophilic archaea withstand harsh environmental conditions with respect to temperature and pH, archaeal biofilms “extremofilms” are of great interest for novel biotechnological applications like whole cell biocatalysis with biofilms.
The goal of this project is to understand the response of bacterial and archaeal biofilms towards process relevant conditions for the biotechnical production of chemical compounds. Main focus is on the influence of solvents involved in biotransformation processes, which are most often toxic to the whole cell biocatalysts, impairing process performance especially of planktonic cultures. Biofilms as new designer biocatalysts offer the advantages of enhanced tolerance to environmental stress factors and long-term stability, allowing for the conversion of biologically challenging compounds and continuous processes.

Contribution of Biofilm Centre to the consortium:
Two model organisms are investigated: the mesophilic bacterium *Pseudomonas* sp. strain VLB120 (TU Dortmund) and the archaeon *Sulfolobus acidocaldarius* DSM 639 (University of Duisburg-Essen). For the investigation of the intrinsic response of *S. acidocaldarius* “extremofilms” when exposed to such solvents like 1-butanol a variety of different static and flow-through incubation systems for biofilm formation and analysis were established. These different incubation systems (e. g. static systems like microtiter plates, and µ-dishes) with different substrata like glass and polystyrene were utilized to analyze *S. acidocaldarius* biofilm formation by different microscopic and biochemical methods. Applied methods are e.g. confocal laser scanning microscopy of biofilms incubated with different dyes and fluorescently labelled lectins, atomic force microscopy, crystal violet staining for biofilm quantification and a newly developed resazurin assay for cell viability.

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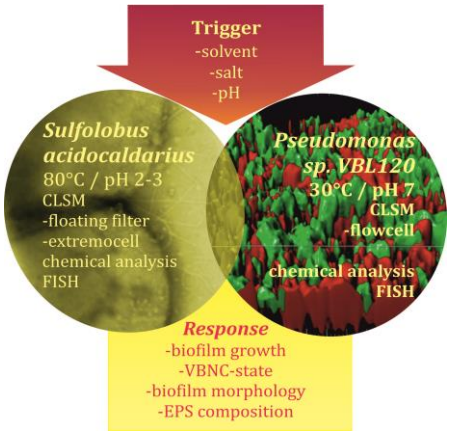

Conceptual theme of the project

HotZyme - Systematic screening for novel hydrolases from hot environments

Funded by: 7th framework program for research and technological development (FP7) of the European Union
Project period: 23.07.2012 - 23.07.2014
Collaborative Project - Project Partners: University of Copenhagen, MicroDish BV, Montana State University, Stiftelsen Norges Geotekniske Institut, Winogradsky Institute of Microbiology, Novozymes A/S, Wageningen University, Department ATV, University of Exeter, Sigma-Aldrich Production GmbH, Consiglio Nazionale Delle Ricerche, National Technical University of Athens, European Centre for Research & Financing
Homepage: http://hotzyme.com/

HotZyme is a large-scale integrating project (EU 7th Framework) on the systematic screening for novel hydrolases from hot environments coordinated by Prof. Xu Peng (University of Copenhagen, Denmark). HotZyme aims to identify such enzymes from hot terrestrial environments using metagenomic screening methods. New bioinformatics tools will be developed to facilitate function prediction of genes from metagenomes that show low or no sequence homology to enzymes of known function. A range of high-throughput screening technologies will be employed to identify novel hydrolases.

Contribution of Biofilm Centre to the consortium:
Biochemical characterization of novel enzymes

Environmental sampling

Aim of this study is to understand the mechanism of action, substrate specificity and feasibility of the use of selected hydrolase enzymes for relevant bio-processes. More recently, a thermophilic lactonase (VmutPLL) with promiscuous phosphotriesterase activity from *Vulcanisaeta moutnovskia* has been characterized. The enzyme converts lactones and acyl-homoserine lactones (AHLs) with comparable activities. Towards organophosphates (OP) VmutPLL shows a promiscuous but significantly lower activity and only minor activity was observed with carboxylesters. The high thermal stability as well as the broad substrate spectrum towards lactones, AHLs and OPs underlines the high biotechnological potential of VmutPLL. Furthermore, a thermophilic multi-domain cellulase (MDC) of *Thermococcus sp*. was identified from a xylan enrichment culture from Kuril Islands hot springs and the recombinant enzyme is currently under investigation.Thermophilic hydrolases, especially cellulases are used in various biotechnology applications, like in textile and paper industry for bleaching processes. A limiting factor in these processes is the thermostability of enzymes therefore the thermophilc MDC is a promising candidate.

