Montana State University

Center for Biofilm Engineering

Bozeman

montana biofilm SCIENCE & TECHNOLOGY meeting

JULY 14–16, 2015

presentation
and poster

Proceedings

K Gorham, MSU News

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Presentation Abstracts

SESSION 1: Oilfield Biofilms

Visualization and quantification of biofilm removal

Presenter: **TJ Tidwell**[1], Principal Microbiologist
Co-authors: Renato M. de Paula[1], Glen P. Nilsen[1], Victor V. Keasler[1]
Affiliation: [1]Nalco Champion Company, Sugar Land, TX, USA.

Biofilms are the predominant antagonists when it comes to microbiologically influenced corrosion (MIC). The removal of this biofilm community is crucial to mitigating the risk of corrosion failures due to MIC. Despite this, there are few current techniques that can accurately address whether a biocide treatment is effectively removing the biofilm, simply lowering its metabolic activity, or killing the cells, thereby leaving the infrastructure intact for new microbes to more easily attach to surfaces. We have developed a technique utilizing confocal laser scanning microscopy to determine the biofilm removal efficiency of commonly used oilfield biocides using thickness and volume calculations. Volume measurements allow us to quantify the ability of the biocide to penetrate throughout the biofilm rather than only seeing a reduction in overall thickness, which can be influenced by a number of factors.

Mechanisms and mitigation of severe microbial corrosion by sulfate-reducing bacteria

Presenter: **Dennis Enning**, Senior Research Engineer
Affiliation: ExxonMobil Upstream Research Company, Houston, TX, USA.

Recent years have seen considerable progress in the understanding of how microorganisms electrically interact with solid surfaces, as well as with each other. One of the areas of particular, applied interest within this emerging discipline of 'electromicrobiology' is the corrosion of iron and steel by anaerobic microorganisms such as sulfate-reducing bacteria (SRB). Several microorganisms have now been demonstrated to corrode metal by direct uptake of electrons, a process recently termed "Electrical Microbiologically Influenced Corrosion (EMIC)." Laboratory-grown cultures of these organisms degrade metal at previously unprecedented rates and have been suggested to play a prominent role in the corrosion of iron infrastructure such as oil and gas pipelines.

Microbial corrosion is indeed one of the main types of corrosion with a profound impact on materials integrity in the oil and gas industry. Of particular interest in this context is the internal corrosion of large carbon steel pipelines, the replacement and repair of which can be very costly as well as disruptive to production.

Pipelines for which a susceptibility to MIC has been identified are usually treated with biocides that are injected periodically (e.g., weekly) into the lines for a designated period of time (usually a few hours). These treatments are intended to kill or inhibit steel-attached corrosive microorganisms with the aim of slowing down overall pipe wall loss and preventing localized 'pitting'.

The comparative effectiveness of different biocides is usually determined in the laboratory. Most commonly this involves microbial 'kill tests' that evaluate the degree to which microbes in a biofilm can be killed or inactivated. However, the distinction between live and dead microorganisms in a

complex biofilm is not always straightforward and results of such 'kill tests' do not necessarily translate directly into effective MIC control.

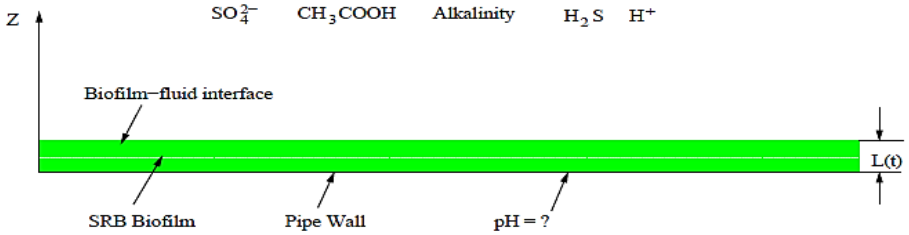
We recently developed a methodology to test biocides with respect to their capability to reduce microbial corrosion rates (rather than kill efficiency) as a function of biocide type, treatment duration and treatment frequency. This presentation elaborates on the novel methodology and compares the effects of glutaraldehyde and THPS batch treatments on microbial corrosion rates, corrosion morphology and microbial community composition. The presentation will further discuss biocide performance in the context of microbial corrosion mechanisms.

It was found that under the tested laboratory conditions, highly corrosive (up to 80 mpy) SRB-dominated biofilms were controlled more effectively and more reliably with weekly treatments of THPS.

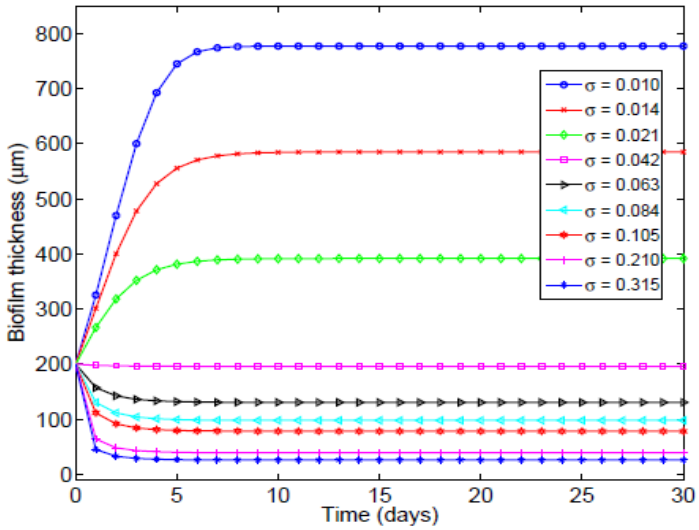
Modeling the growth and chemistry of sulfate-reducing biofilms

Presenter: **Robin Gerlach**[1], professor of chemical and biological engineering
Co-author: Tianyu Zhang[1], assistant professor of mathematical sciences
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

A mathematical model describing the chemical processes in sulfate-reducing biofilms was developed using MATLAB. The model incorporates biofilm growth and detachment, sulfate reduction catalyzed by sulfate reducing bacteria (SRB), and the speciation of dissolved inorganic carbon and sulfide species. Effects of parameters on the model output, especially the pH value, were investigated through numerical simulation of the non-dimensionalized governing equations. Finally, a sensitivity analysis of a number of input parameters on the model output with respect to pH profiles and sulfide concentrations was conducted.



The model provides a theoretical framework for establishing a quantitative relationship between SRB activity and concentration of chemical species in biofilms that might affect corrosion processes in oilfield pipelines and other natural and industrially relevant environments.



Thiosulfate-reducing microbes in oilfield systems: Too often overlooked

Presenter: **Kathleen Duncan**, research associate professor of microbiology and plant sciences
Affiliation: Department of Microbiology and Plant Sciences, OU Biocorrosion Center, University of Oklahoma, Norman, OK, USA.

Several lines of evidence support the importance of thiosulfate reduction for the production of sulfide in oil production facilities. Enumeration of sulfide-producing bacteria often reveal greater numbers of thiosulfate-reducers than sulfate-reducers and molecular surveys find a large proportion of 16S rRNA gene sequences are highly similar to those of organisms known to reduce thiosulfate. Thiosulfate can reach high levels: thiosulfate levels in a highly corroded subsea pipeline reached as high as 0.5 mM, and thiosulfate can be generated by partial oxidation of H_2S by oxygen entering the system. Distinguishing between production of sulfides by thiosulfate reducers versus sulfate reducers is important, as biocides effective against one group of these types of microbes have been reported to be ineffective against the other, and the enzymatic systems of sulfide production can be quite different.

A number of thiosulfate-reducing microbes utilize fermentation to oxidize organic carbon compounds. Today's presentation will focus on two different groups: *Anaerobaculum* (Synergistetes), found in high temperature, moderately saline reservoir conditions and Halanaerobiales (Firmicutes), often dominant in highly saline systems. All described species of *Anaerobaculum* can reduce thiosulfate, sulfur, and L-cystine to sulfide, suggesting a direct link to biocorrosion. *Anaerobaculum* was the dominant bacterium found in enrichments for sulfide-producing microbes inoculated with pig envelope scrapings from Alaskan North Slope samples (Liang et al., 2014). Steel coupons incubated with the enrichment developed pitting corrosion.

Halanaerobium are classified as obligately halophilic, anaerobic fermenters. Several of the *Halanaerobium* species were isolated from oilfield brines. Some *Halanaerobium* have been shown to reduce thiosulfate to sulfide compounds, but none are capable of reducing sulfate.

Among our samples, one set was from a facility processing highly saline production fluids and experiencing high levels of corrosion. Both cultivation and molecular surveys revealed an abundance of fermentative bacteria, primarily *Halanerobium*. Cultivation studies also noted more thiosulfate-reducers than sulfate-reducers. Annette De Capite (OUBC master's student), isolated over a dozen thiosulfate-reducing *Halanaerobium* strains from these sites and two other highly saline oil processing facilities. One strain represents a new species of *Halanaerobium* and another, a novel genus in the Halanaerobiales.

How can we detect these thiosulfate-reducing bacteria? Thiosulfate reduction can occur through respiratory molybdopterin reductase or sulfur-transferase catalyzed by rhodanese-like proteins. In thiosulfate disproportionation, a rhodanese-like enzyme mostly likely catalyzes the formation of sulfide and sulfite from thiosulfate. Part of the sulfite is oxidized to sulfate using the same enzymes used in sulfate reduction but acting in reverse. The remainder of the sulfite is reduced to sulfide by dissimilatory sulfite reductase. Molybdopterin thiosulfate reductase and thiosulfate-specific rhodanese catalyze reactions that differentiate thiosulfate reduction and disproportionation from sulfate reduction. Their genes could serve as functional probes for these processes in oil field facilities, but the basis of thiosulfate-reduction in *Anaerobaculum* and *Halanaerobium* is still the subject of research.

Fate of hydraulic fracturing chemicals under downhole conditions

Presenter: **Genevieve Kahrilas**[1], PhD candidate in chemistry
Co-authors: Jens Blotevogel[2], Thomas Borch[1,3]
Affiliation: [1]Department of Chemistry, Colorado State University, Fort Collins, CO, USA.
[2]Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, CO, USA.
[3]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, USA.

Biocides represent a key class of chemicals within hydraulic fracturing fluids included to prevent microbially induced souring and/or corrosion in wells where underground conditions may favor bacterial growth. Glutaraldehyde (GA)—the most common biocide added to hydraulic fracturing fluids—was used in over half of all wells fractured in the continental U.S. in 2014. Despite this widespread use, very little is known about GA's fate when subjected to the high pressures, temperatures, salinity, and varying pH values experienced in unconventional reservoirs during a hydraulic fracturing event. This knowledge gap not only prevents clarification of chemical behavior downhole, but also makes predicting the presence of GA in flowback water impossible. To clarify this problem, we employed stainless steel high temperature/pressure reaction vessels to simulate various deep subsurface conditions while utilizing various analytical methods to track transformation of GA over time, revealing the relationships between chemical transformation and the underground "matrix parameters" (temperature, pH, pressure, and salinity). Our results show increases in degradation rates corresponding to increased temperature, increased pH, increased pressure, and decreased salinity. Furthermore, several major transformation products are revealed as polymers of GA. The work presented here not only provides a novel look at downhole behavior of GA, but it also provides a framework for other downhole studies focused on the organic chemicals used in hydraulic fracturing. The findings from this study can be used to optimize chemical packages for hydraulic fracturing and to predict chemicals that are likely to return with flowback water.

Enhancement of sustainable biogenic coalbed methane production

Presenter: **Katie Davis**[1,2], PhD Student
Co-Authors: Elliott Barnhart[1,3], Ashley E. Berninghaus[1,2], Matthew W. Fields[1,3], Robin Gerlach[1,2], Al Cunningham[1,4], Randy Hiebert[1,5], Hannah Schweitzer[1,3]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Chemical and Biological Engineering,
[3] Department of Microbiology and Immunology, and
[4] Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[5] MET, Inc, Butte, MT, USA.

Coal is the largest fossil fuel resource in the United States; however, most of this coal is too deep in the subsurface to extract safely and economically. In many of these deep coal seams, methane, the main component of natural gas, has been discovered and successfully harvested. Coalbed methane (CBM) accounted for 6–7% of U.S. natural gas production from 2011 to 2013. Combustion of natural gas produces substantially less CO_2, SO_x, NO_x, and other toxic emissions (e.g., mercury and heavy metals) per kWh than combustion of coal or oil and is therefore a cleaner energy source.

The accumulation of methane gas in these coalbeds occurs thermogenically or biogenically. The Powder River Basin (PRB) in southeastern Montana and northeastern Wyoming is the largest coal reserve in the U.S. and a large source of biogenic CBM. The *in situ* conversion of coal to CBM by the native microbial community is of particular interest for present and future natural gas sources, as it provides the potential to harvest energy from coal seams over time with less environmental impact than mining and burning coal.

The rate of naturally occurring biogenic CBM production is lower than the rate of conventional gas collection, and the typical lifespan of a CBM well in the PRB is only 7–10 years. Once operation of the wells becomes no longer economically viable, CBM wells in the PRB are being abandoned, creating an economic and environmental liability.

Previous long-term batch enrichments investigating yeast, algae, and cyanobacteria extract additions for stimulation have shown the potential for increasing methane production. The exact reasons for this enhancement are still unclear. Current experiments are quantifying the CBM enhancement for each of these additions relative to unamended treatments and are beginning to provide insight into the mechanisms of and possible strategies for biogenic CBM enhancement in the field. The development of CBM enhancement strategies could increase the lifetime of CBM wells and the sustainability of CBM production.

SESSION 2: Regulatory Agency Update

Highlights of the February 2015 regulatory conference

Presenter: **Paul Sturman,** CBE industrial coordinator
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

In February 2015 the CBE organized and hosted our second conference focusing on biofilms in the regulatory context. The *Anti-Biofilm Technologies: Pathways to Product Development* conference was held in College Park, MD, and was attended by 140 representatives from industry, academia and regulatory agencies. The conference included speakers from both EPA and FDA, as well as presentations highlighting new anti-biofilm technologies, methods for assessing the efficacy of such technologies, and presentations geared toward adoption of appropriate standards for regulating anti-biofilm technologies and claims. The conference was organized into a morning session, which was primarily EPA-related, and an afternoon session, which was primarily FDA-related, with a discussion period following each session. Highlights from conference presentations and implications for further regulatory interactions will be discussed.

Update on biofilm claims for antimicrobial products: U.S. EPA regulatory perspective

Presenter: **Steve Tomasino**, Senior Science Advisor
Affiliation: U.S. EPA Office of Pesticide Programs, Microbiology Laboratory Branch, Fort Meade, MD, USA.

