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Center for Biofilm Engineering

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proceedings

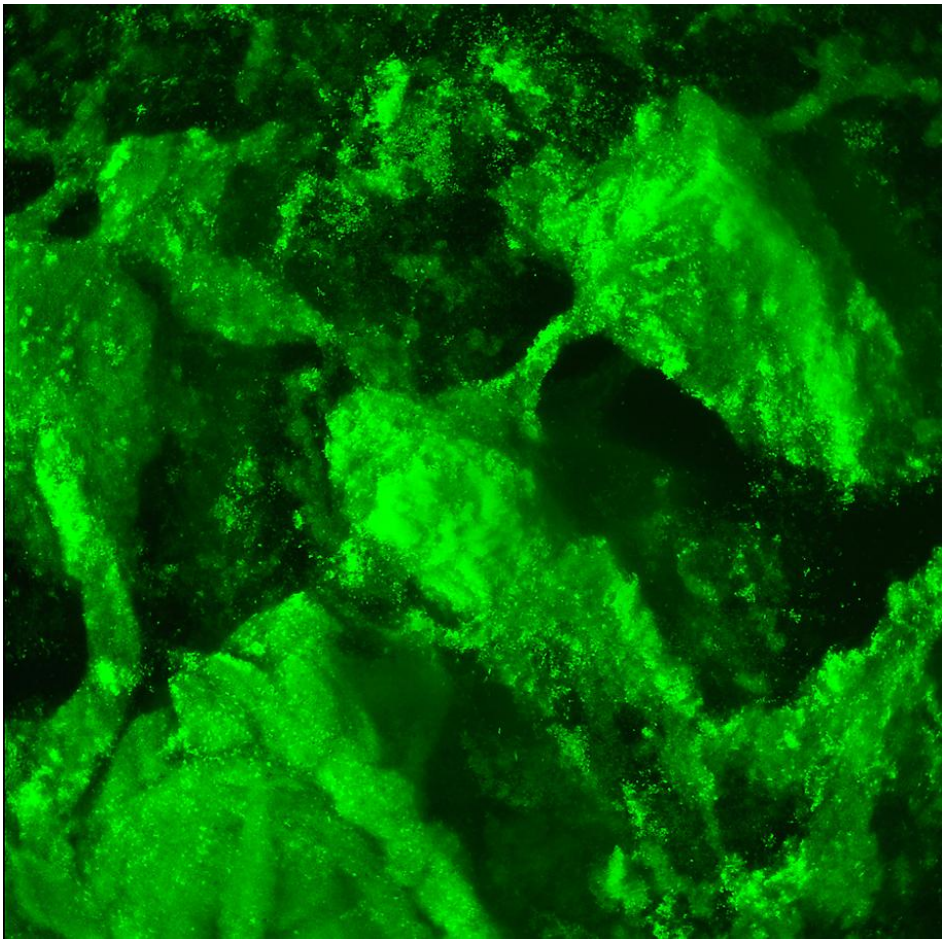

Image of *Staphylococcus aureus* biofilm CBE's newly developed Treatment Flow Cell, by Betsey Pitts and Lindsey Lorenz.

Montana Biofilm Science & Technology Meeting: February 5–6, 2013
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Speaker Abstracts

SESSION 1: Skin Biofilms

Biofilm in comedonal and inflammatory Acne vulgaris: In vivo identification and characterization

Presenter: Manisha J. Patel[1], MD, Assistant Professor
Co-authors: Agostinho A[2], James G[2], Rosenthal I[1], Chang N[1], Leung S[1], Chien A[1], Kang S[1]
Affiliation: [1]Department of Dermatology, Johns Hopkins School of Medicine, Baltimore, MD, USA
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Background: Acne vulgaris is a common multifactorial disorder of the pilosebaceous follicles, involving follicular hyperkeratinization, hormone imbalance, bacterial infection, and immune hypersensitivity. It is the most common skin disease, estimated to affect approximately 40–50 million people in the United States. The role of *Propionibacterium acnes (P. acnes)* in pathogenesis is controversial; however, it is suggested to play a role in inflammation. It is known to colonize the upper pilosebaceous unit in all acne-prone individuals. Yet the development of acne lesions is not universal in this group. Even in a patient with acne, why a lesion develops in one location and not in other areas remains unknown. In addition, why treatments work in some patients and not others, or stop working over time, continues to elude us. It is now well established that bacterial biofilms play an important role in the pathogenesis of many human infections. Most advantageous to a microbial biofilm population, particularly in human disease, is the fact that the biofilm mode of growth enables resistance to a number of removal strategies—namely antibiotics.

Objective: This case-control study aimed to identify and characterize biofilm in skin biopsies from patients with comedonal and inflammatory acne compared to non-lesional skin in the same population.

Methods: Confocal laser scanning microscopy was used to identify and characterize biofilms in the pilosebaceous units from recruited subjects. 14 patients with one comedone, one inflammatory papule, and adjacent uninvolved normal skin were identified, and biopsy specimens were obtained after digital photographs were taken.

Results: A total of 42 tissue specimens were obtained from 14 research participants (14 normal skin, 14 comedones, and 14 inflammatory acne papules). Biofilm, as defined by large micro-colonies of approximately 100 cells or more, was demonstrated in 9 (21%) of all specimens (1 normal skin specimen, 5 comedones, and 3 inflammatory papules). We were not able to identify bacteria or biofilm in 6 (43%) of our subjects (18 specimens).

Conclusions: We have identified biofilm in vivo from lesional skin in patients with acne vulgaris. Our study supports that biofilms are encountered more often in comedonal and inflammatory papules than in normal skin controls. Further study of biofilm in acne may prove central to our understanding the pathogenesis of acne vulgaris and the development of improved therapeutics.

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Imaging biofilms in tissue

Presenter: Garth James, CBE Medical Projects Manager; Associate Research Professor, Department of Chemical & Biological Engineering, MSU-CBE
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Biofilm infections of soft tissue, including skin and lungs, have been increasingly recognized. Although not routinely used for diagnosis, microscopic imaging of tissue can provide direct evidence of biofilm involvement and can enable important insights relative to biofilm architecture and community structure.

Cutaneous wounds provide readily available specimens due to accessibility and the fact that removal of tissue from the wound by debridement is commonly practiced in wound care. Following specimen collection, proper fixation of the samples is important for subsequent microscopic analysis. Embedding and sectioning the specimen enables high-resolution imaging through the depth of the wound sample. A variety of stains for biofilm components, such as protein, polysaccharides, and DNA, are available. However, distinguishing host components from biofilm components can be a challenge. For example, while lectins can be used to stain biofilm extracellular polysaccharides, they also stain polysaccharides associated with the host extracellular matrix. Biofilm community structure can be elucidated to some extent using traditional approaches such as Gram staining and immunofluorescent labeling. Fluorescent in situ hybridization (FISH) techniques have been increasingly used to study biofilms. New techniques, such as combinatory labeling and spectral imaging (CLASI-FISH) and douple labeling of oligonucleotide probes (DOPE-FISH), have expanded the utility of FISH for the analysis of increasingly complex microbial communities. However, the use of FISH techniques for the analysis of biofilms in tissue still presents considerable challenges. Nonetheless, it is important to continue adapting these and other new techniques for elucidating biofilm structure and investigating the role of biofilms in health and disease.

Metagenomic study of the human skin microbiome associated with acne

Presenter: Huiying Li, Assistant Professor
Co-Authors: Fitz-Gibbon S, Tomida S, Chiu B, Nguyen L, Du C, Liu M, Elashoff D, Erfe MC, Loncaric A, Kim J, Modlin RL, Miller JF, Sodergren E, Craft N, Weinstock GM
Affiliation: Molecular & Medical Pharmacology, University of California, Los Angeles, CA, USA

The human skin microbiome plays important roles in skin health and disease. However, bacterial population structure and diversity at the strain level is poorly understood. *Propionibacterium acnes* is a dominant skin commensal, but is also linked to acne vulgaris, one of the most common skin diseases. We compared the skin microbiome, at the strain level and genome level, between acne patients and healthy individuals. Metagenomic analysis demonstrated that while the relative abundances of *P. acnes* were similar, the population structure at the strain level was significantly different in the two cohorts. Certain strains were highly associated with acne and other strains were enriched in healthy skin. By sequencing and comparing a large number of *P. acnes* genomes, we identified potential genetic determinants of various *P. acnes* strains in association with acne or health. Our study underscores the importance of strain level analysis of the human microbiome to define the role of commensals in health and disease.

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SESSION 2: Industrial Biofilms

Manganese sulfide inclusions and pit initiation in carbon steel during microbially influenced corrosion: Pits initiate in the immediate surroundings of the inclusions

Presenter: Recep Avci, Director, ICAL, and Research Professor
Affiliation: Imaging & Chemical Analysis Laboratory (ICAL), and Department of Physics, Montana State University, Bozeman, MT, USA

The propagation of pitting is relatively well understood and is not too sensitive to metallurgical properties; however, pit initiation and pit stability are still challenging problems and are far more dependent on the metallurgical makeup of the material and the environment. It was demonstrated that in a saline environment under anaerobic conditions with dissolved H_2S present, pitting attacks on carbon steel are initiated in the boundary regions of MnS inclusions within a narrow interface less than 50 nm in width. Studies were conducted on finely polished 1018 carbon steel surfaces cut perpendicular to the rolling direction. The tips of MnS inclusions ~1 μm in size were visible on these surfaces. The inclusions extended into the iron matrix, perpendicular to the surface, as thin micro-wires with length-to-width ratios

exceeding 100. At normal pH the slow dissolution rates of MnS inclusions relative to the adjacent matrix result in initial pit morphologies with MnS micro-bars protruding from the centers of pits, while the dissolution of the surrounding iron gives rise to deeper and wider pits. These effects are more noticeable in the presence of biofilms of sulfate-reducing organisms, though pit initiation and growth also take place abiotically. It is hypothesized that the anodic character of the iron in the MnS boundary regions is caused by the disorder and residual strain exerted on the Fe matrix by MnS contamination of the interface due to metallurgical processes.