Person in charge: Verena Kallnik, Dr. rer. nat., and Britta Tjaden, Dr. rer. nat.

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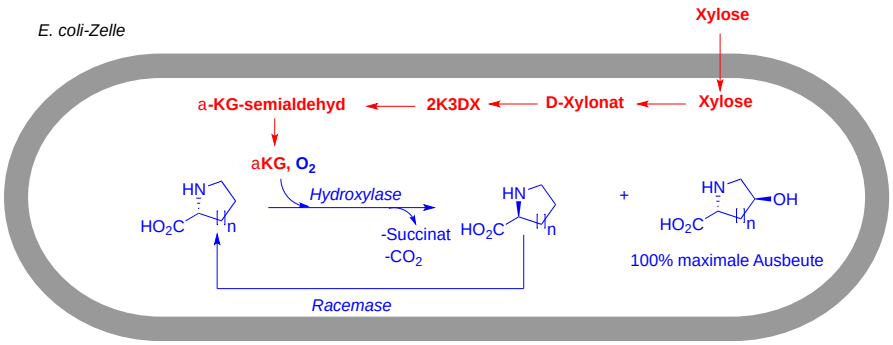
Novel pathways for the synthesis of hydroxylated amino acids (HA)

Funded by: Mercator Research Center Ruhr (MERCUR)
Project period: 01.12.2013 - 31.11.2016
Collaborative Project - Project Partners: Jun.-Prof. Dr. Robert Kourist, Microbial Biotechnology, Ruhr University Bochum (RUB); Prof. Dr. Andreas Schmid, Dr. Bruno Bühler, Dr. Mattijs Julsing, Laboratory of Chemical Biotechnology, Technical University Dortmund (TUD).

Hydroxylated natural and non-natural amino acids are important precursors for the synthesis of various compounds especially in the pharmaceutical industry. However, efficient synthesis pathways for HAs are largely missing. In this project an *E. coli* production strain for HA synthesis shall be developed. An enzymatic system for amino acid hydroxylation via α-ketoglutarate dependent dioxygenase and racemases to enable 100% yield from racemic raw materials will be established (RUB). Furthermore, an enzymatic system for the synthesis of the dioxygenase cofactor α-ketoglutarate from lignocellulosic biomass, particularly from pentoses like xylose, will be developed (MEB, DUE). Both enzymatic systems will be tranfered to *E. coli* host strains to establish and optimize a whole cell biocatalyst for the production of HAs (TUD).



Contribution of Biofilm Centre to the consortium:
In the thermoacidophilic *Sulfolobus* spp. pentoses are degraded via the corresponding sugar acids, the respective 2-keto-3-deoxy sugar acids and α-ketoglutaric semialdehyde finally to α-ketoglutarate using two dehydrogenases and two dehydratases. These enzymes as well as homologues from other - also mesophilic - archaea and bacteria will be cloned and expressed in *E. coli* and characterized with special respect to catalytic efficiencies under mesophilic conditions and to substrate specificity. Furthermore, in some mesophilic bacteria the genes encoding the respective enzymes are organized in a single operon which will entirely be cloned and transferred into *E. coli* for expression. The first milestone will be the *in vitro* constitution of the whole pentose to α-ketoglutarate degradation pathway followed by the transfer in a suitable *E. coli* host strain. A hyperthermophilic, broad substrate spectrum sugar dehydrogenase from *S. solfataricus* has already been characterized showing efficient activities even under mesophilic conditions.