The U.S. Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) Office of Chemical Safety and Pollution Prevention (OCSPP), under the statutory authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), is responsible for the registration of antimicrobial products, including products intended to treat biofilms on inanimate environmental surfaces. Antimicrobial products bearing claims for control of microorganisms on these surfaces that are infectious to man are considered directly related to human health: these are known as public health products. Under FIFRA, the registrant of an antimicrobial product with a public health claim is required to submit product efficacy data to EPA in support of the product's registration. Thus, antimicrobial products with claims to prevent, destroy, repel or mitigate biofilm on an inanimate environmental surface would require registration under FIFRA, including the submission of product efficacy data. In this presentation, EPA's perspective will be provided on the status of biofilm test methodology and the steps being considered to establish regulatory guidance for making biofilm claims on antimicrobial products.

Biofilms express unique characteristics, and therefore require specific and relevant test methods for measuring the efficacy of products designed for treating biofilms. The choice of method will dictate the type of label claim (e.g., log reduction of bacteria in biofilm). Formal guidelines have not been established for biofilm claims; however, the EPA is in the process of collecting the data necessary to develop guidance to inform registrants on the recommended methodology and associated testing criteria. Currently, the EPA is considering the use of ASTM method E2871-13 (Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm using the Single Tube Method) as a regulatory method. This quantitative method was collaboratively developed by the EPA OPP Microbiology Laboratory Branch (MLB) and the Montana State University (MSU) Center for Biofilm Engineering. Over the past three years, the Single Tube Method has been evaluated in two separate collaborative studies (one led by MSU and the other by EPA), each designed to ascertain the method's performance, and to ultimately improve the method. The EPA-led collaborative included a number of modifications, and based on the data, additional improvements to the method are necessary. Additional studies are required to verify the changes to the method. The method's repeatability (within lab variance) and reproducibility (between lab variance) values will be used to inform the EPA on best practices for use of the methodology. Based on the outcome of the additional studies, it may be possible to prepare the regulatory guidance necessary to support a label claim for treating biofilms on inanimate environmental surfaces. Also, the EPA will continue to work with the stakeholder community and ASTM International to reach consensus on revisions to the standard method.

Results from continued optimization of the Single Tube Method

Presenter: **Darla Goeres**, associate research professor of chemical and biological engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

ASTM Method E2871 titled "Standard Test Method for Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor using Single Tube Method" describes a step-by-step protocol for determining the log reduction in viable biofilm bacteria following exposure to a liquid disinfectant. During fall 2012, an ASTM Interlaboratory Study (ILS #853) was conducted to determine the repeatability and reproducibility of the Single Tube Method in order to complete the method's Precision and Bias statement. The repeatability standard deviations ranged from 0.731 to 1.448 and the reproducibility standard deviations ranged from 0.891 to 1.668, falling at the upper end of what is generally acceptable for standardized microbial tests. In 2014, the EPA launched a second collaborative study of the Single Tube Method. For this study, two disinfectants with a presumed high level of efficacy were tested using five coupons per treatment. Both disinfectants tested had a mean log reduction greater than 7, but the variability was still at the high end of acceptable. The repeatability standard deviations were 0.616 and 0.849 and the reproducibility standard deviations were 1.732 and 1.311. After the first collaborative study, it was hypothesized that the variability was attributed to differences in how the laboratories vortexed and sonicated the samples. A ruggedness test of the sonicator settings determined the differences in how the samples were sonicated did not fully explain the variability in the ILS data (results reported in MBM poster titled "Sonication Ruggedness of Efficacy Tests in the Single Tube Method" by Jennifer Summers et. al). For the second collaborative study, changes were made to better define the protocol but the variability did not improve as expected.

During the last three years, researchers using the Single Tube Method noticed that sometimes, as the coupon dropped into the bottom of the conical vial, a splash would land on the side of the vial. The splashing was not predictable and often it was difficult to see with the naked eye. If one or more splashes occurred above the 4 mL mark, the cells contained in the splash were not exposed to the

disinfectant; but these cells were recovered and enumerated once the 36 mL of neutralizer was added to the vial, thus biasing the test results and leading to variability in the data. In collaboration with Kevin Cook from the MSU Mechanical Engineering Department, and Bryan Warwood from BioSurface Technologies, the SBML developed a plastic sleeve that rests on the top of the conical vial while the coupon is being dropped and then is removed immediately before disinfectant is added. The purpose of the plastic sleeve is to guard against splashes on the side of the conical vial.
A parallel study was completed that compared control and treated data from coupons that were dropped into the conical vial as written in the ASTM Single Tube Method to data from coupons dropped with the splash guard in place to data from coupons placed in the bottom of the reactor with a pair of flame sterilized forceps. All the treated coupons were exposed to 5,000 ppm pH adjusted NaOCl and the control coupons were exposed to 4 mL sterile buffered dilution water. The experiment showed that the control counts were not influenced by how the coupon was dropped into the vial: 8.4 +/- 0.15 $Log_{10}CFU/cm^2$ (ASTM method), 8.5 +/- 0.19 $Log_{10}CFU/cm^2$ (forceps), and 8.5 +/- 0.17 $Log_{10}CFU/cm^2$ (splash guard). Dropping the coupon into the conical vial with the splash guard in place decreased the number of treated coupons with viable cell counts to 11%. Using the forceps decreased the number of treated coupons with viable cell counts to 12.5%. Dropping a coupon into the vial with no splash guard yielded 33% treated coupons with viable cell counts, corresponding to a lower mean log reduction. This research confirms the need to modify the ASTM Single Tube Method to specify a different approach for placing the coupons into the conical vials.

SESSION 3: Medical Biofilms

3-D imaging of oral biofilms

Presenter: **Garth James**, associate research professor of chemical and biological engineering
Co-authors: Steve Fisher and Kelly R. Kirker
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

In vitro oral biofilms are useful for screening potential oral care ingredients and formulations. The CDC Biofilm Reactor (CDC-BR) was used to grow biofilms from saliva with a 10% Tryptic Soy Broth medium containing 0.5% sucrose and 7.5mg/l of amphotericin B to inhibit fungal growth. With a 24-hour batch phase and 24-hour flow phase, this method produced repeatable biofilms for evaluation of treatment efficacy using viable plate count methods. The CDC-BR coupons were treated, stained, and imaged or placed in a treatment flow cell for confocal scanning laser microscopy (CSLM) time-lapse imaging during treatment. Hydroxyapatite coupons were used to simulate the tooth surface; however, glass or polycarbonate coupons were also used to enable transmitted light imaging. A wide variety of fluorescent DNA-binding and carbohydrate-binding stains was used. Live/Dead® was useful for imaging biofilms after treatment, in comparison to control treatments. Image analysis was used to quantify the amount of red and green fluorescence from different depths within the biofilms. Sequential Live/Dead® staining was used for time-lapse imaging of treatments. A combination of SYBR® Green and Concanavalin A conjugated to rhodamine B was used to image cells and extracellular polymers during treatment. Image analysis was used to quantify changes in green and red fluorescent volume. Overall, fluorescent staining and CSLM proved to be useful tools for helping to evaluate treatment effects on in vitro oral biofilms.

Clostridium difficile and health care-associated infection

Presenter: **Seth Walk**, assistant professor of microbiology and immunology
Affiliation: Montana State University, Bozeman, MT, USA.

Clostridium difficile is the most commonly identified pathogen among health care-associated infections in US hospitals. Primary cases of *C. difficile* infection (CDI) are a common complication of antibiotic therapy and require additional antibiotic treatment to resolve. Secondary or "recurrent" CDI results from a chronically perturbed GI tract microbiome that remains susceptible to pathogen invasion. In terms of health care dollars, well over $1 billion annually is spent on treatment of primary and recurrent CDI in the U.S. alone. Recent changes in the epidemiology of CDI have raised public and clinical concerns, including the emergence of closely related pathogenic clones. Some studies suggest that these clones may cause more severe disease compared to other commonly circulating genotypes. Other studies find that patient factors are more important in predicting patient outcome. This presentation will introduce the audience to CDI and summarize some of the important aspects of CDI epidemiology. A secondary focus will be on how microbial biofilms may play a role in *C. difficile* pathogenesis and transmission, and how this may provide an opportunity for novel therapeutic approaches.

In vitro analysis of *Clostridium difficile* biofilms: Imaging and antimicrobial treatment

Presenter: **Elinor Pulcini**[1], assistant research professor of chemical and biological engineering
Co-authors: James GA[1] and Chesnel L[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Merck and Co., Inc., Kenilworth, NJ, USA.

Background: *Clostridium difficile* infection (CDI), caused by a spore-forming gram-positive anaerobic bacillus, can result in severe gastroenteritis in humans. Although spore formation helps to protect *C. difficile* during antibiotic treatment, biofilm formation may provide additional protection against antibiotics, potentially resulting in treatment failure and the recurrence of CDI. Fidaxomicin (FDX), a narrow spectrum macrocyclic antibiotic, and surotomycin (SUR), an investigational cyclic lipopeptide antibiotic, are potential new treatments for CDI. In this study, FDX and SUR were evaluated in comparison with vancomycin (VAN) and metronidazole (MET) for in vitro efficacy against established *C. difficile* biofilms.

Methods: *C. difficile* (ATCC BAA-1382) biofilms were grown at 37°C anaerobically using the colony biofilm model (CBM) with polycarbonate filter membranes and BHIS medium. Antibiotic treatments were performed by transferring 48- and 72-hour-old biofilms to BHIS plates containing antibiotics at 25X MIC for 24 hours (total growth and treatment time of 72 and 96 hours, respectively). Both vegetative cells and spores were enumerated. CFU data were log(10) transformed and averaged to determine mean log density (MLD). For antibiotic treatments, log reductions were calculated relative to control biofilms and averaged to determine mean log reduction (MLR). ANOVA was used to compare MLRs. In addition, some colony model biofilms grown for grown for 48 or 72 hours were treated with fluorescently labeled SUR, cryo-sectioned, and examined by confocal scanning laser microscopy (CSLM).

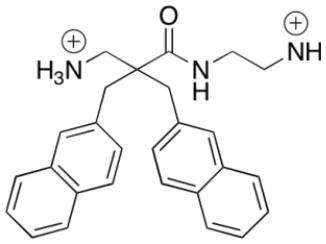
Results: Control biofilms grown for 72 hours had a total MLD for vegetative cells of 8.33±0.41 and a spore MLD of 3.74±0.96. There was not a significant difference in MLRs between biofilms grown for 48 and 72 hours prior to treatment ($p=0.119$). The MLR for treatment with FDX (MLR 2.17±1.20) and with SUR (2.94±0.55) was significantly greater ($p<0.0001$) than for VAN (0.66±0.36) and MET (0.44±0.56). For the spore MLR, the only significant differences were between FDX and MET, with

FDX having a significantly higher MLR ($p < 0.022$) for biofilms grown for both 48 and 72 hours prior to treatment. CSLM imaging indicated that one hour of treatment with fluorescently labeled SUR resulted in fluorescence throughout the biofilm. After 24 hours of treatment, cells within the biofilm exhibited intense fluorescence.

Conclusions: Both SUR and FDX were more effective for killing *C. difficile* vegetative cells in biofilms than VAN and MET. FDX was more effective at reducing spore counts within the biofilms than MET. SUR was also shown to penetrate biofilms and accumulate within biofilm cells.

Antimicrobial and anticancer β^{2,2}-amino acid derivatives: Peptide mimetics with potential for oral administration

Presenter: **Dominik Ausbacher**, CBE visiting postdoctoral researcher in pharmacy
Affiliation: Department of Pharmacy, UiT—The Arctic University of Norway.



The lead derivative **A2**

Drug resistance is a serious problem in infectious and malignant diseases because of the decrease in efficacy of a drug to cure or improve the health status of a patient, which may ultimately lead to death. In order to tackle the burden of resistance development and the need for novel drugs, our group has developed small peptide mimetics called β^{2,2}-amino acid derivatives (Mw < 500 Da). These derivatives resemble surfactant-like compounds with both hydrophilic and hydrophobic properties. The construction principle was derived from cationic antimicrobial peptides, which unfold their activity by destroying bacterial membranes due to their amphipathic conformations. Also, anticancer activity has been reported for cationic antimicrobial peptides based on a similar mechanism of action.

Activity, selectivity and the mechanism of action of these novel β^{2,2}-amino acid derivatives were investigated on bacteria, cancer cells and bacterial biofilms. Several of the derivatives were highly active, displaying MIC values below 4 μM against gram-positive bacteria, including multidrug resistant strains like methicillin resistant *Staphylococcus aureus*. Moreover, we also demonstrated that these derivatives are able to permeate models of biological membranes resembling colon uptake upon oral administration, a novelty within antimicrobial peptide research.

Several of our β^{2,2}-amino acid derivatives are active against cancer cells, and our lead derivative, **A2**, showed potency against 60 different cancer cell lines with IC_{50} values below 5 μM. Recently, we could demonstrate that *Staphylococcus aureus* biofilms are susceptible to β^{2,2}-amino acid derivatives. We observed impaired biofilm viability and reduction in biofilm biomass at concentrations as low as 30 μM.

The β^{2,2}-amino acid derivatives are a promising new class of compounds due to their alternative bactericidal and cytotoxic properties compared to common antimicrobial or anticancer drugs. Currently, we are investigating their anti-biofilm mechanism of action in order to further evaluate their potential as future treatment options for biofilm related infectious diseases.

Staphylococcus aureus biofilms and keratinocyte apoptosis

Presenter: **Kelly Kirker**, assistant research professor of chemical and biological engineering
Co-author: Mary Cloud Ammons Anderson[2]
Affiliation: [1]Center for Biofilm Engineering and
[2]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA.

Recently, it was demonstrated that planktonic *S. aureus* and *S. aureus* biofilms have distinguishable, lethal effects on keratinocytes; planktonic *S. aureus* induced necrosis, while *S. aureus* biofilms induced apoptosis. Because these characteristic effects could be observed when keratinocytes were exposed to soluble factors, we hypothesized that planktonic and biofilm *S. aureus* produce unique small molecule metabolites (≤ 3000 daltons) to induce this result. The goal of this investigation was to identify the molecular pathways mediated by *S. aureus* biofilms that induce keratinocyte apoptosis. By elucidating the apoptosis signaling, we anticipated that targeted interruption of these molecular signals could be applied and the keratinocytes could be rescued from lethal biofilm exposure. To achieve this aim, planktonic-conditioned medium (PCM) and biofilm-conditioned medium (BCM) were fed to the keratinocyte HaCaT cell line. At selected time points cells were assayed for viability and several apoptosis indicators, including key caspases and DNA fragmentation. The viability of both the PCM and BCM groups declined with time and were both significantly lower than control groups ($p \leq 0.019$) by 9 hours of exposure; however, the indicators of apoptosis were significantly higher in the BCM group as early as at 3 hours of exposure (caspase 9, $p < 0.0001$). Caspase 9 activation indicated that BCM induced the intrinsic apoptosis pathway. Attempts to block apoptosis using specific caspase inhibitors were successful, but the cells were not rescued from death. It was suspected that the cells found an alternative death modality, either necrosis or autophagy. Therefore, a molecular therapy for rescuing keratinocytes from *S. aureus* biofilms appears doubtful. Current efforts are focused on characterizing small molecule metabolite profiles of BCM and PCM in order to identify the causative agents in BCM that induce keratinocyte apoptosis.