Characterization of *Desulfovibrio alaskansis* G20 physiology and biofilm metabolism on glass and steel surfaces

Presenter: Greg Krantz, PhD student, Microbiology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

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. *Desulfovibrio alaskansis* G20 (G20) is an SRB isolated from a producing oil well in Ventura, California. This project focuses on the effects of electron donor limitation and electron acceptor limitation on electron flow through metabolic pathways in G20, as well as the role of glass and steel surface materials in biofilm physiology.

Development and implementation of a new treatment for biofilm remediation in industrial systems

Presenter: Adrian Denvir, Manager, Water Treatment Science and Technology
Affiliation: NCH Corporation

It is estimated that there are between 500,000 and 600,000 large- to mid-range cooling towers currently in operation throughout the United States and they are used in a wide range of settings, from industrial manufacturing, to energy production, and heating and cooling systems for buildings and homes. The smaller mid-range systems are designed to handle tens of gallons of water per minute, while the larger systems utilize hundreds of thousands of gallons of water per minute; however, despite the differences in size and application, cooling towers have one thing in common: the need for chemical treatment regimes to control scale build-up and biofilm formation on the critical heat exchangers, pipes, and fill that are necessary for energy conservation and cost efficient operation.

This presentation outlines the development of a new product designed to remediate biofilm in industrial systems. We address the biological testing from bench scale work through field demonstrations, and we identify key reactions and processes that define the operational boundaries for the new treatment process.

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Systems-based analysis of industrially relevant microbes

Presenter: Ross Carlson, Associate Professor, Chemical & Biological Engineering
Abbie Richards, Assistant Professor, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

A physiological and systems biology study of problematic microbes isolated from an industrial process was performed. The physiological study examined a matrix of medium salt and pH values to identify ideal culturing conditions. This was important for i) company testing procedures to best identify conditions needed to screen product for contaminant, and ii) identifying potential product modifications that could reduce bacterial growth. Information from genome sequencing of microbes was used to build an in silico representation of the central metabolism and was used to build RNA microarrays for transcriptomic studies. Using a combination of the in silico models and transcript data, it is hypothesized that the microbes are nitrogen limited in company product, providing a target for further product modifications to control microbial growth.

SESSION 3: Environmental/Mineral Biofilms

Microbial ecology of mine waste environments

Presenter: Lisa Bithell Kirk, Research Scientist
Co-Authors: Brent Peyton, Mark Kozubal
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

The structure and function of microbial communities in mined environments—and industrial systems in general—are very poorly described. The variable acidity, salinity, and metalliferous habitats of mined wastes worldwide indicate that iron, sulfur, and nitrogen cycling are dominant processes. Cycling of these elements, in turn, controls redox conditions, which then significantly affect metal mobility. While environmental managers may be most interested in remediating a particular trace metal or nutrient in mine waste through manipulation of the microbial ecology, design must consider the more complex ecology of the overall system to be effective.

At sites currently under study at the CBE, iron oxidation and reduction, sulfur oxidation, denitrification, and hydrocarbon degradation are dominant biogeochemical processes in mine waste where selenium reduction is a desired means of in situ stabilization and water quality protection. Using molecular and cultivation dependent methods, together with in situ monitoring, the relative influence of these processes on solute release and options for remedial design can be examined. This presentation will review results from ongoing studies and published literature, emphasizing areas of current and future work at the CBE.

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Why the mining industry needs microbiologists

Presenter: Chris Kennedy, PhD, P.Geo., Senior Consultant
Affiliation: SRK Consulting Inc., Vancouver, British Columbia, Canada

The mining of rock to extract commodities (e.g., base and precious metals) results in waste materials that provide unique opportunities for microbial biofilms to colonize and proliferate. The two main waste types from mining operations are waste rock (also known as spoils) and tailings. Waste rock is the material that is removed in order to access the ore (rock containing the commodity of interest), while tailings represent rock that has been processed through a plant to extract the commodity. Waste rock is generally coarse (gravel- to basketball-sized boulders) and stored unsaturated. Conversely, tailings are often stored underwater, and have a very fine particle size (mainly less than 0.15 mm) containing organic compounds from processing the mined rock. As a consequence, material of similar composition can have very different effects on the environment and the type of microbial community that can be supported. This presentation will provide three scenarios to demonstrate how understanding of microbiology is essential for the mining industry.

The first scenario involves arsenic mobility in tailings. Arsenic is often an element of concern (EOC) in tailings, and iron oxides are often used to mitigate arsenic loading to the receiving environment. One of the main concerns is the fate of iron oxides in an anaerobic environment, as reductive dissolution of the iron oxides by a microbial community would likely result in arsenic remobilization. Despite this concern, arsenic remobilization is rarely seen. While many plausible explanations have been put forward, it may be possible that biofilms associated with the iron oxides contribute to inhibit reductive dissolution. It is also likely that the near-surface microbial community utilizes available dissolved organic carbon (DOC) and diffusion processes limit its availability to support an anaerobic community deeper into the tailings.

The second scenario involves treatment of tailings effluent. One method to treat contaminated effluent from tailings is by the installation of a passive treatment system (PTS). The design usually involves optimization of a specific microbial metabolic group for the element of concern. Sulfate reducing bacteria (SRB) constitute one group commonly employed to generate sulfides to scavenge trace metals such as

arsenic. Design of a PTS at an abandoned site in British Columbia failed to recognize that the types of waste present would support numerous microbial groups higher up the redox ladder than SRB. Ultimately an iron oxidizing community may have resulted in some As attenuation; however, this was by chance, not by design.

The third scenario involves predictive test work for new operations. While there has been a substantial increase in the amount of effort put into characterizing solid materials and predicting water chemistry for proposed operations, these studies often do not consider the role of microorganisms. One recent study determined that elemental sulfur in tailings waste would not pose an environmental risk (elemental S will produce acid if oxidized). Testing a pilot plant and disposal of a small amount of elemental sulfur in a field trial produced markedly different results as elemental sulfur oxidized, likely on account of microbial oxidation, and as a result, engineering design will likely need to be re-configured.

Planktonic and biofilm community dynamics in situ

Presenter: Kara De León, PhD student, Microbiology
Co-Author: Matthew W Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Department of Microbiology, Montana State University, Bozeman, MT

Though it is becoming increasingly common in many areas of microbiology to consider biofilm populations in analysis, comparisons of community dynamics between biofilm and planktonic communities are still infrequent. This is especially the case in subsurface analysis, where microbial communities are often analyzed by groundwater filtration because temporal extraction of sediment cores does not allow for keeping the spatial component constant and drilling multiple cores in close proximity becomes expensive and causes hydrological problems. Surrogate sediment samplers filled with core sediment allow for temporal community studies in the subsurface. Temporal and spatial surrogate sediment samples were compared to groundwater during stimulation for Cr(VI) reduction via polylactate injection at the Hanford 100H site in Southeastern Washington. While sediment and groundwater communities were similar at the phylum level, they varied on the genus level. There were many genera unique to sediment or groundwater pre-stimulation; however, few genera were unique to sample type post-stimulation, suggesting possible dispersal of the biofilm from the sediment into the groundwater or invasion of the biofilm of groundwater genera. Using relative abundances of genera and linear discriminant analysis size effect (LEfSe), genera that are statistically significant to one sample type was determined. These results indicated that *Desulfovibrio*—capable of Cr(VI) reduction and one of the genera that was targeted for during stimulation—was significant to groundwater. Examination of the relative abundance of *Desulfovibrio* in sediment indicated that it was rare or absent in the biofilm community even though it is known to form biofilms. Interestingly, many of the genera significant to sediment, such as *Methylibium* and *Thiohalomonas,* are known for heavy-metal resistance but not reduction; thus the function of these genera at the site is unknown. SparCC correlation analyses yielded more correlations in sediment than groundwater. In general, sediment and groundwater correlations tended to be within the heavy metal reduction and nitrogen utilization guilds, respectively. Both sediment and groundwater had many correlations between fermentative genera. With these data, it is possible to make predictions about processes occurring at site, which can be used in further research to better understand and possibly achieve better control of the community dynamics in situ.

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Special Presentations

2012 ASM Biofilms meeting digest

Presenter: Phil Stewart, CBE Director; Professor, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Highlights from the American Society for Microbiology Biofilms meeting of 2012 will be presented. This was the largest international biofilm meeting of the past couple of years. It took place in Miami and was attended by 447 participants from 40 countries. Several interesting presentations addressed biofilm morphogenesis and the roles of specific extracellular polymeric substances. **Munehiro Asally** described the mechanical buckling of a *Bacillus* biofilm that generates wrinkle structures in a process that begins with cell death in certain regions of the biofilm. In a similar model system, **Dave Weitz** presented astonishing video evidence of fluid flow through hollow wrinkles; the flow is driven by evaporation. Analogous wrinkles that form in *E. coli* colonies are dependent on extracellular proteinaceous appendages called curli. **Matthew Chapman** showed that curli genes are differentially expressed in space within these colonies and described new aspects of the biosynthetic pathway for curli. Working with the same bacterium and a similar focus on pili and curli, **Scott Hultgren** proposed interdicting the virulence of the organism by targeting these extracellular structures with "pilicides" or "curlicides." He showed examples of small molecule drug candidates that block the elaboration of pili or curli, a novel approach to interrupting biofilm formation. **Cynthia Whitchurch** played videos of bacterial cells that lyse explosively, releasing a spiderlike cloud of extracellular DNA. Two presentations broke new ground from a methods standpoint. **Jessica Welch** presented a stunning advance in fluorescence in situ hybridization (FISH) technology for simultaneous visualization of the microscale spatial distributions of multiple (up to 15) different microorganisms within complex multi-species biofilms using dental plaque as an example. **Roger Linington** described a robotic microtiter dish biofilm assay system for screening libraries of natural products for anti-biofilm activities. The approach makes use of automated image analysis of biofilm structure. A recurrent theme at the meeting was the central role of cyclic di-GMP in regulating the bacterial switch between sessile and dispersed lifestyles. **Tim Tolker-Nielsen** presented both in vitro and animal model data showing that reducing intracellular c-di-GMP concentration leads to dispersal of *Pseudomonas* biofilm. He argued that this provides proof-of-concept for a strategy of manipulating c-di-GMP levels as an anti-biofilm approach. **Karin Sauer** demonstrated that a specific metabolic pathway, involving pyruvate, is important in the ability of *Pseudomonas* to progress to the microcolony formation step of biofilm development. Finally, **Paul Stoodley** took aim at dental plaque with a high speed camera and a high velocity water jet to produce video of biofilm being mechanically removed from the surface by the impinging fluid flow.