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Bachelor- and Master Thesises in 2013

Bachelor Theses accomplished 2013 within or supervised by the Biofilm Centre

Name	Title	Home Supervisor	Host Supervisor	Host Institution
Wiebke Maria Beysiegel	Entwicklung eines biozidfreien Verfahrens zur Prävention der Biofilmbildung in industriellen Kühlsystemen	Prof. Dr. Flemming	Dr. Gabriela Schaule	IWW Mülheim
Yildiz Dansian	Untersuchung der Korrelation zwischen protein- und respirationsbasierten Aktivitätsmessungen einer Belebtschlammprobe unter besonderer Berücksichtigung der Temperaturabhängigkeit von Respirationsmessungen	Dr. Jost Wingender	PD Dr. Martin Denecke	Uni Duisburg-Essen
Nikolas Ditz	Klonierung, Expression und Charakterisierung von Gylkosyltransferasen aus *Sulfolobus acidocaldarius*	Prof. Dr. Siebers	Dr. Theresa Kouril	Uni Duisburg-Essen
Felicitias Dudziak	Wechselwirkung der fakultativ pathogenen Bakterien Aeromonas hydrophila und *Pseudomonas aeruginosa* mit dem Zooplanktonorganismus Daphnia magna	Prof. Dr. Flemming	Dr. Jost Wingender	Uni Duisburg-Essen
Nadine Hollmann	Charakterisierung einer putativen Phosphotriesterase aus dem Crenarchaeon *Vulcanisaeta mountnovskia*	Prof. Dr. Siebers	Dr. Verena Kallnik	Uni Duisburg-Essen
Julien Holz	Wiederbelebung von hungernden *Pseudomonas aeruginosa* im VBNC-Zustand	Prof. Dr. Flemming	Dr. Gabriela Schaule	IWW Mülheim
Jan Niklas Kazalski	Cytotoxizität und Gentoxizität von *Legionella pneumophila* und Pseudomonas aeruginosa in verschiedenen physiologischen Zuständen	Prof. Dr. Flemming	Prof. Elke Dopp	Uni Klinikum Essen
Eva Kleibusch	Evolving an Archaeabacterium-Optimization of the IPP and DMAPP production by studying the effect of IspDF and the contribution of the promoter region by varying construct make-up	Prof. Dr. Siebers	Melvin Siliakus	Wageningen University, Netherlands
Jens Kuhn	Influence of stainless steel on biofilm formation and EPS composition of *Leptothrix discophora*	Prof. Dr. Sand	Dr. Mario Andres Vera Veliz	Uni Duisburg-Essen

Hendrik Noll	Membrane vesicles as portable modules for transport and protection of QS signals and polystyrene nanoparticles	Prof. Dr. Flemming	Prof. Alan W. Decho	University of South Carolina, USA
Nadine Richter	Signaling molecules and their effect on the microbial community composition in cultures of mesophilic acidophiles growing on pyrite	Prof. Dr. Sand	Dr. Mario Andres Vera Veliz	Uni Duisburg-Essen
Denise Schäfer	Investigation into the biosynthesis and structural biology of the glycan component of pseudomurein of thermophilic methanogens; with emphasis on epimerases	Prof. Dr. Siebers	Dr. Ron Ronimus	NZAGRC, New Zealand
Jaqueline Uphoff	Wasserfraktionenanalyse an *Deinococcus geothermalis* mit Methoden der DSC	Prof. Dr. Flemming	Prof. Dr. Mayer	Uni Duisburg-Essen

Master Theses accomplished 2013 within or supervised by the Biofilm Centre

Name	Title	Home Supervisor	Host Supervisor	Host Institution
Markus Stöckl	Terra Preta - a rediscovered way of nutrient recoveryand sanitation of human faecal matter	Dr. Jost Wingender	Prof. Ralf Otterpohl	TU Harburg
Annika Kruit	Leaching behavior of the moderately thermophilic bacterium *Sulfobacillus acidophilus* in a pure culture and in a consortium with *Leptospirillum ferriphilum* on pyrite	Prof. Dr. Sand	Dr. Mario Andres Vera Veliz	Uni Duisburg-Essen
Simone Kutschki	Characterization and analyses of amyloid beta (Aß) peptides using HPLC, and direct infusion MS towards an Aß quantification method set up for Alzheimer´s disease diagnosis	Prof. Dr. Siebers	Prof.Katrin Marcus	RuB
Jalal-Al-Din Sharabati	Stimuli-Responsive Membranes for Water Treatment: Investigation of fouling and cleaning	Prof. Dr. Flemming	Prof. Dr. Mathias Ulbricht	Uni Duisburg-Essen
Krishna Khanal	Effect of Aluminium on Planktonic cells: A metabolomics approach	Prof. Dr. Flemming	Dr. Sanjay Swarup	National University of Singapor