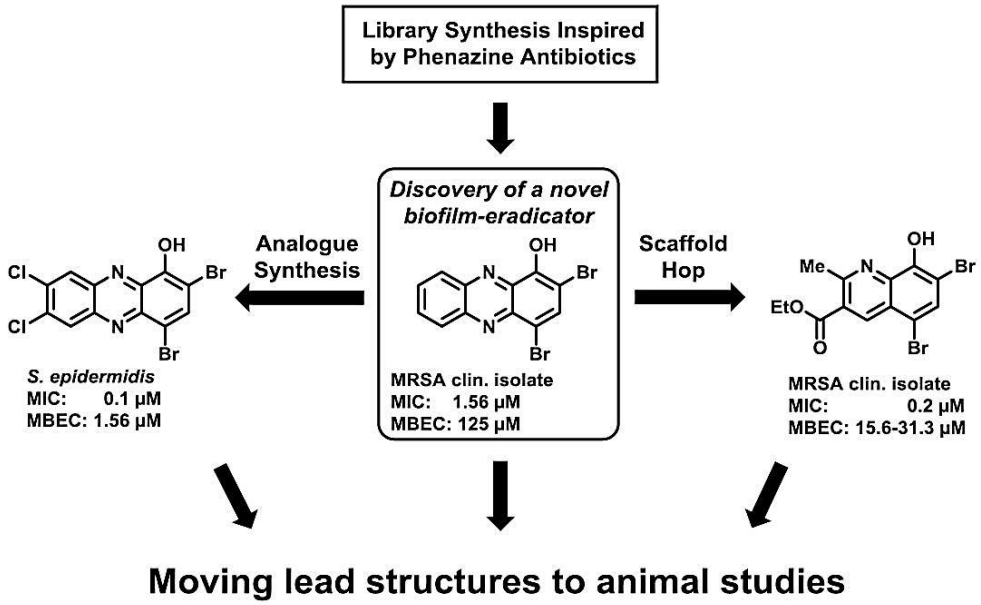
Young Investigators

Phenazine antibiotic inspired discovery of biofilm-eradicating small molecules

Presenter: **Robert Huigens III**, assistant professor of medicinal chemistry
Affiliation: Department of Medicinal Chemistry, University of Florida, Gainesville, FL, USA.

Bacterial biofilms are surface-attached bacterial communities that occur in ~80% of all bacterial infections. Despite the killing effectiveness of bactericidal antibiotics to free-floating planktonic cells, these agents are ineffective at the eradication of bacterial cells within a biofilm. The discovery of novel antibacterial agents possessing dual antibacterial activities against both planktonic and biofilm cells are of clinical importance as our antibacterial pipeline has failed to produce biofilm eradicating therapeutic agents to date. Unfortunately, few classes of small molecules have been identified that eradicate bacterial biofilms. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Acinetobacter baumannii* are major human pathogens notorious for their roles in biofilm-associated infections in humans. Our group recently synthesized a focused phenazine antibiotic-inspired library of diverse phenazine small molecules which were evaluated for antibacterial and biofilm eradication activities against staphylococcal and *A. baumannii* strains. From these studies, we have discovered a series of potent halogenated phenazine (through analogue synthesis) and 8-hydroxyquinoline (through a scaffold hopping strategy) small molecules that collectively demonstrate a broad spectrum of antibacterial and biofilm eradication activities. Currently, we are

obtaining initial pharmacokinetic data as we move our most promising biofilm eradicators forward to animal studies. These discoveries have the potential to provide clinically effective treatment options for drug-resistant, biofilm-associated bacterial infections.



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Coupling multi-scale in situ determination of biofilm mechanical properties to mathematical modeling of biofilm fluid-structure interaction

Presenter: **Juan P. Pavissich**, postdoctoral researcher in biotechnology
Co-authors: Florian Blauert[3], Michael Wagner[3], Harald Horn[3], and Cristian Picioreanu[1]
Affiliation: [1]Department of Biotechnology, Delft University of Technology, The Netherlands.
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[3]Karlsruhe Institute of Technology, Karlsruhe, Germany.

From a material science point of view biofilms are complex entities. Biofilms are structurally heterogeneous and present a wide range of mechanical properties, including elastic to time-dependent viscoelastic rheology. Mathematical modeling of biofilm mechanics is a valuable tool for assessing biofilm deformation, enabling a better understanding of growth dynamics and biofilm detachment under fluid shear stress. In this context, modeling approaches should be able to simulate biofilm constitutive mechanics and fluid-structure interaction (FSI) without heuristic arguments. Here, we combine computational fluid dynamics (CFD) and continuum mechanics principles to develop a FSI model including biofilm viscoelasticity. Moving-mesh and phase-field methods allow capturing biofilm deformation and detachment (Fig. 1). A comprehensive empirical knowledge of biofilm properties is also required. For this we applied novel approaches for in situ and non-destructive rheological characterization of biofilms. Optical coherence tomography (OCT) is used as imaging technique to obtain biofilm structures at the meso-scale, and time-resolved deformation and detachment under fluid-flow (Fig. 2A). On the other hand, information about biofilm mechanical heterogeneity at local scale is determined using microparticle actuation, coupled to confocal laser scanning microscopy (CLSM) (Fig. 2B). The model incorporates structural and mechanical information from these experimental techniques. Simulations show that the FSI model can be used to estimate mechanical properties from OCT analysis results. Also, the model can assess the effect of the structural heterogeneity given by microparticle probing on biofilm deformation and detachment. Coupling biofilm mechanics determined by means of in situ and non-destructive experimental techniques, to appropriate mathematical modeling is well suited for the study of relevant biofilm

problems. Such include biofilm development, biofilm streamer formation, and biofilm removal by mechanical means and biofilm disrupting chemical agents (Fig. 1B).

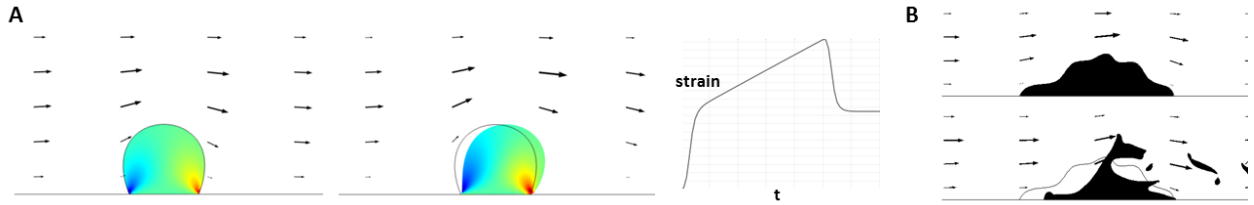

Figure 1: (A) FSI simulation of viscoelastic biofilm (arrows show flow field). Undeformed (left), deformed (middle), and permanent deformation after constant load-unload test (right). Maximum shear stress magnitude (red and blue). **(B)** FSI simulation of biofilm detachment due to flow stress and chemical attack. Initial structure (top), disrupted structure (bottom).

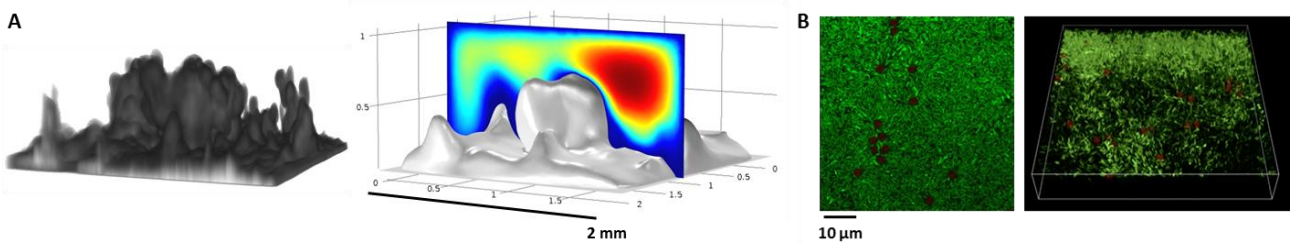

Figure 2: (A) Biofilm structure for measurement of deformation and CFD simulations. 3-d experimental structure from OCT imaging (left) in the computational model (right). **(B)** CLSM images of *Pseudomonas aeruginosa* (green) and biofilm-embedded 2 µm-microbeads (red), for determination of mechanical properties using microparticle actuation.

SESSION 4: Biofilm Mineral Interactions

From speleogenesis to sewer corrosion

Presenter: **Phil Stewart**, professor of chemical and biological engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

What do corrosion of concrete sewer pipes and the formation of certain karst cave systems have in common? Biofilms, of course. This presentation juxtaposes two publications from last year, one in *National Geographic* (July 2014) describing acidogenic biofilms, dubbed snottites, that drip from the ceiling of some caves and an August 2014 *Science* paper analyzing the devastating infrastructure loss that results from very similar biofilms that form in sewer pipes. In both systems, the causative agents are sulfide-oxidizing bacteria that use hydrogen sulfide and oxygen from the air phase and generate sulfuric acid in the biofilm along the ceiling of the cave or pipe. The acid corrodes the calcareous rock or concrete releasing gypsum, water, and carbon dioxide. In the cave systems, the pH of acid dripping from the biofilm can be as low as 1. In sewer systems, the hydrogen sulfide is generated by sulfate-reducing bacteria in the water or sediment at the bottom of the pipe. In cave systems such as that in Frassasi, Italy, the hydrogen sulfide is of geological origin. Pikaar et al. propose a simple, cost-effective switch to reduce the loss of infrastructure in sewers: replace the coagulant used in water treatment, aluminum sulfate, with sulfate-free alternatives. In the Queensland, Australia, systems that they studied, coagulant addition accounts for more than half of the sulfate in wastewater. These vignettes highlight a principle of microbial ecology: environments with similar physical and chemical conditions will support similar ecologies and geochemical processes.

Subaerial biofilms: New horizons in stone biodeterioration research

Presenter: **Federica Villa**[1], postdoctoral researcher
Co-authors: Cappitelli F[1] and Stewart PS[2]
Affiliation: [1]University of Milan, Milano, Italy.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Science and technology interact with art and culture in many ways: the arts draw both inspiration and new materials from science, while the scientific examination of art and artefacts has provided us with important insights into the progress of human civilizations. Now, science—in particular biological science—has an even more important role: to protect and conserve mankind's often fragile cultural heritage for future generations.

The evidence that many of the world's most precious artworks are made of stone and have a finite life drives the interest in this topic. Their irreversible deterioration due to biological attack is a worldwide concern. Microorganisms colonize outdoor lithic surfaces and develop into biofilms at the solid/air interface (subaerial biofilms, SABs), which, in turn might cause aesthetic, chemical and physical decay. Although it has been estimated that at least 99% of the world's microbial biomass exists in biofilms, the role and behavior of microorganisms within the biofilm matrix and their complex interactions with the external environment is still unknown.

In this talk I will approach the complex interaction between SABs and cultural heritage assets from two different angles: 1) the establishment of a laboratory model system of SABs relevant to cultural heritage studies, and 2) the urgent need to develop preventive anti-biofilm strategies.

1) One of the main gaps challenging our understanding of the physiology and the activity of biofilms inhabiting outdoor stone heritage is the lack of a model system of SABs. To overcome this limitation, we developed a methodology to obtain a laboratory model of a dual-species SAB relevant to cultural heritage studies. The results underscore the ability of the dual-species SAB model to underpin functional traits characteristic of biofilms inhabiting lithic substrate such as: i) microcolonies of aggregated bacteria; ii) network-like structures following surface topography; iii) deconstruction of the complexity of environmental samples into their main component parts (e.g., phototroph-heterotroph interactions); iv) ability to change the chemical parameters that characterize the microhabitats; v) survival in harsh environments; and vi) biocide tolerance. To the best of our knowledge, this is the first time that a phototroph-heterotroph association at the stone/air interface has been successfully developed at the laboratory scale starting from two introduced, controlled species and not from an environmental microbial consortium. The present study has the potential to significantly advance our mechanistic understanding of the biofilm-stone-air interplay that has proven difficult to study in field experiments due to the inaccessibility of samples and the complexity of the ecosystem under investigation.

2) SABs inhabiting artistic surfaces, as any other biofilm, show a remarkable resistance to biocides, making them recalcitrant to traditional cleaning procedures. Plants offer a virtually inexhaustible and sustainable resource of very interesting classes of biologically active, low-molecular-weight compounds (parvome). In the past, the plant parvomes were screened mainly for their lethal effects, disregarding concentrations and ecologically relevant functions of these molecules in the natural context. Testing sub-lethal concentrations of plant-derived compounds mimicking environmental levels may be critical to reveal mechanisms subtler than the killing activity, e.g., those influencing multicellular behavior, offering an elegant way to develop novel biocide-free anti-biofilm strategies. In a cross-disciplinary fashion, I will illustrate recent successes of sub-lethal concentrations of the plant-derived compound zosteric acid, its mechanism of action and the structural characteristics responsible for anti-biofilm activity, envisioning applications on cultural heritage assets.

Biomineralization of kidney stones

Presenter: **Logan Schultz**[1], postdoctoral researcher
Co-authors: Trace Hobbs[1], Ellen Lauchnor[1], Dirk Lange[2], Robin Gerlach[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Stone Centre, Vancouver General Hospital, Vancouver, BC, Canada.

Biofilm infections in the urinary tract can cause kidney stones by increasing the pH of urine and forming minerals such as struvite ($MgNH_4PO_4*6H_2O$). Current treatment options for kidney stones are invasive and sometimes ineffective, and in vivo tests are constrained by ethics. Furthermore, technological development is constrained by our incomplete understanding of how pathogens colonize the urinary tract and how microbe-mineral interactions influence the properties of kidney stones.

This presentation describes how we combine flow systems, analytical chemistry, computer models, and microscopy to study how infection stones form. We compare the properties of real kidney stones with *in vitro* stones that were synthesized in flow systems with artificial urine, and we discuss the exciting potential to develop better treatment and prevention strategies using our methods.