Update: Biofilm Methods Index

Presenter: Darla Goeres, Assistant Research Professor, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

In biofilm research the goal is to grow a biofilm that is relevant to the environment of interest. If the research question involves efficacy testing, the disinfectant or antibiotic should also be tested under "real use" conditions. At the Center for Biofilm Engineering, researchers use a host of different methods for growing relevant and repeatable biofilms, treating the biofilms with antibiotics or disinfectants, sampling the biofilm—which includes both removing the biofilm from the growth surface and disaggregating the biofilm into a homogeneous sample—and analyzing the sample for viable cells, total cells, protein concentration, ATP, etc. During the summer 2012 Montana Biofilm Science and Technology Meeting, CBE Industrial Associates asked the CBE to create an index of the methods CBE researchers use to growth, treat, sample, and analyze biofilm bacteria. This presentation will demonstrate the template the CBE is proposing to use for the Biofilm Index.

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SESSION 4: Pathogen Persistence in Biofilms

Pathogen persistence in biofilms

Presenter: Anne K. Camper, Professor, Civil Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

One of the many properties of biofilms is the prevalence of extracellular polymers that act as a bridge between the cells and the substratum. This matrix also has the potential to trap and retain chemicals, other organisms, and inert particles. Previous work has shown that biofilms are capable of physically trapping and retaining particles, and research at the CBE has been collected on the retention of fluorescent latex and polystyrene beads. Further work demonstrated that biofilms entrained other bacterial cells, including potential pathogenic organisms. This work was done using water of drinking water quality, suggesting that even thin biofilms in nutrient-poor environments elicit this behavior. The data provide insight on the accumulation and persistence of pathogens in biofilms.

Root associated biofilms: Physical gradients and nutrient cycling

Presenter: Chris Allen, PhD student, Civil Engineering
Co-Authors: Anne Camper
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Treatment wetlands (TW) are highly engineered natural treatment systems that offer low capital, low energy input treatment solutions for municipal and industrial wastewater. Improvements in water quality are achieved through complex interactions of plant and microbial processes without the maintenance and energy inputs of conventional treatment options. Wetland plants increase the removal rates of organic carbon and nitrogen compounds by a degree that is significantly greater than can be attributed to plant uptake alone. The increase in treatment efficacy is hypothesized to result from a synergistic relationship between plant hosts and the microbial communities they foster. We hypothesize that plant exudates such as simple organic carbon compounds and acids, as well as diffusates such as oxygen, play an important role in generating the environments and biofilms required for the microbial removal of contaminants in TWs. We have shown that specific plant species improve nutrient removal across a variety of nutrient loading environments and further hypothesize that in waters with few available organic carbon sources, plants facilitate nitrogen removal by exuding carbon substrates required for heterotrophic denitrification—while the same plants, when growing in a reduced environment, generate oxic conditions at the root surface, facilitating the degradation of organic carbon compounds and nitrification. These results allude to the influence of the complex environmental gradients surrounding plant roots on the activity of the surrounding biofilm and suggest that biofilm activity on the living tissue has temporal as well as spatial heterogeneity.

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Pathogen-biofilm-root interactions for common constructed wetland plants

Presenter: Rachel VanKempen-Fryling, PhD student, Microbiology
Co-Author: Dr. Anne Camper
Affiliations: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Constructed wetlands treatment systems (CWTS) are important environmental systems to study because they have the ability to remediate common wastewater contaminants, such as carbon load, nitrogen, sulfate, heavy metals, and pathogenic organisms. Previous research involving CWTS has been ongoing at Montana State University (MSU) for almost a decade, giving supporting evidence that CWTS are effective in both summer and winter seasons and can be utilized in northern climates, disproving the assumption that wetlands were only effective in warm, tropical climates. One important characteristic of CWTS is the ability to remove pathogenic organisms from the water. *Escherichia coli* O157:H7 is a standard example of a waterborne pathogen that enters wastewater through fecal contamination from an infected host, the most common being cattle. In order to determine more mechanistically how CWTS remove pathogens from

wastewater, a molecular approach was taken to explore the rhizosphere and gravel biofilm. Molecular techniques and culturing were used to determine the presence, abundance, and persistence of *E. coli* O157:H7 within the root zone of common wetlands plants *Carex utriculata* (sedge) and *Schoenoplectus acutus* (hardstem bulrush).

SESSION 5: Next Generation Biomaterials

A porous biomaterial approach to biofilm infection control

Presenter: Andrew J. Marshall, PhD, Director and Chief Technology Officer
Affiliation: Healionics Corporation, Seattle, WA, USA

The exit sites of percutaneous implantable devices such as catheters are highly vulnerable to infection. When a catheter is implanted, the edge of the epidermis migrates inward; this creates a sinus tract that fills with dead scab tissue, becoming a haven for bacterial biofilm. We are addressing the exit site infection problem with STARcuff™, a porous tissue interface that integrates with the dermis and epidermis to provide protective barriers against bacterial migration and colonization without any added active agents.

STARcuff features STAR Biomaterial, a sphere-templated pore structure with tightly controlled optimized pore size (35-µm) and pore interconnection size (~15µm) with demonstrated biological effects. The STAR pore structure maximizes the number of phagocytic macrophages recruited into the pores, leading to a highly vascularized tissue-biomaterial interface that takes advantage of the body's natural antimicrobial defenses.

In the STARcuff, the tissue-integrating microporous STAR Biomaterial is applied to the catheter surface in submillimeter granules, enabling flexibility and economy in manufacturing devices. And combining the granular macrotexture with the optimized microporosity gives a remarkable reduction in fibrotic scarring in the peri-implant tissue.

We have demonstrated in multiple animal models that the STARcuff provides protection against exit site infection. In a 1-month porcine percutaneous implant model with controlled bacterial challenge, implanted catheter segments treated with STARcuff outperformed untreated bare silicone controls as well as implants treated with on-market silver ion-loaded antimicrobial cuffs. Building on that result, we used a 6-month porcine percutaneous implant model, where we demonstrated that STARcuff-treated catheter segment implants showed significantly reduced frequency of purulent infections compared to untreated controls. Effectiveness of the STARcuff in reducing bacterial colonization in the subcutaneous tissue around the implant was maximized by the use of soft shear-compliant catheter material underlying the tissue-integrating biomaterial.

To commercialize the STARcuff anti-infection technology, we are initially targeting hemodialysis catheters and percutaneous ports for hemodialysis access, device applications where other approaches to long-term exit site infection control have been largely unsuccessful.

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SLIPS—Omniphobic, slippery surfaces to prevent bacterial surface attachment

Presenter: Ben Hatton, Assistant Professor
Co-Authors: Tak-Sing Wong, Joanna Aizenberg, Harvard University
Affiliation: Materials Science & Engineering, University of Toronto, Toronto, Canada

Recent years have shown a development in non-wetting, 'superhydrophobic' surfaces, which can resist the wetting of liquids (typically aqueous) by a mechanism known as the Cassie-Baxter effect. A rough,

hydrophobic surface causes the liquid interface to be stuck at the tips of the rough surface features, and an air layer is maintained underneath. This type of surface—often seen in plant leaves such as the lotus, in insects such as the waterstrider, and in many bird feathers—can be very stable against wetting, and sometimes even for liquids with relatively low surface tension (i.e., alcohols). However, these surfaces often 'fail' (become fully wetted) after some time, or due to surface defects or exposure to surfactants. Therefore, efforts to use such materials to prevent the formation of bacterial biofilms (or marine biofouling) have generally been unsuccessful. Once full wetting of the surface features occurs, the non-wetting, Cassie-Baxter state does not (typically) return, and the bacteria can easily populate the surface to produce a highly-adherent biofilm.

Most recently, however, our lab (Harvard University, School of Engineering and Applied Sciences) has developed a novel 'omniphobic' surface that is highly resistant to the wetting of any liquid—even those with very low surface tension—and over extended periods of time [1]. This material, known as a Slippery Liquid-Infused Porous Surface (SLIPS), incorporates a thin layer of lubricant around the rough microstructure of the substrate surface, which is energetically stable (has a strong chemical affinity for the surface) and is immiscible to other contacting liquids. Therefore, other liquids, including an aqueous medium containing bacteria, in contact with this surface can only 'see' this smooth, stable lubricant layer and there is very little opportunity to develop a physical adhesion. As a result, bacteria grown in contact with SLIPS materials cannot adhere, and there is insignificant biofilm growth even after an extended time. For example, *Pseudomonas aeruginosa* was cultured for 7 d under static and flow conditions, but a SLIPS material (incorporating a perfluorocarbon lubricant) remained 99.6% without bacterial attachment (by area), compared to a fluorinated polymer control (PTFE).