Nadine Krüger	Nachweis und Charakterisierung von Aeromonaden in Oberflächenwasser der Ruhr mit mikrobiologischen und molekularbiologischen Methoden	Prof. Dr. Flemming	Dr. Jost Wingender	Uni Duisburg-Essen
Simon Kellermann	Effectiveness of the removal of pathogens in waste water by constructed wetlands	Prof. Dr. Flemming	Dr. Jochen A. Müller	Helmholtz-Zentrum für Umweltforschung
Maik Csaba Szendy	Molecular analysis of microbial communities and structures in sludge flocs from high loaded membrane bioreactors	Prof. Dr. Flemming	Lena Faust	Wetsus, Netherlands
Saskia Dillmann	DSC measurements of water in biofilms	Prof. Dr. Sand	Prof. Dr. Christian Mayer	Uni Duisburg-Essen
Kishor Acharya	Activity and Spatial dynamics of pesticide degraders in unsaturated porous media	Prof. Dr. Sand	Dr. Arnaud Dechesne	Technical University of Denmark
Inga Vanessa Kirstein	Nutrition optimization of thermophilic-halophilic-phototrophic Cyanobacteria	Prof. Dr. Sand	Dr. Mario Andres Vera Veliz	Uni Duisburg-Essen
Robert Barthen	Abiotic and cell-cell-communication related influences on the biofilm formation process by Acidithiobacillus ferrivorans in comparison to Acidithiobacillus ferrooxidans	Prof. Dr. Sand	Dr. Mario Andres Vera Veliz	Uni Duisburg-Essen
Karolin Spitzer	Biodiversity of terrestrial green algae in tropical mountain rain forest in Podocarpus National Park (Ecuador) using a culture dependent approach	Prof. Dr. Flemming	Prof. Friedl	Uni Göttingen
Martin Mackowiak	Virus inactivation mechanisms by different disinfectants	Prof. Dr. Flemming	Dr. Astrid Paulitsch-Fuchs	Wetsus, Netherlands
Florian Itzel	Deactivation of the Chlordioxide dosage and monitoring the impact on the water quality and the distribution system	Prof. Dr. Flemming	Dr. Rohns	Stadtwerke Düsseldorf

Andrea Bernds	Biochemical experiments with blue-light photoreceptors	Prof. Dr. Siebers	Prof. Wolfgang Gärtner	MPI für Chemische Energiekonversion
Julia Schmitt	Microbiological characterization of iron- and manganese-richmarine sediments, Bothnian Sea	Prof. Dr. Flemming	Prof. Mike Jetten	Radboud University of Nijmegen
Maximilian Wolf	Optimization and verification of an enrichment method for somatic coliphages in water analytics	Prof. Dr. Flemming	Vera Schumacher	Stadtwerke Düsseldorf
Irina Quade	Charakterisierung der antibakteriellen Wirkung von SIlberionen auf planktonische Zellen und Biofilme von *Pseudomonas aeruginosa*	Prof. Dr. Flemming	Dr. Jost Wingender	Uni Duisburg-Essen
Sonja Rani	Resource recovery from source-separated domestic wastewater: Are heavy metals a problem?	Prof. Dr. Flemming	Dr. Lucia Hernandez	Wetsus, Netherlands
Lu Shen	Characterisation of three enzymes from *Sulfolobus acidocaldarius* DSM639: the phosphoglycerate kinase, the D-2-hydroxyacid dehydrogenase and the aldehyde dehydrogenase	Prof. Dr. Siebers	Dr. Theresa Kouril	Uni Duisburg-Essen

Ph. D. theses accomplished in 2013:

Dr. Miriam Tewes: “Association of hygienically relevant microorganisms with freshwater plankton”
Supervisors:
Prof. Dr. Hans-Curt Flemming
Prof. Dr. Bernd Sures
February 13, 2013, Essen