Overview of biofilm mediated mineralization and engineering applications

Presenter: **Adie Phillips**[1,3], assistant professor of civil engineering
Co-authors: Robin Gerlach[2,3] and Al Cunningham[1,3]
Affiliation: [1]Department of Civil Engineering,
[2]Department of Chemical and Biological Engineering, and
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

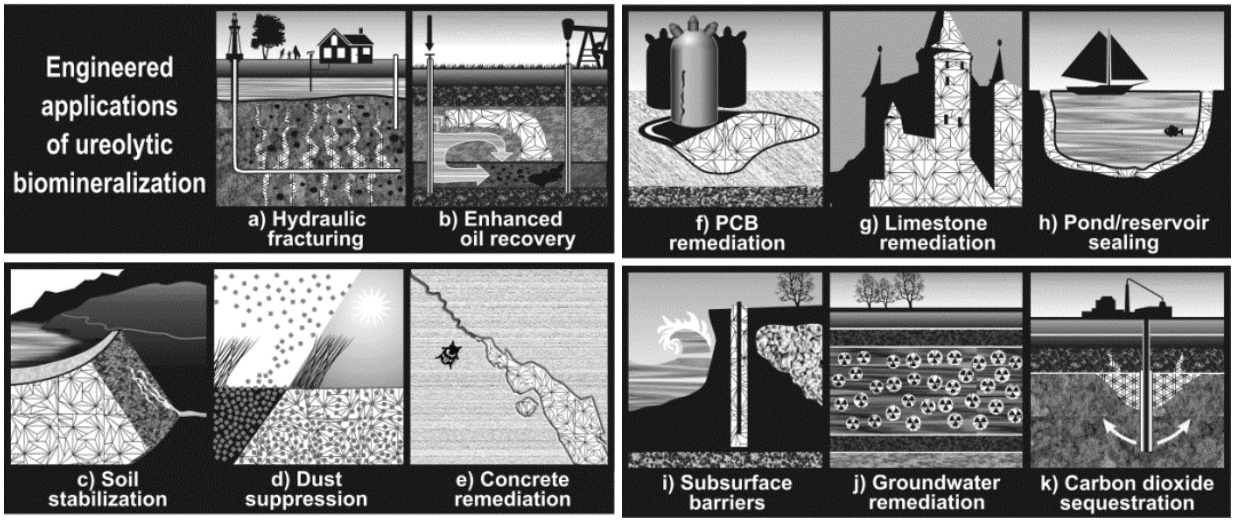

Figure 1. Several proposed ureolysis-driven MICP engineering applications (white crystal hatch pattern represents $CaCO_3$) (a, b, k) sealing fractures or cementing preferential flow paths to improve oil recovery or mitigate subsurface fluid leaks, (c, d) consolidating porous materials to strengthen dams or minimize dust, (e) remediate concrete fractures, (f, g) coat surfaces such as PCB-oil contaminated concrete to prevent contaminant migration or limestone to minimize acid erosion, (h, i) sealing ponds or controlling salt water intrusion, and (j) remediating subsurface groundwater.

Detrimental effects of biofilms in industrial and medical environments are commonly reported. However, biofilms may also be used for beneficial engineering applications[1]. In this overview, ureolytic biofilms are discussed for their ability to induce calcium carbonate ($CaCO_3$) precipitation (MICP) for beneficial use in construction materials, cementing porous materials, and environmental

remediation (Figure 1). Also discussed is the spatial MICP distribution and how that is controlled by manipulating three factors, the (1) ureolytic activity (of microorganisms) (2) reaction and transport rates of substrates and (3) saturation conditions of carbonate minerals. This presentation will discuss the applicability of MICP engineering technologies toward commercial scale applications.

[1] Phillips AJ, Gerlach R, Lauchnor E, Mitchell AC, Cunningham AB, Spangler L "Engineered applications of ureolytic biomineralization: A review," *Biofouling* 2013; 29(6):715–733.

SESSION 5: Biofilm Fundamentals

Novel extracellular membrane structures in a sulfate-reducing biofilm

Presenter: **Lauren Franco**[1,2], PhD candidate in microbiology and immunology
Co-authors: Wu S[3], Jahmb K[3], Joo M[3], Ivanisevic J[4], Siuzdak G[4], Auer M[3], Fields MW[1,2]
Affiliation: [1]Center for Biofilm Engineering and
[2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
[3]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
[4]The Scripps Research Institute, La Jolla, CA, USA.

Desulfovibrio vulgaris is a sulfate-reducing bacterium commonly observed in anaerobic subsurface environments associated with metal-reducing conditions. Biostimulation of heavy metal-reducing organisms by injecting electron donors into the subsurface can create unbalanced ratios of electron donor to acceptor and here we show that these ratios affect biofilm structure and activity. Samples were analyzed for protein, carbohydrate, and sulfide content; imaged using electron microscopy; and compared via metabolomics. Microscopy revealed the presence of membrane vesicles, extracellular filaments, and extracellular membranous structures. Membranous structures create geometrical pockets in the biofilm that are devoid of bacteria, or are sheet-like structures that are heterogeneously distributed throughout the biofilm. Uranyl acetate-stained biofilm (non-osmicated) revealed an unstained thin core structure, which, upon osmication, becomes black, indicating that the thin structure is lipid-based. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers. EDS imaging revealed presence of Fe, O and P, but not sulfide, and these results suggested the metal deposits are not solely the result of inorganic chemistry interactions of metals ions with hydrogen sulfide. The biofilm and metal deposition was visualized in 3-D with SBF/SEM, and showed a heterogeneous distribution of metal precipitates away from cells. Metabolomic analysis revealed an up-expression of particular fatty acids under electron acceptor-limited conditions compared to balanced conditions and a down-expression of metabolites involved in DNA turnover, N-cycling, and peptidoglycan turnover, and these results indicated that electron acceptor-limitation may induce an overall stress response that is coordinated with alternative electron transfer mechanisms.

Antimicrobial tolerance in biofilms

Presenter: **Phil Stewart**, professor of chemical and biological engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The tolerance of microorganisms in biofilms to antimicrobial agents is examined through a meta-analysis of literature data. A numerical tolerance factor comparing the rates of killing in the planktonic and biofilm states is defined to provide a quantitative basis for the analysis. Tolerance factors for biocides and antibiotics range over three orders of magnitude. This variation is not explained by taking into account the molecular weight of the agent, the chemistry of the agent, the

substratum material, or the species of the microorganisms. Tolerance factors do depend on the areal cell density of the biofilm at the time of treatment and on the age of the biofilm as grown in a particular experimental system. This suggests that there is something that happens during biofilm maturation, either physical or physiological, that is essential for full biofilm tolerance. Experimental measurements of antimicrobial penetration times in biofilms range over orders of magnitude, with slower penetration (>12 min) observed for reactive oxidants and cationic molecules. These agents are retarded through the interaction of reaction, sorption, and diffusion. The specific physiological status of microbial cells in a biofilm contributes to antimicrobial tolerance. A conceptual framework for categorizing physiological cell states is discussed in the context of antimicrobial susceptibility. It is likely that biofilms harbor cells in multiple states simultaneously (e.g., growing, stress-adapted, dormant, inactive) and that this physiological heterogeneity is an important factor in the tolerance of the biofilm state.

Biofilms formed by the archaeon *Haloferax volcanii* exhibit cellular differentiation and social motility, and facilitate horizontal gene transfer.

Presenter: **R. Thane Papke**, associate professor of molecular and cell biology
Affiliation: Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA.

Archaea share a similar microbial lifestyle with bacteria, and not surprisingly then, also exist within matrix-enclosed communities known as biofilms. However, the biology of archaeal biofilms is only now being explored. We investigated the development, composition and dynamics of biofilms formed by the haloarchaeon *Haloferax volcanii* DS2. Analysis by confocal scanning laser microscopy showed that *H. volcanii* cells formed microcolonies within 24 h, which developed into larger clusters by 48 h and matured into flake-like towers often greater than 100 µm in height after 7 days. To visualize the extracellular matrix, biofilms formed by GFP-expressing cells were stained with concanavalin A, DAPI, Congo red and thioflavin T. Stains colocalized with larger cellular structures and indicated that the extracellular matrix may contain a combination of polysaccharides, extracellular DNA and amyloid protein. Following a switch to biofilm growth conditions, a sub-population of cells differentiated into chains of long rods sometimes exceeding 25 µm in length, compared to their planktonic disk-shaped morphology. Time-lapse photography of static liquid biofilms also revealed wave-like social motility. Finally, we quantified gene exchange between biofilm cells, and found that it was equivalent to the mating frequency of a classic filter-based experimental method. The developmental processes, functional properties and dynamics of *H. volcanii* biofilms provide insight on how haloarchaeal species might persist, interact and exchange DNA in natural communities. *H. volcanii* demonstrates some biofilm phenotypes similar to bacterial biofilms, but also has interesting phenotypes that may be unique to this organism or to this class of organisms, including changes in cellular morphology and an unusual form of social motility. Because *H. volcanii* has one of the most advanced genetic systems for any archaeon, the phenotypes reported here may promote the study of genetic and developmental processes in archaeal biofilms.

Biofilm formation mechanisms of *Pseudomonas aeruginosa* predicted via metabolic models

Presenter: **Francisco Vital-Lopez**, Research Scientist
Co-authors: Anders Wallqvist, Jaques Reifman
Affiliation: DoD Biotechnology High-Performance Computing Software Applications Institute (BHSAI), Frederick, MD, USA.

Pseudomonas aeruginosa is one of the most frequently found bacterial pathogens in patients with chronic infections such as non-healing wounds and cystic fibrosis. The persistence of *P. aeruginosa* in these infections is enabled by its ability to form biofilms. Standard antibiotic treatments, effective

against bacteria living as single cells, are generally unsuccessful against biofilms. Understanding the physiological adaptations associated with biofilm formation could make it possible to design treatments for biofilm-based persistent infections. In this talk, we will present insights about the underlying mechanisms of biofilm formation generated from a genome-scale kinetic model of the *P. aeruginosa* metabolic network. Thus, our analysis provides *1*) an understanding of how *P. aeruginosa* regulates its metabolism to synthesize molecules that are important for biofilm formation and *2*) identification of enzymatic reactions that can be specifically targeted to develop anti-biofilm drugs. This approach will be important in identifying metabolic mechanisms and strategies adapted by pathogenic bacteria refractory to conventional antibiotic treatments.

SESSION 6: New Tools & Methods

Quantifying biofilm characteristics over time from 3-D confocal microscope movies

Presenter: **Al Parker,** assistant research professor of mathematical sciences; CBE bio-statistician
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The decades-old process of counting viable cells in an agar plate remains a primary method for quantifying microbial abundances, as when submitting antimicrobial efficacy data to the U.S. EPA. Rapid advances in imaging technology may change this paradigm. Confocal scanning laser microscopy (CSLM) has provided beautifully rendered qualitative measures of microbial populations for years, such as 3-D movies of pathogenic biofilms before and after application of antimicrobials. State-of-the-art algorithms and the power of modern computers have made quantitative statistical analyses of the massive data sets obtained by CSLM more feasible. Biofilm characteristics such as volume and surface area coverage, and standard errors (i.e., error bars), can be estimated over time from CSLM movies. An important component in the analysis of biofilms is empirically modeling attenuation of CSLM light as it passes through the biofilm. A fast, yet simplified (and limited), analysis of these movies utilizes a Bayesian linear model of biofilm heights. Limitations of the linear approach can be overcome by solving a more computationally intensive pixel-based Bayesian non-linear model.

Drop-based microfluidics for high-throughput biological assaying

Presenter: **Connie B. Chang**, assistant professor of chemical and biological engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Using drop-based microfluidics, emulsion drops can be created one at a time in microscale channels. These drops have volumes ranging from picoliters to nanoliters and are created at high-throughput rates, up to thousands per second. I will describe the use of drop-based microfluidics as a method for high-throughput assaying and sensing for biological applications.

Biofilms as materials

Presenter: **Jim Wilking,** assistant professor of chemical and biological engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Bacillus subtilis is a historically well-studied soil bacterium and a model organism for biofilm formation. Many of the genes responsible for biofilm formation have been identified and the primary components of the extracellular matrix established. Such knowledge has enabled a materials-based

approach to understanding *B. subtilis* mechanical properties. Here, bulk rheology and microrheology measurements on biofilms and reconstituted matrix components will be presented and the mechanical roles of the primary matrix components discussed. Through these studies we hope to address the role of mechanics in biofilm physiology and develop an understanding of how biofilms modulate their mechanical properties.

Applications of microelectrodes for biofilm kinetics and inhibition studies

Presenter: **Ellen Lauchnor,** assistant professor of civil engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The transport limitations within biofilms are well known and a key characteristic of the biofilm lifestyle. Diffusion limited transport of substrates and products results in chemical gradients that have been investigated in biofilms using micro-scale devices such as microelectrodes. The use of microelectrodes allows for spatially resolved measurement of species such as dissolved oxygen, pH and other chemical species inside biofilms. Ongoing research at CBE utilizes microelectrodes as powerful tools to elucidate conditions such as dissolved oxygen within biofilms and the changes in those conditions with biofilm depth. Concentration profiles generated from these measurements can also be used as data for reactive transport model calibration and evaluation of biofilm kinetics.

Our previous and ongoing work uses microelectrode measurements and a 2-D reactive transport model to evaluate kinetic parameters in nitrifying biofilms containing ammonia oxidizing bacteria. Ammonia oxidizers contribute to nitrogen removal in wastewater treatment systems and the environment. To effectively model nitrifying biofilms, the kinetic parameters for ammonia oxidation were evaluated in biofilms using integrated microelectrode measurements and modeling. Microelectrode measurements have been used to evaluate changes in microbial activity in ammonia oxidizing biofilms exposed to the inhibitor, phenol. Current research is working toward correlating this microelectrode data to a kinetic inhibition model. A similar 2-D modeling approach can be used in other systems to determine biofilm kinetic and inhibition parameters using microsensor profiles, where the fluid phase hydrodynamics can be characterized.

Poster Abstracts

Academic Posters (non-CBE)

Title: **The corrosion of carbon steel in suboxic/sulfidogenic fuel/seawater environments: The role of metallurgy**
Date: 07/2015
Authors: **Recep Avci[1]**, Martin J[1], Wolfenden M[1], Davis B[1], Lucas K[1], Beech I[2]
Affiliation: [1]Montana State University, Department of Physics, Bozeman, MT, USA.
[2]University of Oklahoma, Department of Microbiology, Norman, OK, USA.