We show that SLIPS-based antibiofilm surfaces are stable in submerged, extreme pH, salinity, and UV environments. They are low-cost, passive, simple to manufacture, and can be formed on arbitrary surfaces. We anticipate that these approaches could enable a broad range of antibiofilm solutions in clinical and industrial environments.

[1] Wong TS, Kang SH, Tang SKY, Smythe EJ, Hatton BD, Grinthal A, Aizenberg J. "Bioinspired self-repairing slippery surfaces with pressure-stable omniphobicity." *Nature* **477**, 443 (2011).

[2] Epstein AK, T. Wong S, Belisle RA, Boggs EM, Aizenberg J. "Liquid-infused structured surfaces with exceptional anti-biofouling performance." *Proceedings of the National Academy of Sciences* **109**, 13182 (2012).

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In vivo analysis of a novel antimicrobial coating to prevent biofilm implant-related infection

Presenter: Dustin Williams, PhD, Postdoctoral Fellow
Affiliation: Department of Orthopaedics, University of Utah; George E. Wahlen Department of Veterans Affairs, Salt Lake City, UT, USA

Active release antimicrobial coatings for medical devices have been developed to prevent and treat biofilm implant-related infections. To date only a handful of coatings have been put into clinical use, with limited success. In this study, a novel antimicrobial compound was incorporated into a silicone (polydimethylsiloxane or PDMS) polymer to develop a novel active release coating that addressed several limitations of current device coatings. The efficacy of this coating was optimized using an in vitro flow cell system, then translated to an animal model of a simulated Type IIIB open fracture, wherein well-established biofilms were used as initial inocula. Results indicated that the novel coating was able to prevent infection in 100% (9/9) of animals that were treated with biofilms and the novel coating (treatment group). In contrast, 100% (9/9) of animals that were inoculated with biofilms and not treated with the coating (positive control), did develop infection. Nine animals were used as negative controls, i.e., those that were not treated with biofilms, and showed a rate of infection of 11% (1/9). Eight animals were treated with the novel coating only to determine its effect on host tissue. Results indicated that the novel active release coating may have significant promise for future application to prevent biofilm implant-related infections in patients.

Inhibiting bacterial biofilm formation on stainless steel 316L using self-assembled monolayers

Presenter: Kristen M. Kruszewski[1], Research Chemist I
Co-Authors: Luanne Hall-Stoodley[2], Ellen S. Gawalt[3]
Affiliation: [1]PPG Industries, Allison Park Coatings Innovation Center, Pittsburgh, PA, USA
[2] Southampton Wellcome Trust Clinical Research Facility, Southampton General Hospital, Southampton, UK
[3]Duquesne University, Pittsburgh, PA, USA

Stainless steel 316L (SS316L) is commonly used for orthopedic implants, which can fail due to biofilm infection. Since infection typically occurs surrounding the time of implant surgery, it is important to reduce bacterial adhesion early on. To address this, self-assembled monolayers (SAMs) were used to modify the SS316L surface. Initially, SAMs with long alkyl chains presenting hydrophobic (-CH_3) or hydrophilic (oligoethylene glycol) tail groups were used to form passive coatings.

In another approach, active antimicrobial coatings were formed by using SAMs to immobilize the antibiotics gentamicin or vancomycin individually and in combination. Modified surfaces were characterized using surface infrared spectroscopy, contact angles, MALDI-TOF mass spectrometry, and AFM. *Staphylococcus aureus* biofilm growth on modified surfaces was monitored using confocal microscopy, SEM, and colony forming unit analysis. Neither hydrophobic nor hydrophilic SAMs inhibited biofilm development, but antibiotic-linked films significantly reduced biofilm growth by 99% up to 48 hours. Gentamicin-linked films were shown to be effective from 2–24 hours while vancomycin-linked films significantly inhibited biofilm growth at longer time points (6–48 hours). Combining the antibiotics resulted in a synergistic effect and limited biofilm development from 2–24 hours.

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abstracts

Poster Abstracts

Center for Biofilm Engineering posters

CBE Poster #563

Date: 02/2012

Title: **Dissolved organic matter in the WAIS Divide ice core**

Authors: ***Juliana D'Andrilli***[1,2], Foreman C[1,2], McConnell J[3] and Priscu J[1]

Affiliation: [1] Department of Land Resources and Environmental Sciences, and
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
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Sponsored by: National Science Foundation

The glacial environment of the West Antarctica Ice Sheet (WAIS) Divide contains an active microbial community and serves as a reservoir for organic carbon accumulation. We compare the dissolved organic matter (DOM) character and source material by Excitation Emission Matrices (EEMS) from early Holocene ice below the brittle ice zone (1300–1700m) obtained from the WAIS Divide ice core. Approximately 90% of the DOM in these ice cores was dominated by the presence of both tyrosine-like and tryptophan-like protein fluorescence signatures. Proteinaceous fluorophores are believed to reflect the production of amino acids during microbial metabolism and are typically more labile than DOM with significant humic signatures. Some humic-like components were detected in both terrestrial and marine fluorescent regions by EEMS, which denotes the commonly detected fluorescing material in those types of environments. However, fluorescence in those regions was far less prevalent than the protein-like fluorescent contributions. Even with low dissolved organic carbon concentrations in the WAIS Divide ice core, sufficient fluorescing material is present to characterize the different fluorophores present in the ice core DOM.

We will compare the 484 EEMS of the DOM collected from 1300–1700m of the WAIS Divide ice core with the co-registered geochemical datasets, which will allow us to better understand the DOM trends throughout the southern hemisphere historical record: i.e., how does the DOM chemical character change after a volcanic event, how does DOM relate to other environmental nutrients/elements, what periods in history correlate to low and/or high concentrations in DOM and its corresponding fluorescent nature? A small percentage (~3%) of DOM from these ice cores show a strong shift to more humic material present in the DOM and represent areas of potential geochemical interest. Currently, we are working on a new statistical model based on parallel factor analysis (PARAFAC) to explicitly analyze the DOM components specific to glacial/ice core environments that are not commonly found in existing global PARAFAC models. This further characterization will not only contribute to the importance of recognizing DOM reservoirs in glacial regions, but will also be a significant addition to our understanding of global carbon cycling.

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CBE Poster #566

Date: 01/2012

Title: **Imaging biofilm and microbially induced $CaCO_3$ precipitation in porous media reactors**

Authors: ***James Connolly***[1,2], Iltis G[4], Wildenschild D[4], Cunningham A[1,3] and Gerlach R[1,2]

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Sponsored by: National Science Foundation and the U. S. Department of Energy

Biological processes in the subsurface environment are important to understand in relation to many engineering applications including, but not limited to: groundwater remediation, geologic carbon sequestration, and petroleum production. Two biological processes studied here are biofilm formation and

microbially induced calcium carbonate precipitation. Many analytical tools are available to researchers for the study of these processes, but microscopic imaging provides additional information and validation to these data sets. For example, visualization of biofilm geometry in the pore space is important for the characterization of hydrodynamic changes in a porous medium affected by biofilm growth.

Confocal laser scanning microscopy (CLSM) and field emission scanning electron microscopy (FEM) were used to study processes in two dimensional (2D) reactors with regular etched pore structures. Two different reactors were used. The first has uniform 1.0mm square pore structures and is designed for direct observation with ordinary photography, stereoscopy or microscopy after destructive sampling. The second reactor is a micro-model flow cell with 100µm pore structures and is specifically designed for CLSM imaging. Samples imaged under CLSM are generally prepared by staining the biofilm with various fluorescent stains. However, since staining may cause deleterious changes to metabolic processes, organisms that produce fluorescent protein are also imaged with CLSM so as to study basic biofilm behavior. Two-dimensional systems are convenient for high resolution imaging with CLSM and traditional light microscopy. However, high resolution imaging of undisturbed biofilm formation in 3D systems cannot be accomplished with traditional microscopy because light cannot penetrate deeply into the sample. Synchrotron-based x-ray computed microtomography (CMT) is capable of producing three-dimensional images with similar resolution to CLSM; however, due to the highly hydrated nature of biofilms, novel x-ray contrast agents must be used. Two contrast agents that use particle size exclusion to capture 3D features of biofilms (neutrally buoyant, silver-coated, glass micro-spheres and barium sulfate suspensions) were compared in this work. Biofilms grown in 2D micro-model flow cells were imaged using both CMT and CLSM in order to validate the use of these contrast agents in 3D systems. Images from this comparative study will be presented.

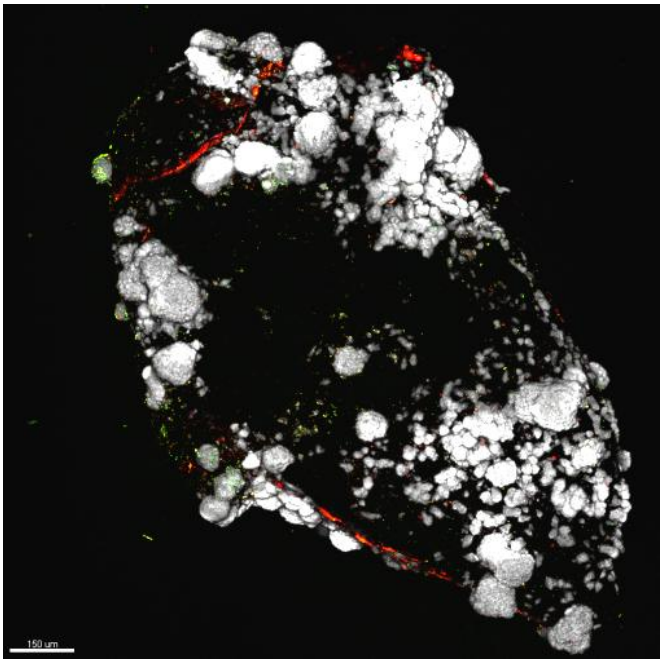

Figure 1. A CLSM reconstruction of a sand grain colonized by *Sporosarcina pasteurii* under ureolytic conditions where calcium carbonate (shown in white) has been precipitated. The sample was stained with Invitrogen LIVE/DEAD so areas with healthy cells are shown in green. Regions with cells that have compromised membranes or contain extracellular nucleic acids are shown in red. *S. pasteurii* is common model organism for the study of ureolysis-driven calcium carbonate precipitation. Scale bar = 150µm.