Dr. Anja Bauermeister: “Characterization of stress tolerance and metabolic capabilities of acidophilic iron-sulfur-transforming bacteria and their relevance to Mars!
Prof. Dr. Hans-Curt Flemming
Prof. Dr. Wolfgang Sand
April 23, 2013, Essen

Dr. Anna Hagemann: „Trehalose Metabolismus in (hyper-) thermophilen Archaea“
Supervisors:
Prof. Dr. Bettina Siebers
Prof. Dr. Jochen Stefan Gutmann
July 24, 2013, Essen

Dr. Dominik Esser: “Protein Phosphorylation in Archaea: Central Carbohydrate Metabolism in *Sulfolobus* species*”*
Supervisors:
Prof. Dr. Bettina Siebers
Prof. Dr. Jochen Stefan Gutmann
Prof. Dr. Phil Craig. Wright
November 5, 2013, Essen

Dr. Silke Jachlewski: “Biofilm formation and EPS analysis of the thermoacidophilic Archaeon *Sulfolobus acidocaldarius”*
Supervisors:
Prof. Dr. Bettina Siebers
Prof. Dr. Hans-Curt Flemming
November 20, 2013, Essen

Dr. Bernadette Rauch: „Functional analysis of multiple general transcription factors in *Sulfolobus acidocaldarius*”
Supervisors:
Prof. Dr. Bettina Siebers
Prof. Dr. Perihan Nalbant
Dezember 12, 2013, Essen

Dr. Agata Joanna Wikiel: “Role of extracellular polymeric substances on biocorrosion initiation or inhibition”
Supervisors:
Prof. Dr. Wolfgang Sand
Prof. Dr. Jose J. G. Moura
August 31, 2013, Essen

Dr. Claudia Janosch: “Sulfur oxidation in moderately thermophilic leaching bacteria”
Supervisors:
Prof. Dr. Wolfgang Sand
Prof. Dr. Bettina Siebers
September 19, 2013, Essen

Dr. Nanni Noel: “Attachment of acidophilic bacteria to solid substrata”
Supervisors:
Prof. Dr. Wolfgang Sand
Prof. Dr. Axel Schippers
Dezember 18, 2013, Essen

Publications of Biofilm Centre

Books

Flemming, H.-C. (Hrsg.): Vermeidung und Sanierung von Trinkwasser-Kontaminationen durch hygienisch relevante Mikroorganismen aus Biofilmen der Hausinstallation „Biofilme in der Hausinstallation“. IWW Schriftenreihe, Mülheim 2010, 258 pp.

Flemming, H.-C., Wingender, J., Szewzyk, U. (eds.): Biofilm Highlights. Springer Int. Heidelberg, New York, 2011, 243 pp

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Berndt, E., Behnke, S., Dannehl, A., Gajda, A., Wingender, J., Ulbricht, M. (2010) Functional coatings for anti-biofouling applications by surface segregation of block copolymer additives. Polymer 51, 5910-5920

Exner, M., Christiansen, B., Flemming, H.J., Gebel, J., Kistemann, T., Kramer, A., Martiny, M., Mathys, W., Nissing, W., Pleischl, S., Simon, A., Trautmann, M., Zastrow K.D., Engelhart, S. (2010): Pseudomonas aeruginosa – Plädoyer für die Einführung eines technischen Maßnahmewertes in die Novelle der Trinkwasserverordnung. Hyg. Med. 35, 370 – 379

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Flemming, H.-C. u. Wingender, J. (2012): Die Stärke der Gemeinsamkeit. BioSpektrum 18, 714-716

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Bellenberg S, Barthen R, Vera M, Guiliani N, Sand W (2013) Biofilm Formation, Communication and Interactions of Mesophilic Leaching Bacteria during Pyrite Oxidation. Adv Mat Res 825: 107-110

Bellenberg S, Kalin M, Sand W (2013) Microbial Community Composition on Lignite before and after the Addition of Phosphate Mining Wastes. Adv Mat Res 825: 42-45