This research focuses on the role that the metallurgical preparation of carbon steel plays in its corrosion. When a micro- or nano-sized phase (such as pearlite or MnS) is introduced into a pure Fe matrix, it is expected that the immediate surroundings of these islands will distort the pure Fe lattice, giving rise to localized dislocations, which in turn cause localized plastic strain. We hypothesize that this strain increases carbon steel's propensity toward corrosion. This hypothesis was verified experimentally using carbon steel (1018) coupons cut and polished parallel or perpendicular to their rolling direction. The coupons were exposed to sulfidogenic environments under anaerobic or suboxic conditions. The cultures of interest were *D. alkanenexedens*, a hydrocarbon-degrading organism, in anaerobic artificial seawater and mixtures of *Marinobacter* and *D. indonensiensis* in a suboxic fuel/seawater environment. Predetermined areas were mapped using backscattered electron diffraction before corrosion. After corrosion the same areas were analyzed with and without the corrosion products using electron microscopy and atomic force microscopy. Corrosion was monitored in situ with electrochemical measurements. Matlab codes were written to compare corrosion rates of strained areas and unstrained areas. Predicted carbon steel corrosion rates were compared to actual corrosion rates determined from AFM depth measurements.

A close correlation was observed between corrosion rate and plastic strain remaining from metallurgical processes. Areas containing a carbide phase (pearlite) within the iron lattice appeared to be the most strained. Quantitative agreement was found between predicted and observed corrosion rates of strained areas on carbon steel. A galvanic potential difference of about 10–20 mV was predicted by the model calculations. This is in agreement with experiments carried out with 1018 carbon steel and pure Fe subjected to the same MIC environment.

Carbide and MnS phases in carbon steel introduce local strains within the carbon steel matrix, creating a 3-D network of galvanic cells between the strained and unstrained areas of the Fe lattice, which increases the metal's propensity toward localized corrosion. Dissolution of MnS at low pH conditions gives rise to localized high concentrations of H_2S, HS^- and S, which fuels the localized corrosion.

Title: **Determining the effects of *Pseudomonas aeruginosa* biofilms on chronic non-healing diabetic wounds in a murine model**
Date: 06/2015
Authors: **Jacob A. Gibson**[1], Larrivee CL[2], Hunt AMA[1], Navitskaya S[2], O'Reilly S[2], Busik JV[2], Waters CM[1]
Affiliation: [1] Michigan State University Department of Microbiology and Molecular Genetics, Lansing, MI, USA.
[2] Michigan State University Department of Physiology, Lansing, MI, USA.
Sponsored by: American Diabetes Association and the Beckman Foundation

Diabetes and obesity have reached epidemic proportions. According to 2012 CDC reports, diabetes affects 29 million people—9.3% of the U.S. population. Diabetes leads to impaired wound healing and chronic non-healing wounds resulting in tens of thousands of amputations annually. It is important to understand the causes of chronic non-healing diabetic wounds in order to develop novel treatment approaches. Bacterial biofilms have recently been implicated as an underlying cause of chronic non-healing wounds; however, the molecular mechanisms responsible for chronic wound formation and wound severity remain to be defined. Using a Streptozotocin (STZ)-induced diabetic murine model, we examined the impact of *Pseudomonas aeruginosa* biofilms in wound healing of diabetic and non-diabetic mice. Our results indicate that uninfected diabetic wounds showed a delay in healing compared to wild type mice. To assess the impact of biofilm formation on the rate of wound healing, diabetic and non-diabetic mice were wounded prior to inoculation of bioluminescent

P. aeruginosa biofilms. The rates of healing and biofilm viability were then monitored via the In Vivo Imaging System (IVIS) and microscopy until complete recovery. IVIS imaging revealed a decrease in biofilm viability from day-one to day-five in the non-diabetic mice, in contrast to a general increase in biofilm in the diabetic mice; however, the study established that biofilms halt healing in both models. Ultimately, specific mutations in chemical signaling known to regulate *P. aeruginosa* biofilm formation will be examined in these models, leading to a better understanding of molecular mechanisms that contribute to diabetic chronic wounds.

Title: **Nitrification for a drinking water source water contaminated with ammonia using BioNET**
Date: 05/2015
Authors: **Wu Y-J**[1,2],
Co-Authors Liu Y-W[2], Cheng H-H[1], Ke C-W[1], Lin T-F[1,2,3], Chang C-H[1,2] Whang L-M[1,2,3]
Affiliation: [1] Global Water Quality Research Center, National Cheng Kung University (NCKU), Tainan, Taiwan.
[2] Department of Environmental Engineering, NCKU, Tainan, Taiwan.
[3] Sustainable Environment Research Laboratory (SERL), NCKU, Tainan, Taiwan.
Sponsored by: Taiwan Water Corporation

Global climate change causes an uneven rainfall distribution situation in Taiwan, resulting in severe drinking water security problems. Even in the rainy season, the extremely heavy rain accompanied by high turbidity may shut down drinking water treatment systems. However, river water with stable water levels in the dry season and low turbidity after heavy rains in southern Taiwan mostly are contaminated by ammonia, and cannot meet source water quality standards in Taiwan.

A pilot-scale biological pre-treatment process was applied in this study to examine the capability and efficiency of ammonia removal in the contaminated river water. The biological pre-treatment process applied porous polyurethane carriers (BioNET) for microorganisms, including heterotrophs

and nitrifiers, to grow on and retain in the bioreactor. Three different hydraulic retention times (HRT), 1.33, 0.81, and 0.5 hours, were examined in this study. Terminal restriction fragment length polymorphism (T-RFLP) was applied to monitor ammonia oxidizing archaea and bacteria (AOA and AOB) community dynamics in the bioreactor. Archaeal and bacterial ammonia monooxygenase subunit A (amoA) gene and 16S rRNA genes of total bacteria were quantified using real time quantification polymerase chain reaction (qPCR).

Contaminated river water was directly used as raw water for the BioNET process. Ammonia concentration in the river water increased from below 1 mgN/L (August/September) to 8–9 mgN/L (April/May). Under HRT 0.5 hours, average ammonia removal efficiency and rate could reach 81% and 0.37 kg-N/Day/m^3, respectively. The amoA gene based T-RFLP results showed that the microbial diversity of AOA increased after HRT decreased; while AOB showed relatively stable microbial diversity through the operation period. The quantification results showed that AOB abundance was 2–3 orders of magnitude higher than that of AOA. The ratio of AOB over total bacteria varied between 0.1% to 8.6%. Besides, AOB abundance higher than 1.5×10^8 copy/BioNET provides nitrification removal efficiency more than 70%. In addition, both reactor performance and batch tests indicated denitrification occurred in the biological pre-treatment process. It is suggested that BioNET provided an anoxic zone for the growth and functioning of denitrifiers. Our results suggest a promising strategy of nitrogen removal from slightly polluted river water using porous polyurethane carriers.

Figure 1. BioNET carrier used in this study

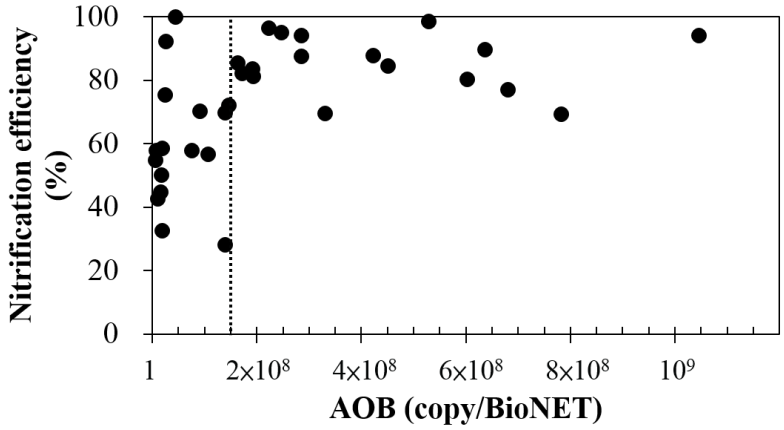

Figure 2. Relationship between abundance of ammonia oxidizing bacteria(AOB) and nitrification efficiency

Center for Biofilm Engineering Posters

CBE Poster #631

Title: **In-situ detection of subsurface biofilm using low-field NMR: A field study**
Date: 07/2015
Authors: **Catherine M. Kirkland**[1], Herrling MP[2], Hiebert R[1], Bender A[1], Grunewald E[3], Walsh DO[3], Codd SL[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chair of Water Chemistry and Water Technology, Karlsruhe Institute of Technology, Karlsruhe, Germany.
[3]Vista Clara Inc., Mukilteo, Washington, USA.
Sponsored by: U.S. Department of Energy, National Science Foundation, Carl-Zeiss Foundation, and the Karlsruhe House of Young Scientists (KHYS)

Subsurface biofilms are central to bioremediation of chemical contaminants in soil and groundwater, whereby micro-organisms degrade or sequester environmental pollutants.[1] Conventional methods to monitor subsurface biofilm growth are indirect, destructive, and potentially hazardous. Previous laboratory research conducted at MSU has indicated that low-field NMR is sensitive to biofilm growth in porous media, where biofilm contributes a polymer gel-like phase and enhances T_2 relaxation.[2-3] Here we show that a small-diameter NMR well logging tool[4] can detect biofilm accumulation in the subsurface. T_2 relaxation distributions were measured over a 17-day experimental period by two NMR probes, operating at approximately 275kHz and 400kHz, installed in 4-inch (10.2 cm) wells in an engineered field testing site. The mean log T_2 relaxation times were reduced by 62% and 43%, respectively, while biofilm was cultivated in the soil surrounding each well. Biofilm growth was confirmed by bleaching and flushing the wells and observing the NMR signal's return to baseline. This result provides a direct and non-invasive method to spatio-temporally monitor biofilm accumulation in the subsurface.

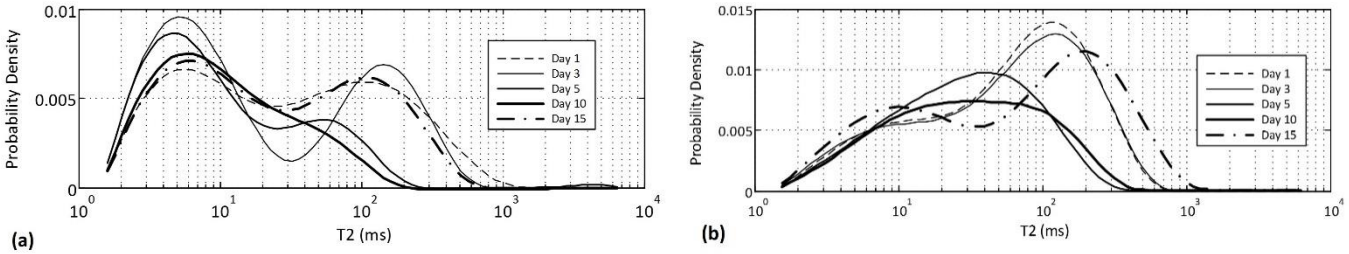

Figure 1. T_2 distribution for ~275kHz well (a) and ~400kHz well (b) at inoculation (short dash line), during biofilm growth (solid lines), and after flushing and bleaching each well (dash-dot lines).

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CBE Poster #649

Title: **Growth of a native algal species in coal bed methane water for biofuel and biomass accumulation**
Date: 06/2015
Authors: **Logan Hodgskiss**[1,4], Cunningham AB[1,4], Gerlach R[2,4], and Fields MW[3,4]
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Sponsored by: U.S. DOE Advancements in Sustainable Algal Production (ASAP) DE-EE0005993; NSF Sustainable Energy Pathways (SEP) Program, NSF CHE-1230632

Coal bed methane (CBM) production in the Powder River Basin is associated with large amounts of production water. The water is typically stored in shallow ponds and is generally high in sodium and low in divalent cations, such as magnesium and calcium, making them an undesirable water source for agriculture, irrigation, and drinking. However, there is potential to exploit the water as a medium for biofuel production. A native green alga species, Alga A, has been isolated from CBM production water and is being evaluated for the potential to produce lipids and biomass that could be used as a biofuel source. Alga A was routinely grown in controlled conditions in axenic cultures of Bold's Basal Medium (BBM). Growth and lipid production in this medium is compared to growth of the species in sterile CBM water with and without added nutrients. Small, bench-scale growth experiments were done at 20°C in incubators with a 14:10 light/dark cycle. Cultures were monitored for pH, chlorophyll levels, biomass concentration, anion species, and lipid production. High levels of growth and lipid production were not observed in the tested CBM water without amendments due to low levels of nitrate, phosphate, and microelements. Addition of these nutrients increased growth to a comparable level of that observed in the defined BBM. When only nitrate was added to CBM production water, biomass levels increased and elevated levels of lipids were accumulated. The combination of nitrate with phosphate and other micronutrients also caused an increase in biomass and lipid accumulation, but to a lesser extent. The results indicated that a native algal species could be cultivated in CBM production water with nutrient amendments and that nitrate alone promoted the highest levels of accumulated lipids.

CBE Poster #650

Title: **Elementary flux mode analysis of irradiance-induced stress acclimation strategies in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1**
Date: 07/2015
Authors: **Ashley E. Beck**[1,2], Bernstein HC[2], Carlson RP[2]
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Sponsored by: PNNL, NSF

Irradiance plays a central role in regulating phototrophic metabolisms, including the metabolism of photoautotrophic cyanobacteria. Oxygenic cyanobacteria are critical primary producers in most aquatic ecosystems and have become industrially relevant as bioprocess hosts for biofuels and secondary metabolite synthesis. Here, the model thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 was studied for metabolic acclimation strategies to irradiance-induced stress using elementary flux mode analysis. Metabolic stress was considered in conjunction with the availability of dissolved inorganic carbon and fixed nitrogen as well as the inhibitory effects

of metabolic byproducts. Physiologies and their associated byproduct secretion profiles were analyzed over a gradient of irradiances. Formate was predicted to be the most competitive fixed carbon byproduct under stress conditions, a result interpreted in terms of metabolic pathways. Additionally, this work details the experimental determination of biomass macromolecular composition (carbohydrate, DNA, lipid, protein, RNA) for stoichiometric models, which is an often undervalued activity.