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CBE Poster #568

Date: 01/2012

Title: **Temporal transcriptomic analysis during bio-oil accumulation in *Pheaodactylum tricornutum:* Importance of C4-mediated carbon flow**

Authors: ***Jacob Valenzuela***[1,5,6], Mazurie A[2,3], Carlson RP[4,6], Gerlach R[4,6], Cooksey KE[2], Bothner B[1], Peyton BM[4,6], and Fields MW[2,6*]

Affiliation: [1] Department of Biochemistry and Chemistry, [2] Department of Microbiology, [3] Bioinformatics Core, [4] Department of Chemical and Biological Engineering, [5] Molecular Biosciences Program, and [6]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Sponsored by: U. S. Department of Defense, U. S. Department of Energy, Molecular Bioscience Program

Phaeodactylum tricornutum is a unicellular diatom that belongs to the class *Bacillariophyceae*. The full genome has been sequenced (<30 Mb), and approximately 25 to 30% TAG accumulation has been reported under different growth conditions. In order to elucidate gene expression profiles of *P. tricornutum* during

nutrient-deprivation and lipid-accumulation, cell cultures were grown with nitrate and phosphate at a ratio of 20:1 (N:P) and whole-genome transcripts were monitored over time. The specific NR fluorescence (NR fluorescence per cell) increased over time; however, the increase in NR fluorescence was initiated before external nitrate was completely exhausted. Phosphate was depleted before nitrate, and *P. tricornutum* appears to accumulate and store external phosphate under the tested growth conditions. Three transcriptomic time points were selected based upon different growth phases with dynamic NR fluorescence. The first sample (Q1) represented exponential growth with high external nitrate, phosphate, and DIC levels and low NR fluorescence. The second sample (Q2) represented the transition between exponential and stationary phases—with depleted nitrate and phosphate levels and low DIC, but increasing NR fluorescence. The third sample (Q3) represented extended stationary phase induced by depleted nitrate and phosphate, rebounding DIC but high NR fluorescence. RNA-seq analyses assembled 30,373 transcripts to 10,124 mapped loci and 1,812 genes were differentially expressed at statistically significant levels between phases. Of all significant genes, approximately 180 genes were differentially expressed between all three time points, 546 genes between any two time points, and 177 genes between only two time points. With a focus on nitrogen and carbon metabolism, the expression trends for key genes were determined. The up-expression of both putative nitrate (469- and 808-fold) and phosphate (199- and 507-fold transporters were observed during exponential growth as nitrate and phosphate were depleted. Both nitrate (NADH-dependent) and nitrite reductase (Fd-dependent) were up-expressed (over 200-fold) as nitrate levels were depleted. In conjunction with the nitrate assimilation, glutamine synthetase, glutamate synthase, asparagine synthetase, glutamate dehydrogenase, and carbamoyl-phosphate synthetase were up-expressed (3-fold to 175-fold). The highest overall up-expression was observed in the cytosolic glutamate dehydrogenase, but the largest increase from basal levels was observed in the chloroplastic glutamine synthetase. All of these genes displayed a down-expression in prolonged stationary-phase during sustained increases in NR fluorescence. Many of the genes associated with the C3 pathway for photosynthetic carbon reduction (PCR) were not significantly altered; however, genes involved in the C4 pathway for photosynthetic carbon assimilation (PCA) were up-expressed as the cells depleted nitrate, phosphate, and DIC levels. Gene products involved in C4-PCA were up-expressed and included PEP carboxylase, PEP carboxykinase, and pyruvate carboxylase; however, PEP carboxykinase and one form of the pyruvate carboxylase displayed the highest up-expression during DIC depletion. The malate dehydrogenase, malic enzyme, and pyruvate-P dikinase were up-expressed 6-fold, 8-fold, and 3-fold respectively, and could be responsible in recycling oxaloacetate, malate, and pyruvate for delivery of CO_2 for PCR. *P. tricornutum* has multiple, putative carbonic anhydrases, but only two were significantly up-expressed (2-fold and 4-fold) at the last time point when DIC levels had increased. The results indicated that during nitrate and phosphate depletion, *P. tricornutum* depleted external DIC levels and initiated lipid accumulation. Based upon transcript levels, C4 based carbon assimilation was used in response to depleted DIC during presumptive lipid accumulation.

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CBE Poster #569

Date: 01/2012
Title: **Design and testing of a flow cell for microscopy of biofilm during treatment**
Authors: ***Betsey Pitts, Lindsey Lorenz***, Sturman P, Buckingham-Meyer K, Warwood B*, and Stewart PS
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*BioSurface Technologies Corporation, Bozeman, MT, USA

Fully hydrated, time-lapse microscopy of biofilms has been a strength at the CBE since its inception, and some of the most stunning and insightful observations about biofilms have come from use of this technique. In particular, with the appropriate flow-cell system, this technique allows us to visualize the impact of a treatment on existing biofilm as it is applied under flow conditions. Flow cells are generally designed with the desired type of image collection and analysis in mind, and existing systems are fairly specific. For example: the capillary flow cell allows for imaging of penetration of agents into isolated biofilm clusters,

but clusters must be viewed from the back; the coupon evaluation flow cell is designed for monitoring of biofilm growth on a surface over time, but is not useful for treatment; flat plate flow cells are best for comparison of biofilm architecture, but provide only one sample per flow cell. We set out to design a flow cell specifically tailored to accept biofilm-covered coupons grown in a CDC reactor, and to allow high throughput, top-down imaging of biofilm clusters under flowing treatment application. Some design priorities for this system included: ease of coupon insertion and removal; small treatment volume requirements; top-down, fully hydrated imaging; material compatibility; and objective magnification and working distance limitations. We have tested numerous designs, treatments and image collection protocols which will be detailed on this poster and will also be available as movies. Our prototype testing has produced a simple flow cell design that allows for high volume coupon testing and efficient collection and production of biofilm treatment movies.

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CBE Poster # 575

Date: 06/2012
Title: **Convection around biofilms**
Authors: ***Phil Stewart***
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Water that flows around a biofilm influences the transport of solutes into and out of the biofilm and applies forces to the biofilm that can cause it to deform and detach. Engineering approaches to quantifying and understanding these phenomena are reviewed in the context of biofilm systems. The slow-moving fluid adjacent to the biofilm acts as an insulator for diffusive exchange. External mass transfer resistance is important because it can exacerbate oxygen or nutrient limitation in biofilms, worsen product inhibition, affect quorum sensing, and contribute to the development of tall, fingerlike biofilm clusters. Measurements of fluid motion around biofilms by particle velocimetry and magnetic resonance imaging indicate that water flows around, but not through biofilm cell clusters. Moving fluid applies forces to biofilms resulting in diverse outcomes including viscoelastic deformation, rolling, development of streamers, oscillatory movement, and material failure or detachment. The primary force applied to the biofilm is a shear force in the main direction of fluid flow, but complex hydrodynamics including eddies, vortex streets, turbulent wakes, and turbulent bursts result in additional force components.

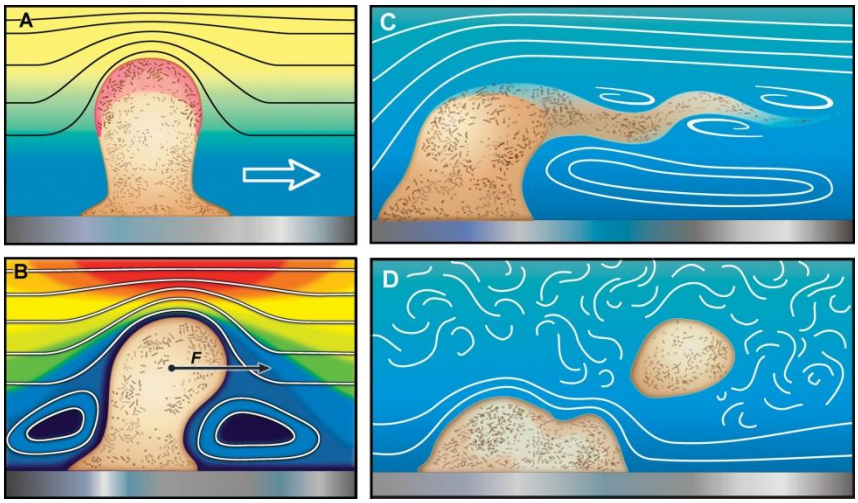

Figure 1. Effects of fluid flow on microbial biofilm. The four panels illustrate different phenomena, and correspond to increasing fluid velocity from A to D. The direction of fluid flow (block arrow) is from left to right. Solid lines are pathlines, the trajectory of an individual fluid particle. (A) Especially in slow flows, the fluid can pose resistance to diffusive transport of solutes (metabolic substrate, e.g. oxygen, indicated in yellow) exacerbating limitations within the biofilm. Rapid cell growth (pink) is restricted to the regions of the biofilm with access to substrate. These regions expand preferentially, leading to fingering of biofilm structures. (B) Fluid moves around biofilm cell clusters, but not through them. Hotter colors (red) indicate high fluid velocity, cooler colors (blue) indicate slower fluid velocity. Moving fluid applies a force to the biofilm (arrow). Complex secondary flows, such as eddies, can occur even under laminar flow conditions. (C) Fluid flow can induce deformation and movement of the biofilm, such as the formation of oscillating streamers on the downstream edge of a cell cluster. (D) When the force applied by the fluid exceeds the cohesive strength, the biofilm can fail leading to a detachment event. Turbulent flow produces bursts that penetrate to the immediate environs of the biofilm and result in brief, but intense force excursions. The dimension of biofilm structures like those cartooned here typically range from tens of microns to millimeters.