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Wikieł A, Datsenko I, Vera M, Sand W (2013) Impact of Sulfate Reducing Prokaryotes Biofilms on Corrosion Behaviour of Carbon Steel in Marine Environment. Bioelectrochemistry http://dx.doi.org/10.1016/j.bioelechem.2013.09.008

Zhang R, Vera M, Bellenberg S, Sand W (2013) Attachment to Minerals and Biofilm Development of Extremely Acidophilic Archaea. Adv Mat Res 825: 103-106

Invited Lectures

Hans-Curt Flemming:

6.-7. 6. 2013 “How dead is dead? Progress” International Conference “How dead is dead”, Berlin

14. 6. 2013 “Microbiology of water lines” Royal Society for Public Health Conference, Copenhagen

15. 7. 2013 “Viable but nonculturable bacteria in drinking water installations” Conference on Drinking Water Quality, Singapore

28. 8. 2013 „Schwachunkt Hausinstallation“ bpt-Kongress Mannheim

9. 9. 2013 „ Relevance of biofilms on surfaces and techniques for biofilm sensing”

10. 9. 2013 „Pathogens in drinking water – hide and seek“ Eurobiofilms 3, Gent, Belgien

23.-25.10. „Ecology of aquatic biofilms“ Congress „Microbial Diversity“ Italian Society of Microbiology

30. 11. 2013 “Biofilme und Implantate” Congress Dental Prophylaxe, Innsbruck

3. 12. 2013 “Detection of hygienically relevant microorganisms in drinking water” Preventing Pseudomonas Conference

20. 12. 2013 “Extracellular polymeric substances – the key to the biofilm life style” Belgian Interdiciplinary Biofilm Research Conference

Wolfgang Sand:

January - TU Dresden - Biokorrosion und Bioleaching zwei Seiten einer Medaille?

March- Geokompetenzzentrumn Freiberg - Geobiotechnologie in der DECHEMA

April/May - Univ. Baia Mare - Erasmuskurs Biocorrosion

May - BAM Berlin - Grenzflaechenprozesse bei der Biolaugung

September - Eurocorr Istanbul - Interfacial processes in Bioleaching and Biocorrosion

October - Geohannover, Hannover - Mechanismen des Bioleaching

October - Freiberg Workshop Molekulare Methoden in der Geobiotechnologie - Molecular processes in Bioleaching

November - Changsha, China - Short course Biocorrosion

December - Amira & CSIRO, Melbourne - Bioleaching and Biocorrosion mechanisms

Bettina Siebers:

January 1, 2013: Lightening Talk: Kick-off Meeting ERASysAPP – Systems Biology Application (Brussels, Belgium, Dr. Klaus-Peter Michel, Projektträger Jülich)
„Systems Biology Application – SulfoSYS: Life in hot acid"“

January 15,.2013: Gastseminar Physiologie der Mikroorganismen, Ruhr Universität Bochum, (Bochum, Prof. Dr. Nicole Frankenberg-Dinkel): “Life in the hot lane”: Central carbohydrate metabolism and regulation in hyperthermophilic Archaea”

January 24,.2013: SulfoSYS^{BiOTEC} Kick-off Meeting (Essen): “Applied Sulfolobus Systems Biology: Exploiting the hot archaeal metabolic potential for Biotechnology”

July 3, 2012: Molecular Biology of Archaea III (MPI für Terrestrische Mikrobiologie, Marburg) “Sulfolobus Systems Biology: Challenges of life at high temperature

September 25, 2012: Vortrag Evonik (Dr. Martin Schilling) “Life at high temperature: Challenge and potential“

October 9, 2012: V Internatonal Symposium on Biochemistry and Molecular Biology (within the VIII International Congress on Chemistry and Chemical Engineering) (Havana, Cuba) “Archaeal carbohydrate metabolism at high temperature”

November 28,.2013: Igniococcus Treffen (Frankfurt): “*Ignicoccus hospitalis -Nanoarchaeum equitans-*“

Jost Wingender

April 26 and June 14, 2012: Die letzten Meter auf dem Weg zum Wasserhahn: BMBF-Verbundprojekt – Legionellen und *Pseudomonas aeruginosa* im Biofilm. Pall Webinar