CBE Poster #651

Title: **Fluorescent staining and imaging of the *Pseudomonas aeruginosa* PA01 biofilm matrix material**

Date: 07/2015

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Sponsored by: National Institute of General Medical Sciences of the National Institutes of Health
Montana INBRE—IDeA Network of Biomedical Research Excellence

Pseudomonas aeruginosa is an opportunistic pathogen, capable of forming biofilm infections on pulmonary tissue and on artificial implant devices. Biofilms form complex communities with three-dimensional structures which are maintained by extracellular matrix materials. The matrix material of biofilms likely consists of a combination of extracellular polysaccharides, secreted proteins, and nucleic acids. However, the matrices of most bacterial biofilms are not well defined, in part because they are difficult to image by conventional microscopic techniques. In this study we used fluorescent staining and confocal scanning laser microscopy to characterize the extracellular matrix material of *P. aeruginosa* PA01. *P. aeruginosa* has the genetic capacity to produce three secreted polysaccharides, termed Psl, Pel, and alginate (Alg), each with different chemical and physical properties. *P. aeruginosa* PA01 primarily produced the Psl polysaccharide. Four fluorescent probes bound to the extracellular matrix of this strain. Three-dimensional images of the biofilms indicated that matrix material is a fibrous network that appears to coat the substratum, then extend into the biofilm, forming its structural architecture. Rather than being embedded in a gel-like matrix, the bacteria are attached to the fibers. Interestingly, when two of the stains, CellMask Orange (CMO) and Bodipy X-SE 630/650 (BOD) (both Invitrogen) were used simultaneously, each stain appeared to bind separate matrix components. To investigate the fibrous and polysaccharide matrix components, we analyzed PA01 strains containing an arabinose-inducible operon for the Psl polysaccharide and a strain with *pslA, pilA, and cupA1* gene deletions. In the absence of Psl induction with arabinose, the matrix material stained with CMO, but little staining was seen with BOD, suggesting that BOD may bind the Psl polysaccharide. Both stains bound to the matrix of the *pilA* mutant, but the biofilms showed impaired structure and a staining pattern that differed from the matrix material of the wild-type strain. The *cupA1* mutant biofilms were thin, and showed very little matrix staining. The results indicate that specific fluorescent stains bind the matrix material of *P. aeruginosa* PA01 biofilms, and that the Psl polysaccharide, the pilus, and curli are necessary for this strain to produce a fibrous extracellular matrix structure.

CBE Poster #652

Title: **Effects of nutrient limitation on *Desulfovibrio vulgaris* biofilm composition, structure, and metal deposition**

Date: 07/2015

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Sponsored by: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Desulfovibrio vulgaris is a sulfate-reducing bacterium that is commonly observed in anaerobic subsurface environments associated with metal-reducing conditions. Biostimulation of heavy metal-reducing organisms by injecting electron donors into the subsurface can create unbalanced ratios of electron donor to acceptor and here we show that these ratios affect biofilm structure and activity. Samples were analyzed for protein, carbohydrate, and sulfide content, imaged using electron microscopy, and compared via metabolomics. Microscopy revealed the presence of membrane vesicles, extracellular filaments, and extracellular membranous structures. Membranous structures create geometrical pockets in the biofilm that are devoid of bacteria or are sheet-like structures that are heterogeneously distributed throughout the biofilm. Uranyl acetate-stained biofilm (non-osmicated) revealed an unstained thin core structure, which, upon osmication, becomes black, indicating that the thin structure is lipid-based. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers. EDS imaging revealed presence of Fe, O and P, but not sulfide, and these results suggested the metal deposits are not solely the result of inorganic chemistry interactions of metals ions with hydrogen sulfide. The biofilm and metal deposition was visualized in 3-D with SBF/SEM, and showed a heterogeneous distribution of metal precipitates away from cells. Metabolomic analysis revealed an up-expression of particular fatty acids under electron-acceptor limited conditions compared to balanced conditions and a down-expression of metabolites involved in DNA turnover, N-cycling, and peptidoglycan turnover, and these results indicated that electron acceptor-limitation may induce an overall stress response that is coordinated with alternative electron transfer mechanisms.

CBE Poster #653

Title: **Lipid accumulation with mixed photoautotrophic cultures from municipal wastewater**

Date: 06/2015

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Sponsored by: U.S. Department of Energy-Advancements in Sustainable Algal Production (ASAP) program under contract DE-EE0005993 and the National Science Foundation Sustainable Energy Pathways (SEP) Program under NSF CHE-1230632.

Microalgae have emerged as a potential resource for the sustainable production of biomass and biofuel due to high biomass production, bio-oil accumulation, and capability to utilize low-quality nutrient and water sources. While nutrient and water demands challenge large-scale biomass and biofuel production, wastewater offers a potential solution as a low-quality but high-nutrient water source that might be exploited for the production of microalgal biomass. Concentrated algal biomass being used to treat municipal wastewater was used as an inoculum for Bold's Basal Medium (BBM) and non-sterile primary clarified water (PCW) from municipal wastewater. The cultures were grown with a 14:10 light:dark cycle at 20°C. The cultures were characterized with respect to cell biomass, pH, chlorophyll, nitrate, and Nile Red fluorescence over time. In addition, microbial community dynamics were tracked via small-subunit rRNA paired-end sequencing and distribution-based clustering of OTUs. Higher levels of lipids were observed in PCW cultures, while higher biomass levels were observed in BBM cultures. Both cultures had diverse microbial communities but differed with respect to community structure and composition. Photoautotrophic population dynamics were largely diatom and cyanobacteria with few green algae observed. An overall depletion of nitrate and phosphate observed in PCW water appeared to result in an increase in lipid production but lower levels of growth. These results offer an indication of the potential that wastewater possesses for biofuel production with mixed algal communities. It also indicates a more dynamic role of in situ community interactions in contributing to biomass and bio-oil accumulation of mixed algal communities.

CBE Poster #654

Title: **"Species" filter effects on sediment biofilms and groundwater source diversity**

Date: 07/2015

Authors: **Anna Zelaya**[1,2], Bailey K[3], Zhang P[4], Preheim SP[5], VanNostrand J[4], Elias DA[3], Alm EJ[5], Zhou J[4], Adams PD[6], Arkin AP[6], Fields MW[1,2]

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Sponsored by: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Understanding the factors that determine microbial assembly, composition, and function in subsurface environments is critical to assessing contributions to biogeochemical processes such as carbon cycling and bioremediation. However, these factors are still not fully understood. In this study, surrogate sediment samples were incubated for 3 months in 3 wells (FW301, FW303, FW305) within the background site of the Oak Ridge Field Research Center in Oak Ridge, TN. Local sediment biofilm communities were compared to those of the groundwater (source diversity). Groundwater samples from each well were collected approximately 3 times a week. Multiple sediment samples (n=12) were used per well to determine inter- and intra-well variation. Spatial and temporal community analysis of local and source samples via ss-rRNA paired-end sequencing and distribution-based clustering revealed higher richness, diversity, and variability in source groundwater communities compared to sediment-associated communities. Ordination analysis grouped the newly formed local communities as more similar to each other than to groundwater communities of the same well, with the exception of FW305, a younger well. The predominant groundwater sequences share closest relation *Curvibacter* and *Aquabacterium* for FW-301, *Aquabacterium*, *Burkholderiales, and Gammaproteobacteria* for FW-303, and *Curvibacter*, *Acidovorax, Dechloromonas,* and for FW-305. Other sequences displayed transitory predominance for different wells. The community composition was different between wells, and the percentage of populations abundant at less than 5% ranged from 10–95% over time. FW-305 (a younger well) showed greater variability in relative abundances of OTUs over time. In sediment samples, 30–60% of the communities consisted of populations that were abundant at less than 5% of the total sampled diversity. The sediment communities from each well were also distinct from each other. Intra-well sediment samples showed much less variability, with the exception of FW305. Sediment biofilm communities were distinct from corresponding groundwater communities, with some populations becoming predominant in the biofilm (e.g. *Aquabacterium*, *Pseduomonas*, *Moraxellacaea*, and *Paraperlucidibaca*); however, different OTUs were respective to each well. These results indicate a shift in local community structure that is influenced by the available source community as well as hydrology.

CBE Poster #655

Title: **Chemotaxis toward hydrogen gas by *Methanococcus maripaludis***
Date: 07/2015
Authors: **Kristen A. Brileya**[1,2], Connolly JM[1,3], Gerlach R[1,3], Fields MW[1,2]
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Sponsored by: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231
NSF

Chemotaxis toward hydrogen gas (H_2) or "hydrogenotaxis" represents a potential strategy of motile microorganisms that compete for H_2 in subsurface environments. Although the ability for biological cells to sense and swim toward H_2 has been hypothesized for many years, this capacity was only recently demonstrated. H_2 is a crucial substrate for methanogens, and is a common source of energy for other archaea and bacteria in both anaerobic and aerobic environments. After a brief starvation, average swimming velocity of *Methanococcus maripaludis* increased toward a H_2 source, in a modified capillary assay with anoxic gas-phase control and time-lapse microscopy. This indicates that a methanogen couples motility to H_2 concentration sensing and is the first direct observation of hydrogenotaxis in any domain of life. The ability to move toward higher concentrations of H_2 could incur an advantage to methanogens that are otherwise outcompeted by those that are able to use H_2 at lower concentrations and/or utilize terminal electron acceptors that are more energetically favorable. The demonstrated chemotactic response would also allow cells to maintain desirable positions with respect to the major energy source as well as allow for proximity to H_2-producers in mixed communities. Thus, hydrogenotaxis could play a crucial role in the establishment and maintenance of microbial interactions at the population- and community-level. The observed hydrogenotaxis could represent a widespread eco-physiological strategy of methanogens and potentially other hydrogen-utilizing microbes that are important to subsurface processes such as bioremediation, and to overall carbon, sulfur, and nitrogen cycling.

CBE Poster #656

Title: **Urea reduction and the biochemical capacity for biomineralization by novel alkaliphilic, halotolerant, enrichment cultures from Soap Lake, Washington (USA)**
Date: 07/2015
Authors: **Arda Akyel**[1], Skorupa DJ[1], Schultz L[1], and Gerlach R[1,2]
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Sponsored by: U.S. Department of Energy, Grant No. DE-FG02-13ER86571

Microbially induced calcite precipitation (MICP) is recognized as a developing research area aimed at producing biocement, which might be useful in preventing undesired leakages in wells associated with the oil and gas industry. These leakages are well known to cause economic loss, as well as to pose significant threats to sensitive ecosystems. Biomineralization sealing technologies might be able to seal undesired fractures without the use of harsh chemicals and other harmful treatments. One challenge identified with developing this technology is the tolerance of biomineralizing microbes to the pressure, temperature, pH, and salinity in subsurface environments. In this research

aerobic and anaerobic ureolytic microbes were enriched from sediment slurries taken from Soap Lake, an alkaline (pH ~10.0) and saline (1-14% w/v) lake located in Washington State. Growth of both aerobic and anaerobic enrichments was tracked using cell densities at OD_{600nm} to determine maximum growth parameters. Aerobic enrichments were found to grow at NaCl concentrations ranging between 0–100 g/L with optimal growth observed at 50 g/L; aerobic enrichment pH values ranged from 6.0 to 11.0, with highest densities observed at pH 9. Aerobic cultures also tolerated variations in temperature, ranging from 22–40°C, with optimal growth observed at 30°C. Anaerobic enrichments displayed lower tolerance to increased salinity and pH. Here growth was observed across salt concentrations ranging between 0–100 g/L (optimal NaCl was 25 g/L), and pH values from 6.0 to 9.5 (highest OD values were noted at pH 7.0). Anaerobic temperature tolerance was similar to the aerobic enrichments; growth was observed from 22–40°C, with highest densities detected at 30°C. Following optimization of growth, the enrichments were inoculated into ureolytic media containing $CaCl_2*2H_2O$, to track rates of calcium carbonate ($CaCO_3$) precipitation. Using cation ion chromatography the disappearance of dissolved calcium was used to assess $CaCO_3$ precipitation levels. Results found that both aerobic and anaerobic enrichments completely precipitated the $CaCl_2*2H_2O$ after eight hours. Work is currently underway to assess urea hydrolysis kinetics, where comparisons between the Soap Lake enrichments will be made to that of the current MICP model organism, *Sporosarcina pasteurii*. Results from this work will help in determining the applicability the Soap Lake microbes to large scale seal fracturing applications.

CBE Poster #657

Title: **Microbially induced calcite precipitation in radial flow**

Date: 04/2015
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Sponsors: NSF, DOE

Bacterial ureolysis is a well-studied bacterial process that can induce carbonate precipitation. Ureolysis—the degradation of urea—increases alkalinity and in the presence of calcium, calcium carbonate can precipitate out of solution. The series of reactions that take place during the overall process of ureolysis-driven MICP is shown in equations 1-4:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_3 + HCO_3^- + H^+ \quad [1]$$
$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad [2]$$
$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad [3]$$
$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_{3(s)} \quad [4]$$

Microbially Induced Calcite Precipitation (MICP) can be employed in environments such as porous media to reduce leakage through lowering porosity and permeability. For effective use of MICP as a field technology, it is important to study it under conditions relevant to potential application environments. For example, radial flow through porous media is important to study since it is typical of flow encountered around wells. To study MICP under radial flow conditions, a radial flow reactor was built at MSU wherein glass beads were packed between two circular plates to form a 1 mm high packed porous bed (Figure 1).

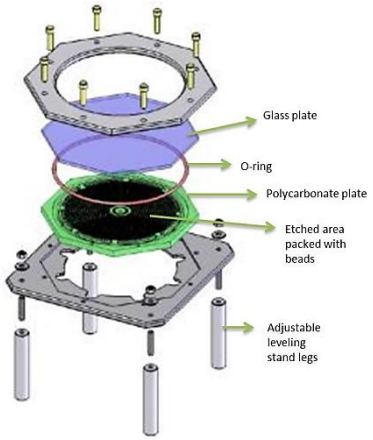

Figure 1. Radial Flow Reactor used to study MICP.

Bacterial growth of *Sporosarcina pasteurii* was promoted in the bed prior to injecting urea and calcium. After MICP was allowed to occur, the flow was not uniformly radial compared to the flow in a clean reactor as shown in Figure 2, suggesting that the calcium carbonate on and between the glass beads cements the beads together influencing flow path.

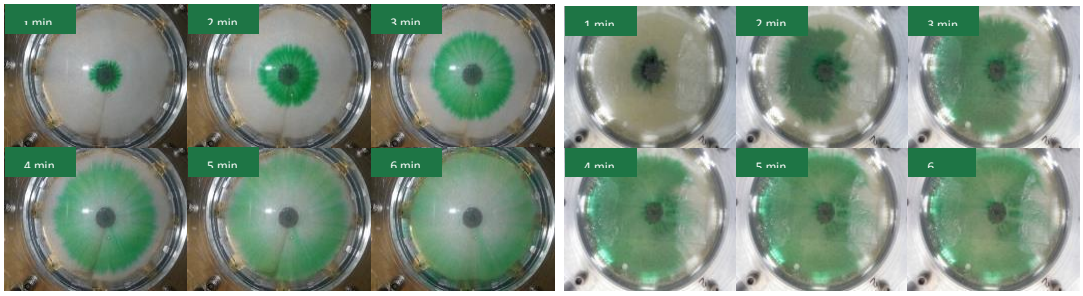

Figure 2. LEFT: Clean Reactor Flow. Fluid travels uniformly in all directions. RIGHT: Flow after MICP. Preferential flow path formation is seen due to precipitation. MICP on these beads was studied under varying medium flow rates as well as varying calcium concentrations. The amount of spatial precipitate formation increased with distance from the influent in all experiments. MICP efficiency in the reactor showed an inverse relationship to the flow rate and calcium concentration of the influent medium.