CBE Poster #576

Date: 07/2012

Title: **Chromium responses and biofilm formation in *Desulfovibrio vulgaris* RCH-1, a sulfate-reducing bacterium isolated from 100H chromium-contaminated groundwater, are temperature-dependent**

Authors: ***Lauren Franco***[1,2], Gorby YA[3], and Fields MW[1,2]

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[3]Department of Geology, University of Southern California, USA

Sponsored by: US Department of Energy

Desulfovibrio vulgaris RCH-1 is a sulfate-reducing bacterium that was isolated from chromium-contaminated groundwater at the 100H Hanford Site. Reduction of chromium(VI) to the insoluble and less toxic chromium(III) could help prevent migration of chromium-contaminated groundwater to the Colombia River, a valuable drinking water source. Biostimulation of chromium-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 *D. vulgaris* RCH-1 ability to reduce Cr(VI). Additionally, growing *D. vulgaris* RCH-1 at a temperature that is relevant to the in situ subsurface temperature affects chromium tolerance, reduction rates, and presence of extracellular filaments. Growth experiments were initiated in batch growth mode with electron donor-limited, electron acceptor-limited, and electron donor/acceptor balanced ratios. Washed *D. vulgaris* RCH-1 cells were exposed to 0, 20, 50, and 100 μM K_2CrO_4 and chromium(VI) levels were monitored during growth. Growth in electron acceptor-limited and electron donor-limited cultures was effected and had increased lag-times compared to cultures with electron donor/acceptor balanced ratios. *D. vulgaris* RCH-1 grows optimally at 30°C, but to understand if the chromium response is different at in situ temperatures, experiments were also carried out at 20°C. *D. vulgaris* RCH-1 was more susceptible to chromium at 20°C than at 30°C and cells could only tolerate 50 μM as opposed to 100 μM K_2CrO_4. *D. vulgaris* RCH-1 was also grown as a biofilm under electron acceptor-limited conditions at 30°C and 20°C and extracellular filaments were observed at 20°C, but not at 30°C. The presence of extracellular filaments at a field-relevant temperature suggests that the filaments play a role in situ. Current studies are focused on the determination of function and composition for extracellular structures. Studies of recent field isolates provide valuable insights into the metabolic potential of organisms that are present in the environment of interest as opposed to a model organism. Assessing chromium reduction at in situ temperatures rather than optimal growth temperatures and under electron donor- and acceptor-limitation provides field relevant insight into chromium toxicity and reduction for respective field sites.

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CBE Poster #578

Date: 01/2012

Title: **Metabolic network analysis of an anaerobic microbial community: Potential for syntrophic methane and hydrogen production**

Authors: ***Kristopher Hunt***[1,6], Lohman E[1,6], Adam Z[2,6], Bell T[3,6], Camilleri L[3,6], Connolly J[1,6], Michaud A[4,6], Smith H[4,6], Tigges M[5,6], Carlson R[1,6], Fields M[3,6], Foreman C[4,6], Gerlach R[1,6], and Inskeep W[4,6]

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[6]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Sponsored by: National Science Foundation, IGERT

Microbial community interactions represent a research area of growing interest to many scientific and engineering disciplines, including ecologists, geobiologists and bioprocess engineers. Community-level behavior is a complex result of both system members and their interactions, which can complicate the development of testable hypotheses for ex vivo microbial systems even when individual microbial community components are well understood. In the absence of suitable natural analogs, computer (in

silico) models of interactions between distinct microbial metabolic groups can assist the characterization of complex microbial ecosystem behavior. Due to increases in sequencing speed and decreases in sequencing costs, relevant genomic data for many microbial systems are readily available. This genomic information can be translated into collections of biochemical reactions that capture the organism's metabolic potential, permitting a "systems based" analysis of relationships between environmental parameters, genome/metagenome content and community functioning. By assessing metabolite flux between individual community members and their effects on community-level behavior, in silico methods can be used to generate hypotheses related to environmental conditions and community structures that achieve useful outcomes. These outcomes include understanding and optimizing both natural and industrial processes of pressing societal importance, such as increased biofuel production or more efficient waste remediation. This presentation discusses in silico techniques used to build models representing six fully sequenced anaerobic organisms, and their combined community potential for predicting syntrophic methane and hydrogen production.

CBE Poster #579

Date: 02/2012

Title: **Fungal bioconversion of cellulose to hydrocarbons**

Authors: ***Natasha Mallette***[1,2], Peyton BM[1,2], Carlson R[1,2], Strobel G[3], Mitchell Smooke M[4], Strobel S[5], Hunt K[1,2], Pankratz E[1,2], and Tosatto L[4]

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[4]Department of Mechanical Engineering & Materials Science, and
[5]Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT, US

Sponsored by: NSF-EFRI (Emerging Frontiers in Research and Innovation)—0937613
NSF-CBET (Chemical, Bioengineering, Environmental, and Transport Systems)—0802666

The goal of our project is to develop fundamental engineering bioprocess knowledge for *direct* conversion of waste cellulose to produce a range of usable fuel hydrocarbons. *Ascocoryne sarcoides* (NRRL 50072) is an endophytic fungus, isolated from Northern Patagonia by Gary Strobel, that has been shown to excrete "mycodiesel," an extensive series of straight chained medium chain-length hydrocarbons, including heptane, octane, and undecane (Strobel et al., 2008). The project challenges the current archetype for fuel production from waste cellulose. Clearly, a novel technology that could *directly* convert waste biomass into fuel grade hydrocarbons would be a significant paradigm shift in current renewable fuel strategies. In contrast to ethanol systems, by potentially eliminating separate saccharification processing, this proposed fungal technology can bypass one of the most costly and energy intensive steps of waste cellulose conversion. Further, while much national effort has focused on ethanol production, beyond characterization of cellulolytic fungal enzymes, very little research has examined the potential role of fungi in renewable fuel production. The objectives and recent results of the project are presented.

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CBE Poster #580

Date: 07/2012

Title: **Characterization of new siderophores produced by a Soda Lake isolate**

Authors: ***Luis O. Serrano Figueroa****, M. S.*[1, 2, 4], Shwartz B[1], Richards AM[2 & 3]

Affiliation: [1]Molecular Biosciences Program, [2]Center for Biofilm Engineering,
[3]Department of Chemical and Biological Engineering, and [4]Department of Microbiology, Montana State University, Bozeman, MT, USA

Sponsored by: National Science Foundation

Soap Lake, located in Washington State, was the subject of an NSF-funded Microbial Observatory and is a naturally occurring saline and alkaline lake. Several organisms inhabiting this lake have been identified as producers of siderophores that are unique in structure. An isolate most closely related to *Halomonas*

variablis was found to produce a unique suite of amphiphilic siderophores. Bacterial isolates, enriched from Soap Lake sediment and water samples, were screened for siderophore production. Siderophore production was confirmed through the chrome azurol S agar plate method. Bacterial isolate SL01 was found to produce relatively high concentrations of siderophores in liquid medium. The Csaky and Arnow assays classified the siderophores as possessing hydroxamate moieties and lacking catecholate moieties, respectively. Siderophores from SL01 were separated from the culture supernatant using solid phase extraction and purified by HPLC. Siderophore structure was determined using LC/MS/MS. Partial sequences, approximately 900 base pairs, of the 16s rDNA genes of this isolate was compared to those in the NCBI database using the BLAST search to determine its closest phylogenetic neighbors. A distinct, new family of amphiphilic siderophores was produced by isolate SL01, a microbe that was found to be most closely related to *Halomonas variablis*. The siderophores comprising this suite ranged in size from 1050 to 1100 amu and consist of a conserved peptidic head group, which coordinates iron, coupled to fatty acid moieties. These siderophores resemble the amphiphilic aquachelin siderophores produced by *Halomonas aquamarina* strain DS40M3, a marine bacterium as well as siderophores from another Soap Lake isolate that was found to produce amphiphilic siderophores. Bacteria thriving under saline and alkaline conditions are capable of producing unique siderophores resembling those produced by microbes inhabiting marine environments.

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CBE Poster #582

Date: 06/2012
Title: **Artificial syntrophic binary biofilm cultures of *Escherichia coli* MG1655 and *Synechococcus* PCC7002**
Authors: ***Alissa Bleem***, Bernstein H, and Carlson R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: National Science Foundation and National Institutes of Health

Biofilm cells typically interact in environments with much higher local cell densities than those found in liquid environments, leading to significantly elevated levels of localized metabolic by-products. Such metabolites have the potential to play a key role in heterogeneous biofilms via syntrophy, in which one type of microbe utilizes the by-products of another for its own proliferation. This project examined the metabolic characteristics of microbial consortia by engineering a biofilm comprised of two organisms. These artificial communities utilized an autotrophic cyanobacteria, *Synechococcus* sp., as a primary producer and *Escherichia coli* as the corresponding consumer strain. Benefits of syntrophic metabolite exchange were characterized through growth rate data, vitamin exchanges, and comparison of biomass productivity under applied and control conditions. The artificial biofilm binary cultures displayed an approximate increase of 40% in biomass productivity and nearly a 1.5-log increase in colony forming units per biofilm over the control *Synechococcus* mono-cultures under various vitamin B12 sufficient conditions. Current work on this system seeks to better understand the role of oxygen production and scavenging between the *Synechococcus* and *E. coli* as well as species-dependent spatial portioning with in the biofilm.