CBE Poster #658

Title: **Regulation of hibernation promoting factor (*hpf*) and ribosome modulation factor (*rmf*) of *Pseudomonas aeruginosa* includes transcriptional and post-transcriptional mechanisms**

Date: 05/2015

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Sponsored by: National Institutes of Health

Pseudomonas aeruginosa biofilms consist of cellular subpopulations with distinct physiological states, including metabolically active cells as well as dormant, non-replicating cells. During dormancy, cellular integrity must be maintained. Previously, we reported on the high abundance of transcripts encoding the ribosome-associated proteins, hibernation promoting factor (HPF) and ribosome modulation factor (RMF) throughout *P. aeruginosa* biofilms, including in the dormant subpopulation. In addition, a *P. aeruginosa Δhpf* mutant strain was compromised in its ability to resuscitate following nutrient starvation, suggesting that HPF is required for the maintenance of cellular integrity during dormancy. Since interactions of HPF and RMF with ribosomes reduce protein synthesis, expression of *hpf* and *rmf* is likely regulated tightly during the cell growth cycle. In this study, we characterized the regulation of *rmf* and *hpf* using single-copy fusions to the yellow fluorescent protein (YFP). The results indicate that both HPF and RMF negatively regulated their own expression. In addition, cellular levels of the secondary messenger molecule, guanosine tetraphosphate (ppGpp) influenced expression of *hpf* and *rmf* during stationary phase. Sequence analysis of *hpf* indicated that it may be cotranscribed with the upstream *rpoN* gene. A reporter construct that included the upstream *rpoN* promoter showed maximum expression of *hpf*. However, a reporter construct that excluded *rpoN* also showed *hpf* expression, suggesting that *hpf* expression is controlled transcriptionally by multiple promoters. Sequence analysis of 5' untranslated region (5'UTR) upstream of *rmf* revealed the presence of extensive RNA secondary structure, predicting six hairpin loops. The putative hairpin loops were modified either by deletion of individual loops or by base-pair changes that affected loop secondary structure. The modifications affected *rmf* expression

either positively or negatively, with a base pair change that disrupted the hairpin containing the ribosomal binding site significantly increasing *rmf* expression. The results indicate that *hpf* and *rmf* expression are regulated at the transcriptional level, but also post-transcriptionally through auto-feedback, RNA folding, and ppGpp levels.

CBE Poster #659

Title: **Shockwave disruption of biofilms**
Date: 07/2015
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Sponsored by: SANUWAVE Health, Inc., Alpharetta, GA, USA.

Pulsed Acoustic Cellular Expression (PACE) technology utilizes high-energy acoustic pressure waves in the shock wave spectrum to produce compressive and tensile stresses on cells and tissue structures, with the ultimate goal to regenerate healthy tissue. This technology was evaluated in vitro for effects on difficult to remove biofilms created by both a gram-negative bacterial species (*Pseudomonas aeruginosa*) and a gram-positive species (*Staphylococcus aureus*). Biofilms were grown on polycarbonate coupons in a CDC Biofilm Reactor using methods similar to ASTM E2562-12. Following two days of biofilm growth, coupons were placed in a testing fixture and treated with focused shockwaves generated by an e44 applicator, which propagated shockwaves through a liquid until they hit the targeted coupon placed in the applicator's focal volume. In this way, the treatment was performed without any contact between applicator and coupon. The coupons were treated with 500 to 8,000 pulses, at a frequency of 4 Hz and at the highest energy setting (E6) for the PACE control console (SANUWAVE Health, Inc.). The amount of remaining biofilm on the coupons was then assessed using plate counts and confocal scanning laser microscopy (CSLM). Treated coupons were compared to sham-treated controls that were placed in the testing fixture for the same time duration as a normal shockwave treatment, but not exposed to shockwaves. For 500 pulses a two- (2) log reduction and for 8,000 pulses greater than three- (3) log reduction in colony forming units per square centimeter were achieved relative to the control coupons for both test species. CSLM indicated that the shockwave treatments removed biofilm from the surfaces exposed to shockwave treatment. Overall, these results indicated that this is a promising technology for removing biofilms from surfaces using only mechanical stresses produced by the shockwaves on biofilms, without the direct contact or the need of any chemicals.

CBE Poster #660

Title: **Chemotaxis of Antarctic and Arctic microorganisms toward various carbon sources using a capillary motility method**
Date: 04/2015
Authors: **Shu Ying Wee**[1,2], Smith H[1,3], Chang CB[1,2], Foreman C[1,2]
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Sponsored by: MSU Undergraduate Scholars Program; NIH-Montana (INBRE) IDeA Network of Biomedical Research; NSF Office of Polar Programs, NASA Planetary Science

The global carbon cycle is significantly dependent on microorganisms that process and cycle carbon. The transformation of organic matter (OM) by the microbial communities in the environment is integral to this relationship. Heterotrophic bacteria obtain carbon for growth from organic

compounds, and as a result of utilization, these organisms can make carbon available to other living organisms. This project aims to investigate the chemotactic activity of motile heterotrophic bacteria from the Arctic and Antarctic, toward a suite of environmentally isolated carbon sources. A *Pseudomonas sp.* isolated from Antarctica has successfully been labeled with a green fluorescent protein (GFP) through electroporation. The GFP label was introduced to aid in the visualization of bacterial movement toward each chemoattractant, as the GFP label provides better visibility for quantification. By identifying which carbon sources specific organisms are attracted to, we are able to gain insight about the in situ organization of psychrotolerant microbial communities. Knowledge gained by this study can be applied to strengthen our understanding of the metabolism of different OM source materials, and aid in the prediction of carbon fate for a changing environment. We have modified a chemotaxis method from Adler et al. (1973), using a microfluidics based approach. A triple-reservoir PDMS microfluidic capillary device was designed following Ahmed et al. (2008). The chamber consists of a reservoir of cells that are exposed to two other reservoirs containing either the chemotaxis media (control) or the chemoattractant of choice. The organisms are incubated and imaged for 10–20 minutes using a confocal microscope to determine the rate of movement as well as the number of bacteria surrounding the carbon source. The amount of cells that migrated to the reservoir with chemoattractants is compared to that in the chemotaxis media in order to obtain the quantity of cells attracted to a certain attractant and to compare the strength of the attraction. The selected carbon sources represents the different types of carbon sources that the isolates would typically be exposed to in their natural environment. Preliminary results have shown positive chemotaxis of motile heterotrophic bacteria toward organic carbon sources isolated from different environments; and that different carbon sources act with varying strengths as chemoattractants for different microorganisms.

CBE Poster #661

Title: **Electron donor limitation promotes metal corrosion by *Desulfovibrio alaskensis* G20 biofilm**

Date: 08/2014

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Sponsored by: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Microbially induced corrosion (MIC) is a major concern for industrial ferrous metal pipelines and can result in pipeline failure. Sulfate-reducing bacteria (SRB) have been implicated in contributing to MIC due to their production of corrosive H_2S gas and elemental sulfur along with metal-microbe interactions. This project focuses on the effects of electron donor limitation and electron acceptor limitation on biofilm physiology and corrosion rate on carbon steel versus stainless steel and glass surface materials. *Desulfovibrio alaskensis* G20 was grown under steady-state conditions in sulfate-reducing biofilm reactors. Batch cultures grown under EAL and EDL conditions had similar maximum growth rates, but differed significantly in final cell yields at 37°C. Under EAL conditions, biofilms on glass had elevated biomass levels, and higher protein levels were detected on 316 steel compared to 1018 steel. At later time points, the 1018 steel had an elevated carbohydrate to protein ratio. Under EDL conditions, biofilms on glass had the highest protein levels; 316 and 1018 steel had similar biofilm protein levels. Hexose-equivalents were similar for the three tested surfaces under EDL conditions and slightly elevated for 1018 steel compared to glass and 316 steel. In addition, under EDL conditions, 1018 steel also had an elevated carbohydrate to protein ratio. Differential

corrosion rates were observed between electron donor limiting (EDL) and electron acceptor limiting (EAL) conditions on 1018 carbon steel and 316 stainless steel. The results indicated that different ratios of respiration substrates contributed to altered rates of corrosion, and the difference in corrosion rates could not be explained solely by sulfide, acetate, or carbohydrate levels. The presented results are the first report of increased mass loss under EDL conditions using a defined medium under steady-state conditions.

CBE Poster #662

Title: **Investigation of the relationship between biofilm and mineral formation in a clinically relevant model flow system of the kidney**

Date: 07/2015
Authors: **Trace Hobbs**[1,2], Schultz L[1], Lauchnor E[1,3], Gerlach R[1,4]
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Sponsored by: National Science Foundation, Howard Hughes Medical Institute

Kidney stones form when ions in urine become supersaturated, resulting in mineral precipitation and aggregation. Struvite ($MgNH_4PO_4 \cdot 6H_2O$) precipitation can be induced by bacteria associated with urinary tract infections, which often consist of *Proteus mirabilis* biofilms. *P. mirabilis* is a ureolytic bacterium; it produces urease, an enzyme that catalyzes the hydrolysis of urea ($CO(NH_2)_2$), producing ammonium (NH_4^+) and increasing the pH of the urine. As the pH rises and ammonium concentrations increase, struvite precipitation can occur in the presence of magnesium (Mg_2^+) and phosphate (PO_4^{3-}).

A model flow system has been developed to simulate biofilm formation in the kidney, ureters, and bladder. The system is being used to investigate the process of microbially induced struvite formation. The flow system is filled with artificial urine and inoculated with ureolytic bacteria to simulate an infected kidney. Liquid and mineral samples have been analyzed to demonstrate that biofilm growth resulted in struvite formation based on mineral analyses and stoichiometric changes of the dissolved ions in the bulk fluid.

The model flow system can be used to investigate initial formation of biofilm and minerals, the relationship between biofilm growth and mineral formation, and migration of bacteria from the bladder to the kidney, among other possibilities.

CBE Poster #663

Title: **Exploring novel subsurface fracture sealing mechanisms through ureolytic biomineralization in Ludox® gels**
Date: 06/2015
Authors: **Abby A. Thane**[1], Phillips AJ[1], Schultz L[1]
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Sponsored by: U.S. Department of Energy

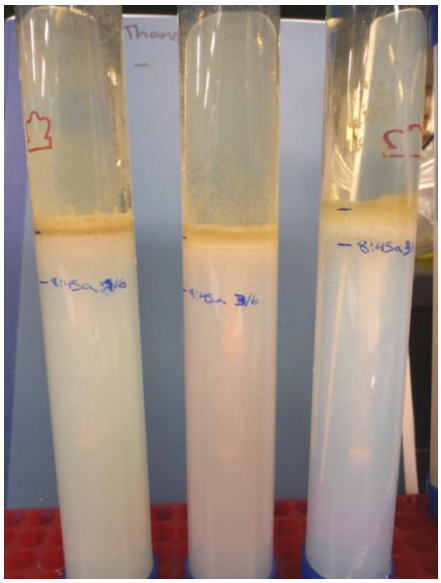

Figure 1. Experimental samples with a visible "crust" at the interface between the gel and the liquid.

Fractures in the subsurface can cause unwanted leakage of natural gas during extraction or of CO_2 during sequestration. There is a need for environmentally friendly ways to seal fractures in situ, and ureolytic biomineralization is one proposed method that produces biocement with $CaCO_3$. In this study, we examined a novel delivery system by combining Ludox® colloidal silica gel with the ureolytic microbe *Sporosarcina pasteurii*. We prepared columns by pouring a layer of liquid calcium media over a layer of Ludox® gel. As the media diffused through the gel, we observed changes in cell activity and population with time, as well as the distribution of $CaCO_3$ precipitation. We also compared the precipitation efficiency of *S. pasteurii* with dissolved urease from jack beans. In all experimental samples, a dense layer of $CaCO_3$ "crust" was formed at the interface of the gel and the liquid (Figure 1). The successful formation of $CaCO_3$ within the gel suggests the possibility of applying the biomineralization process to larger fractures using microbes or enzymes in colloidal silica gel.

CBE Poster #664

Title: **Sustainable coal bed methane (CBM) and biofuel production from algae grown in CBM produced water**
Date: 02/2015
Authors: **Katherine J. Davis**[1,5], Hodgskiss L[2,5], Schweitzer HD[3,5], Corredor L[3,5], Hiebert R[4], Barnhart E[5], Cunningham AB[2,5], Fields MW[3,5]
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Sponsored by: Montana Board of Research Commercialization and Technology (MBRCT); U.S. Department of Energy (DOE); National Science Foundation (NSF)

The Powder River Basin (PRB), located in southeast Montana and northeast Wyoming, is the largest coal mining region in the United States, but the majority of the coal in this region is too deep to be conventionally mined. Within the deep coal beds, microbial ecosystems produce methane that can be used for electrical generation, heat, and transportation fuel; compared to other hydrocarbon fuels, methane produces less carbon dioxide per unit of heat released.

The goal of this project is to conduct research on enhancement of coal bed methane (CBM) production using a mixture of algal extracts and other nutrients. The algae, which can be grown on-

site in CBM production water ponds, can be harvested and converted into nutrients capable of stimulating and sustaining in situ coal bed methane production. If the algae are grown in sufficient quantities in CBM produced water storage facilities, it may be possible to convert much of the biomass directly into biofuels (and other algae-related products) in addition to production of the extracts needed to stimulate CBM production. Large-scale algal production in CBM ponds will also substantially increase CO_2 uptake from the atmosphere, thereby decreasing the carbon footprint of conventional CBM operations. Additionally, re-injection of amended CBM water reduces the quantity of this non-potable water, which needs to be treated and/or discharged.

CBE Poster #665

Title: **Renewable biogas production from algal biomass using anaerobic digestate cultures acquired from municipal wastewater**

Date: 04/2015
Authors: **Ashley Berninghaus**[1,2], Davis K[1,2], Peyton B[1,2],Gerlach R[1,2]
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Sponsored by: Undergraduate Scholars Program; U.S. Department of Energy Efficiency and Renewable Energy (EERE) Biomass Program under Contract No. DE=EE0005993

The global population is growing rapidly, increasing the demand for energy, especially for fossil fuel production. This has created an increased interest in alternative sources of energy that show potential to be carbon neutral as well as economically comparable to current fossil fuel sources. Biogas is a mixture of gases produced by the breakdown of organic matter through an anaerobic process. Growth of algae for biodiesel production is an advancing technology and conversion of waste algal biomass using anaerobic digestion is an attractive process to treat waste, control pollution, and produce energy. Anaerobic digestate acquired from the Bozeman Water Reclamation Facility was used to anaerobically digest lyophilized algal biomass to produce biogas through various modes of methanogenesis. Lyophilized biomass of a cyanobacteria, as well as low-lipid and high-lipid *Chlorella* species, were used as carbon substrates and their rates of methane production compared. Methane production was determined using a gas chromatograph. The high lipid algae allowed for larger amounts of methane to be produced, while the low lipid *Chlorella* species produced the lowest amount. Calorimetry has been used to determine the energy contents of each biomass type and these values will be compared to the energy created via methane production. These results show potential for the use of microalgae biomass as an alternative substrate for methane production, a promising technology which may help treat waste while producing energy.

CBE Poster #666

Title: **A comparison of microbiologically and abiotically induced calcium carbonate precipitation**
Date: 06/2015
Authors: **Ana Paula Braga Coelho**[1,2], Phillips A[1], Kirkland C[1]
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[2]CAPES Foundation, Ministry of Education of Brazil, Brasilia – DF.
Sponsored by: U.S. DOE; NSF; Brazil Scientific Mobility Program

The beneficial use of biofilm-forming microorganisms has increased significantly in recent years. Different applications such as industrial byproduct building materials, remediation of cracks in concrete, biodeposition in soil and sand materials, carbon dioxide sequestration, and removal of heavy metals, among others, have gained emphasis in the scientific community. One of the reasons for this recent emphasis—especially on the biodeposition area—is due to the carbonate production and subsequent precipitation in the presence of calcium. One example is the production of calcium carbonate via ureolysis, a process where the activity of the urease enzyme (present inside some microorganisms) results in proper chemical conditions that induce calcium carbonate precipitation in biofilm systems. However, it is possible to extract and isolate the urease enzyme and use it to realize the same process of calcium carbonate precipitation made by biofilm-forming microorganisms, following an abiotic path instead. To compare the abiotic and microbiologic paths, an experiment was performed in the laboratory using the isolated urease enzyme and the bacteria *Sporosarcina pasteurii* and *Bacillus globisporus*. The studies were taken to analyze the efficiency of calcium carbonate precipitation resulted from ureolysis by comparing the two paths in the process of mineralization in highly porous sand filled columns. The results are going to be used to develop experimental protocols for future testing in a meso-scale radial flow bioreactor designed to model the near wellbore environment.

CBE Poster #667

Title: **Sonication ruggedness of efficacy tests in the Single Tube Method (STM) (ASTM E2871)**
Date: 07/2015
Authors: **Jennifer Summers**[1], Walker DK[1], Parker AE[1], Goeres DM[1]
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An inter-laboratory study of ASTM Method E2871, titled "Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm Grown in the CDC Biofilm Reactor using Single Tube Method," revealed that the repeatability and reproducibility for this method were at the upper end of acceptability. After consulting with researchers who participated in the study, variations in sonicator settings were hypothesized to be a possible explanation for the variability in the data. A ruggedness test was conducted to determine if these deviations from the method would have a significant effect on the treated and control biofilm log densities. A *Pseudomonas aeruginosa* (ATCC 15442) biofilm was grown following ASTM Method E2562, and treated and sampled according to the STM, which calls for a vortex (30s, high)/sonicate (45 kHz, 30s, 10% power) series to remove and disaggregate the biofilm. The ruggedness test was conducted over 24 experiments, with each experiment consisting of 3 treated coupons and 2 control coupons, and each coupon sampled according to the STM with an altered removal and disaggregation series. The coupons were subjected to different combinations of sonication settings, including time (30s/60s), power (10%/100%), placement (suspended in the water bath/ directly on the bottom of the bath), and degas (Y/N). The data was analyzed by a linear regression analysis of each of the log reductions and the control log densities. Although this analysis found that there is not a statistically significant difference in the log reductions ($p \geq 0.554$) and control log densities ($p \geq 0.191$) that resulted from

varying the sonicator settings, there is a practical effect when degas and placement are varied, resulting in a difference of as much as half of a log. These results show that the STM is rugged for a range of sonication parameters but also suggest there is another source of variability within the method.

CBE Poster #668

Title: **Promoting lipid accumulation in *Chlorella vulgaris* UTEX395 using nitrogen limitation and bicarbonate amendment**
Date: 06/2015
Authors: **Matthew Jackson**[1,2], Pedersen T[1,2], Berninghaus A[1,2], Gardner R[3], Gerlach R[1,2]
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Sponsored by: U.S. Department of Energy

Both the sufficient supply of water and nutrients (N and P) have been identified as potentially limiting factors in the large scale production of algal biofuels. Thus, the use of low quality water and nutrient sources for algal biofuel production is of interest. In order to promote lipid accumulation using the nitrogen depletion and bicarbonate amendment strategies outlined by us and others, one needs to understand the effect of the different nitrogen species present in various waste streams, such as municipal and agricultural wastewaters. Enhanced lipid accumulation resulting from nitrogen stress and bicarbonate amendment has been demonstrated for various algal species including *Chlorella vulgaris*. We will present results comparing the growth and lipid accumulation in *C. vulgaris* UTEX 395 in the presence of nitrate and urea. The effect of adding bicarbonate to the cultures at the point of nitrogen depletion will be demonstrated. Understanding the role of different nitrogen species on the growth and lipid accumulation of microalgae may aid in optimizing the utilization of existing low quality water and nutrient streams for industrial algae cultivation and may provide insight into challenges and opportunities for water and nutrient recycling in industrial algal production facilities.

CBE Poster #669

Title: **Determination of oxygen distribution in a *Staphylococcus aureus* biofilm using ^{19}F magnetic resonance oximetry**
Date: 07/2015
Authors: **Jeffrey W. Simkins**[1,2], Seymour JD[1,2], Stewart PS[1]
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Sponsored by: National Institutes of Health

The biofilm-forming bacterium *Staphyloccus aureus*, normally a benign constituent of the human nares microbiome, can in certain situations become pathogenic and colonize wounds, leading to chronic infections[1]. Because biofilms exhibit increased resilience against host defenses and antimicrobials relative to their planktonic counterparts, clinical treatment is often difficult or intractable, resulting in costly and often unsuccessful drug regimes, compromised quality of life, and in some cases, death. One of the mechanisms by which *S. aureus* biofilms evade extermination by the immune system of the host is through manipulation of local oxygen levels. Cells near the biofilm

surface rapidly consume oxygen, resulting in an oxygen sink that immobilizes phagocytic host cells and hinders the ability of neutrophils to initiate oxidative burst[2]. Simultaneously, bacterial cells in the biofilm's anoxic zone transition to a state of decreased activity and altered metabolism, conferring wide-spectrum resistance to antibiotics[3,4]. For this reason, chronic wound oxygenation has been shown to be a significant predictor of clinical outcome, and some treatments such as hyperbaric oxygen therapy (HBOT) even manipulate local oxygen levels in the hopes of improving prognosis[5]. Thus, there is significant clinical interest in characterizing oxygen distribution within an *S. aureus* biofilm. ^{19}F magnetic resonance has become popular in the medical field as a method for quantifying oxygenation in blood, tissues, and tumors[6]. The technique exploits the linear dependence of the ^{19}F spin-lattice relaxation rate R_1 on local oxygen concentration. In the current study, we describe progress made in repurposing this oximetry method toward oxygen quantification within an *S. aureus* biofilm, and discuss future objectives and challenges to this end.

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CBE Poster #670

Title: **Monitoring community ecology in wastewater treatment lagoons for the production of algal biodiesel**

Date: 06/2015
Authors: **Tisza Bell**[1], Doig L[1], Gerlach R[1], Peyton BM[1], Fields MW[1]
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Sponsored by: National Science Foundation and the Department of Energy

Large-scale production of algae for biofuel may help with current energy problems. Most research has focused isolates in costly closed systems. In contrast, open ponds are estimated to be significantly less expensive to run. However, cultivation in open systems presents several challenges one of which is susceptibility to colonization by other microbes. Currently, realistic methods do not exist to simultaneously control positive and negative microbial interactions in large, open systems. In an effort to understand possible interactions during algal cultivation for lipid production in large, open systems, we monitored the microbial community, geochemistry, and FAME content of 5 wastewater treatment lagoons over a year. DNA was sequenced from all three domains. Correlations were observed between community members, chemical variables, and FAME concentrations. In contrast to previous findings, we found high levels of FAME (up to 48% w/w) in some lagoons. Concentrations were significantly correlated to ammonia, nitrite, TKN, pH, phosphate, and phosphorus (R^2= 0.87). Bacterial production of indole-3-acetic acid, a known plant hormone, was also detected. Our findings suggest the feasibility of algal biofuel production using wastewater lagoons and shows both positive and negative interactions within the diverse microbial community. The resulting data will provide important insight into control and optimization for biomass and/or lipid accumulation in an open pond system. (Figure 1, below.)

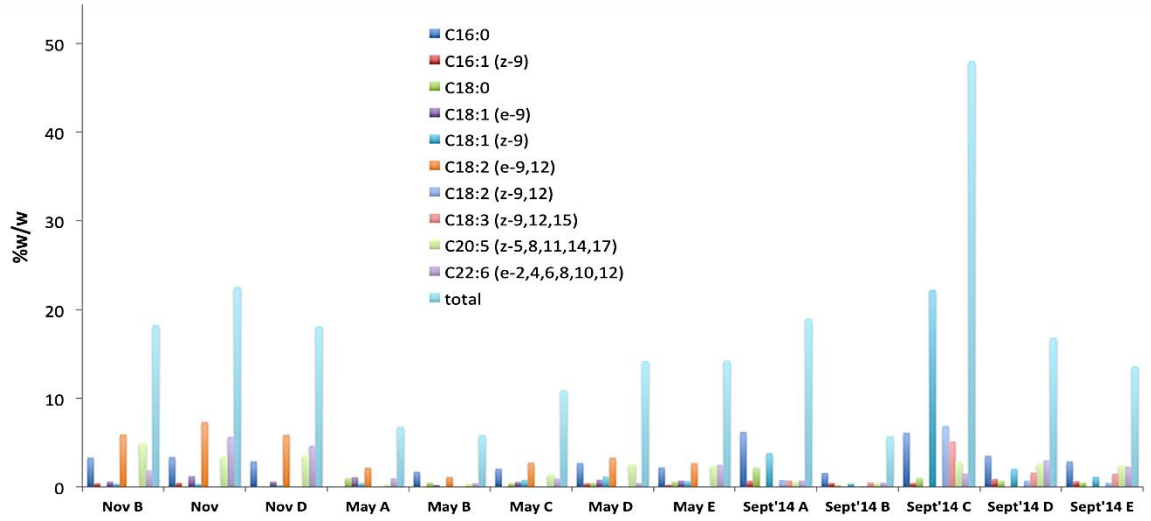

Figure 1. Dominant FAME compounds, identified via GC-MS, that were transesterified from lagoon biomass in Nov. '13, May '14, and Sept. '14. The highest total FAME was observed in lagoon C Sept '14 at 46%w/w. This value is noteworthy because under highly controlled conditions, often using nutrient starvation to trigger lipid accumulation, FAME values are in the mid 50%w/w. Gaining an understanding of the interactions behind the increased FAME in this open uncontrolled system utilizing wastewater has industrial relevance for algal biodiesel production.

CBE Poster #671

Title: **Stoichiometric analysis of primary autotrophy and biomass turnover in a thermoacidophilic iron-oxidizing archaeal community**

Date: 06/2015
Authors: **Kristopher A. Hunt**[1,2], Jennings R deM[3,4], Inskeep WP[1,3,4], Carlson RP[1,2,3]
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Sponsored by: National Science Foundation

Microbial communities are responsible for the majority of global nutrient cycling, making them prime targets for controlling greenhouse gas production and eutrophication. However, the complexity of most naturally occurring microbial communities limits their tractability due to the large number of species and interactions. Extreme temperature and pH environments, like those found in Yellowstone National Park geothermal springs, typically reduce community species diversity; these relatively simple communities represent ideal model systems for studying primary and secondary nutrient fluxes through multiple trophic levels. An aerobic, thermoacidiphilic archaeal biofilm community, which grows at 60–70°C and pH 2–4, was modeled using metagenomics data, direct in situ measurements, and novel stoichiometric modeling approaches. The most abundant autotroph in the system, *Metallosphaera yellowstonensis* MK1, was modeled as an obligate aerobe that oxidizes iron(II) and various reduced sulfur species while respiring on limiting oxygen; MK1 primary productivity was modeled to constrain the potential community compositions and fluxes. The most abundant heterotroph in this system, Geoarchaeota archaeon OSPB-1, modeled recycling of nutrients acquired by MKI via primary producer biomass degradation.

This study represents the first stoichiometric analysis of nutrient/biomass recycling in a natural microbial community. Characterization of this geothermal system illustrates constraints of electron donors and acceptors on community energetics and nutrient recycling.

CBE Poster #672

Date: June/2015
Title: **Biofilm market survey**
Authors: **Sean Downey**[1], Gummer S[1], Kerins F[1], Shehryar O[1], Nile J[1], Shubert J[1] Sturman P[2]
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Sponsored by: MSU Research Expansion Fund

Microbial biofilms are the cause of a wide variety of industrial and medically-relevant problems. These include corrosion, product loss and increased downtime on the industrial side and increased costs as well as negative patient outcomes (including mortality) in the healthcare environment. Because of this, the potential markets for products intended to eliminate or mitigate the effects of biofilms has expanded dramatically over the past decade. While there are many sources of data available online to assess the value of these markets, the information has heretofore been unavailable from a single source. This collaborative project with MSU's College of Business seeks to assess the market value of many biofilm-related and anti-biofilm products in order to provide industry and researchers with easy access to market-related information. Research methods for this survey include review of online governmental, private, and published data as well as selected interviews with knowledgeable industry sources. To date, the markets for anti-biofilm agents in the following product areas have been evaluated:

- Cosmetic implants
- Fresh produce
- Hernia repair mesh
- Hospital-acquired infections
- Oil and gas production and transmission
- Oral care products
- Orthopedic implants
- Pool and spa antimicrobials
- Pulp and paper
- Urinary atheters
- Woundcare (including chronic wounds)

The final results of this ongoing effort will be published on the CBE website. We currently envision periodic (annual or semi-annual) updates to the market study, and the continued collaboration between the CBE and MSU's College of Business.