CBE Poster #585

Date: 06/2012
Title: **Field Emission Microscopy and growth modeling of a *Desulfovibrio alaskansis* G20 biofilm**
Authors: ***Gregory Krantz,*** Fields MW, Gerlach R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: ENIGMA, Molecular Biosciences Program, Center for Biofilm Engineering

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. *Desulfovibrio alaskansis* G20 (G20) is a SRB isolated from a

producing oil well in Ventura, California. This study evaluates whether G20 pure culture can form a biofilm on steel substrate, and attempts to characterize the G20 biofilm with the Biological Accumulation Model (BAM).

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CBE Poster #589

Date: 11/2012
Title: **Genetic basis of *Pseudomonas aeruginosa* biofilm antibiotic tolerance**
Authors: ***Phil Stewart,*** Folsom JP, Williamson KS, Franklin MJ, Boegli L, James GA
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

A transcriptomics approach was pursued to discover the physiological and genetic basis of reduced susceptibility of *P. aeruginosa* in biofilms to killing by the antibiotics tobramycin and ciprofloxacin.

Biofilms cultured for 3 days in drip-flow reactors were less susceptible to both antibiotics when compared to planktonic bacteria challenged with the same dose. Differences in gene expression between biofilm and planktonic cultures were surveyed using microarrays, resulting in a list of 293 genes that were expressed at higher levels in the biofilm cultures. We hypothesized that some of these genes contribute to reduced antibiotic susceptibility in the biofilm state.

We tested for statistically significant overlap between the list of biofilm-induced genes and independently compiled gene lists corresponding to specific hypothesized protective mechanisms. These lists included genes associated with: 1) planktonic susceptibility to either tobramycin or ciprofloxacin, 2) drug efflux pumps, 3) acyl homoserine lactone quorum sensing, 4) adaptive responses to the two antibiotics, 5) oxygen limitation, and 6) stationary phase growth.

Only genes associated with oxygen limitation and stationary phase were significantly enriched in the set of genes upregulated in the biofilm. This suggests that the other protective mechanisms are unlikely to contribute broadly to the biofilm defense in this system. Oxygen concentrations measured by microelectrodes and physiological heterogeneity visualized by induction of a GFP were consistent with oxygen gradients and growth limitation. We therefore cultured biofilms of mutant strains deficient in genes associated with starvation (rpoS, relAspoT) or hypoxia stress response (anr) and challenged these biofilms with antibiotics. All three mutants, when grown as biofilms, were statistically significantly more susceptible to ciprofloxacin than the wild type strain. The mutant biofilms showed log reductions in viable cells of 2.4 to 2.9 compared to a 0.9 log reduction measured for wild-type bacteria. Interestingly, none of the mutants exhibited a statistically significant alteration in tobramycin susceptibility compared to wild type biofilm. These results are consistent with a model in which multiple genes controlled by overlapping starvation/stress responses contribute to the protection of these biofilms from ciprofloxacin, whereas a distinct, as yet undiscovered, mechanism protects from tobramycin.

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CBE Poster #590

Date: 07/2012
Title: **Biofilm-induced calcium carbonate precipitation: Application in the subsurface**
Authors: ***Adie Phillips,*** Eldring J, Lauchnor E, Gerlach R, Mitchell AC, Esposito R, Cunningham A, Spangler L
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: U.S. Department of Energy

We have investigated mitigation strategies for sealing high permeability regions, like fractures, in the subsurface. This technology has the potential, for example, to improve the long-term security of geologically stored carbon dioxide (CO_2) by sealing fractures in cap rock or to mitigate leakage pathways to prevent contamination of overlying aquifers from hydraulic fracturing fluids. Sealing technologies using low-viscosity fluids are advantageous since they potentially reduce the necessary injection pressures and

increase the radius of influence around injection wells. In this technology, aqueous solutions and suspensions are used to promote microbially induced mineral precipitation in subsurface environments. To this end, a strategy was developed to twice seal a hydraulically fractured, 74 cm diameter Boyles Sandstone core with biofilm-induced calcium carbonate ($CaCO_3$) precipitates under ambient pressures. *Sporosarcina pasteurii* biofilms were established, and calcium and urea containing reagents were injected to promote saturation conditions favorable for $CaCO_3$ precipitation followed by growth reagents to resuscitate the biofilm's ureolytic activity after inactivation due to cell entombment. Then, in order to evaluate this process at relevant deep subsurface pressures, a novel high pressure test vessel was developed to house the 74 cm diameter core under pressures as high as 96 bar (1400 psi). After determining that fracture permeability was not influenced by increasing overburden pressure, the fractured core was sealed under subsurface relevant pressures relating to 457 meters (1500 feet) below ground surface (45 bar [650 psi] overburden pressure). After fracture-sealing under both ambient and subsurface relevant pressure conditions, the sandstone core withstood three times higher well bore pressure than during the initial fracturing event, which occurred prior to biofilm-induced $CaCO_3$ mineralization. These studies suggest that biofilm-induced $CaCO_3$ precipitation technologies may potentially seal and strengthen high permeability regions or fractures in the subsurface.

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CBE Poster #591

Date: 01/2013

Title: **Improving control of microbially induced mineral precipitation in flow systems—Experiments and modeling**

Authors: ***Robin Gerlach***[1], Phillips A[1], Lauchnor E[1], Ebigbo A[2], Connolly J[1], Mitchell AC[1,3], Helmig R[2], Cunningham AB[1], and Spangler LH[4]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[2]University of Stuttgart, Germany
[3]Institute of Geography and Earth Sciences, Aberystwyth University, UK
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Sponsored by: U.S. Department of Energy–EPSCoR program, ZERT program; National Science Foundation

Batch and flow experiments at atmospheric and geologic CO_2 storage-relevant pressures in our laboratories have demonstrated the ability of microbial biofilms and biofilm-produced calcium carbonate precipitates to decrease the permeability of natural and artificial porous media as well as to improve the stability of unconsolidated porous media.

Two overarching challenges in effectively implementing microbially induced calcium carbonate precipitation (MICP) are controlling (1) the spatial and temporal distribution of the formed precipitates and (2) the inactivation of microbes during the calcium carbonate precipitation process. Failure to control either one of those could result in injection well plugging or the necessity to implement costly cell-reinjection or -resuscitation strategies.

Our recent work has focused on optimizing strategies for MICP in small capillaries and micro-models, small columns (1 to 2.5 cm diameter, up to 5 cm in length), meso- (2 ft columns and 4 cm x 8 cm 2-d reactors) and large-scale (75 cm diameter, 38 cm high sandstone radial flow) systems.

Results of these experiments have been modeled using two different approaches: (1) a microscale phase-field approach, and (2) a large scale volume averaging approach. Close interaction between experimenters and modelers has resulted in improved injection strategies and the models are currently being used as experimental design tools.

This presentation will focus on recent efforts that combined 2 ft column experimentation with Darcy-scale modeling to calibrate and validate a model before utilizing the model for the optimization of biomineralization strategies in radial flow demonstrations in meso-scale sandstone cores at ambient and high pressures.

CBE Poster #592

Date: 12/2012
Title: **Localized gene expression, protein production, and antibiotic tolerance patterns within *Pseudomonas aeruginosa* biofilms**
Authors: ***Michael J. Franklin***, Williamson KS, Compton KD, Richards L, McInnerney K, Pitts B, and Stewart PS
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: National Institutes of Health

Bacterial biofilms develop into three-dimensional structures that include the bacteria and their secreted extracellular matrix materials. Since the bacteria adapt to their local microenvironments, biofilms contain cells in a variety of physiological states. One hypothesis for the increased resistance of biofilm bacteria to antimicrobial agents is that a subpopulation of metabolically distinct cells is able to tolerate treatments. To test this, we characterized heterogeneity in *P. aeruginosa* biofilms using three approaches: (i) laser capture microdissection (LCM) combined with transcriptomics to study localized gene expression, (ii) yellow fluorescent protein (YFP) fusions to track protein expression patterns, and (iii) fluorescent activated cell sorting (FACS) to identify the antibiotic resistant cells. Our results indicate that *P. aeruginosa* biofilms contain at least two distinct subpopulations, an actively growing cell fraction and a population of cells that is likely dormant. The active cells contain high mRNA levels for genes regulated by quorum sensing (QS), RpoS, and Anr. Expression profiles suggest that these cells are stressed and in transition to a stationary phase-like state. The dormant cells have very low mRNA abundances for most genes and are tolerant to ciprofloxacin and tobramycin. Although inactive, these cells contain mRNA for genes that may be required for cell dormancy, including the molecular chaperone, *ibpA*, and the ribosome hibernation factors, *rmf* and *hpf*. To visualize these proteins within the biofilms, we constructed *P. aeruginosa* RMF-YFP and IbpA-YFP protein fusions. Interestingly, the RMF-YFP fusion showed uneven vertical distribution, with the greatest protein abundance at the top of the biofilms. In contrast, the IbpA-YFP protein was distributed uniformly throughout the biofilms, but only observed in a small subpopulation of the cells. The low IbpA-YFP levels in most cells may be due to post-transcriptional regulation of *ibpA* by its 5'UTR riboswitch. To test this, we deleted one or both RNA hairpin loops from the *ibpA* mRNA 5'UTR. When both hairpins were deleted, *ibpA* mRNA and IbpA-YFP were reduced, indicating this riboswitch influences *ibpA* gene expression or mRNA stability. Overall, the results indicate that gene expression and antibiotic tolerance in biofilms are heterogeneous. Individual proteins are only translated in certain cell subpopulations, regulated in part by post-transcriptional processes.

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CBE Poster #593

Date: 12/2012
Title: **Laboratory-scale column studies to evaluate ureolytically driven $CaCO_3$ mineralization**
Authors: ***Ellen Lauchnor***, Phillips A, Cunningham AB, and Gerlach R
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Sponsored by: U.S. DOE and the National Science Foundation

Calcium carbonate mineralization as a result of the microbial process of ureolysis is being studied for multiple applications in the subsurface. One such potential application is the prevention of near well-bore CO_2 leakage by microbially induced carbonate precipitation in small fractures and leakage pathways around wells in CO_2 injection sites. The microbially catalyzed hydrolysis of urea increases alkalinity and pH, thus promoting $CaCO_3$ precipitation in the presence of dissolved calcium. While the enzyme urease is widespread among microorganisms, we are studying the kinetics of this process in porous media using the model organism *Sporosarcina pasteurii* in two-foot long, sand-filled columns. The columns contain five sampling ports for spatio-temporal observation of ureolysis and calcium precipitation kinetics. We have evaluated the rates of these reactions under different conditions to optimize the timing of fluid injection

and to evaluate the effect of different media components on the mineralization process. Additionally, the columns have been operated using an optimized injection strategy of fluids to minimize mineral plugging in the column inlet region. Thus far, these experiments have shown that an economical source of urea (i.e., fertilizer) can be used for this process. To quantify reduction of porosity and plugging in the column, computed x-ray microtomography was performed on the column after mineralization and on an untreated sand-packed column. The reduction in porosity from 48% in clean sand to about 30% in the inlet region and 24% in the rest of the column after mineralization, as determined by CT image analysis, agreed with calculations of the calcite volume occupying the column pore space, determined from destructive measurements of the precipitates. The goal of these experiments is to better understand the factors involved in kinetics of ureolytically induced mineralization, which can be applied in subsurface environments such as using introduced or native ureolytic microorganisms to induce $CaCO_3$ mineral formation for leakage mitigation in subsurface CO_2 injection sites.

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CBE Poster #594

Date: 01/2013

Title: **In situ and laboratory enriched microbial community composition and function associated with coal-bed methane from Powder River Basin coals**

Authors: ***Elliott P Barnhart***[1,2,4], Clark AC[4], Orem WH[4], Cunningham AB[1,3], and Fields MW[1,2]

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Sponsored by: U.S. Geological Survey, U.S. DOE–ZERT

Natural gas from coal (coal-bed methane) is becoming increasingly important worldwide as a result of the need to provide lower carbon emitting energy sources while meeting the rising energy demand. Most coal-bed methane is microbial in origin, but little is known about the in situ microbial community or the environmental conditions conducive to coal-bed methane formation. Currently, extraction of methane from subsurface coal seams is not sustainable, partly due to a slow in situ methane production rate. An increased understanding of this microbial system, and the biotic and abiotic parameters that control its activity, may expedite development of strategies to stimulate in situ, microbially enhanced coal-bed methane production.

The ecology and physiology of the in situ methane-producing microbial community was determined by examining subsurface samples of strata and coal-utilizing microbes from the Powder River Basin, USA. Core samples obtained above, within, and below a methane-producing coal seam were analyzed using 454-pyrosequencing to identify and determine the vertical distribution of specific members of the in situ microbial community. An inoculum that could be studied in the laboratory was collected in a diffusive microbial sampler that was loaded with coal and deployed at the bottom of a methane-producing well for approximately 90 days. The composition and structure of this inoculum were investigated by 454-pyrosequencing and microscopy as well as cultivation techniques (with and without nutrient supplementation) that maximized methane production in batch, bench-scale incubations. DNA analysis of microbes in the cores, diffuse microbial sampler coal, and laboratory enrichments identified predominant small subunit ribosomal DNA sequences closely related to microorganisms within the domains Bacteria and Archaea, indicating in situ methane production was predominantly hydrogenotrophic, while laboratory-based nutrient additions induced acetoclastic methane production. This information provides new insight into in situ and laboratory-based, stimulated microbial coal-bed community composition and physiology, which may lead to strategies to stimulate in situ, microbially enhanced coal-bed methane production.

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CBE Poster #595

Date: 02/2013
Title: **Microscopic evidence of difference in *Pseudomonas aeruginosa* biofilm architecture between the front and back surface of a CDC coupon**
Authors: ***Lindsey Lorenz***, Buckingham-Meyer K, and Goeres D
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In ASTM Method E2562-12, a Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using the CDC Biofilm Reactor, a biofilm is grown on 24 coupons placed in eight rods. The rods are inserted into the reactor top so that the inside surface of the rod faces a baffle that rotates at 125 RPM. The method specifies sampling the side of the coupon that faces the baffle to determine the biofilm viable cell density after 48 hours of growth. The baffle side of the coupon was chosen because it experiences higher fluid shear—due to the fluid dynamics present in the reactor—than does the back side of the coupon. This poster depicts the microscopic differences between the *Pseudomonas aeruginosa* (ATCC 15442) biofilm on the front and back side of the CDC biofilm reactor coupons and qualitatively demonstrates the importance of fluid dynamics in influencing biofilm architecture.

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CBE Poster #596

Date: 02/2013
Title: **Removal of bacterial spore contamination from needleless connectors using disinfection devices**
Authors: ***Elinor deLancey Pulcini*** and James G
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Bard Access Systems

A variety of disinfection devices has recently become available to replace standard alcohol preparatory pads for the disinfection of needleless connectors. These devices contain isopropyl alcohol (IPA) and apply various levels of mechanical friction (scrubbing) during use. To evaluate the effect of scrubbing on surface cleaning, we performed in vitro experiments with various devices using needle-free connectors contaminated with bacterial spores that were not susceptible to the IPA disinfectant. The injection ports of MaxPlus® connectors were inoculated with approximately 10^5 Colony Forming Units (CFU, mean = 5.6±0.1 Log10 CFU) of *Bacillus cereus* spores. The connectors were then cleaned with various devices according to the manufacturer's instructions for use, and 5 ml of sterile PBS was flushed through each connector. The number of spores (Log10 CFU/connector) was determined in the flush solution by plate count. The ranking of the devices from lowest to highest mean Log10 CFU/ml (±repeatability standard deviation) in the flush was: Site-Scrub® (1.1±0.49), IPA preparatory pad (2.1±0.99) and Curos® (3.2±0.82). All of the devices significantly reduced the number of spores in the flush relative to the untreated control (p=0.0000-0.0003). Flush counts from connectors cleaned with the Site-Scrub® device were significantly lower than the other devices tested (p=0.0000-0.0079). Cleaning with a standard IPA preparatory pad resulted in lower spore counts than the Curos® device (p=0.0049). This analysis of spore ingress through needleless connectors cleaned with various devices resulted in a ranking that correlated with ranking based on the amount of mechanical friction applied with each device. These results suggest that mechanical friction may result in better cleaning of needleless devices, particularly for microorganisms resistant to the chemical disinfectant applied.

CBE Poster #597

Date: 02/2013
Title: **An investigation of the gel properties of microbial alginate using magnetic resonance**
Authors: ***Matthew L. Sherick***[1,2], Sarah J. Vogt[1,2], Hilary T. Fabich[1,2], Varsha V. Rao[1,2], Joseph D. Seymour[1,2], Sarah L. Codd[2,3], Jennifer R. Brown[1,2], and Michael J. Franklin[2]
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Sponsored by: Undergraduate Scholars Program, Montana INBRE

Alginate is a biopolymer isolated from brown algae and certain genera of bacteria, such as *Pseudomonas aeruginosa*. Research involving alginate is relevant to biotechnology and biomedical applications due to its ability to form a physical gel with divalent cations such as Ca^{2+} and Cu^{2+}. In this work, the gelation properties of algal alginate and alginate from two mucoid *P. aeruginosa* strains are examined using magnetic resonance (MR) techniques. Each type of alginate studied differs in molecular structure and/or molecular weight, and differences in gel properties can be associated with these molecular variations. Gelation under homogeneous and diffusive reaction conditions is examined, which allows for molecular scale characterization as well as analysis of microscale structure formation, such as capillaries. 2D correlation experiments performed on homogeneous gels give insight into the effect of O-acetylation on T_2 relaxation in solution and the gel phase. Imaging and 1D experiments performed on diffusive gelation of alginate show that, under certain conditions, bacterial alginates have a higher reaction rate than algal alginate. Imaging techniques have been used to observe capillary formation in each type of alginate gel, with algal alginate gel forming highly ordered capillaries in the presence of low NaCl concentration. Capillaries in algal alginate gel are also shown to coalesce under certain conditions.

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CBE Poster #598

Date: 01/2013
Title: **Taxis toward hydrogen in *Methanococcus maripaludis***
Authors: Kristen Brileya[1,2,3], ***James Connolly***[1,4], Robin Gerlach[1,4], and Matthew Fields[1,2,3]
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Sponsored by: US DOE Office of Biological and Environmental Research

Anaerobic microbial communities play important roles in a broad range of applications and environments including waste water treatment, corrosion, oil souring, medical and dental biofilms, coal bed methane, acid mine drainage, biofuel and biogas production as well as bioremediation. *Methanococcus maripaludis* is an anaerobic, motile archaeum that can use hydrogen or formate as electron donor to reduce carbon dioxide to methane. *M. maripaludis* has been shown to grow as a pellicle at the hydrogen-liquid medium interface in static batch tubes and to be attracted towards syntrophic, hydrogen-producing partners, such as sulfate-reducing bacteria, in continuous culture. Although it has long been suspected that motile Archaea exhibit taxis toward hydrogen gradients, it has never been observed directly. The goal of this study was to subject starved *M. maripaludis* cells to a hydrogen concentration gradient and track cell movement. Square glass capillary tubes (1.0 mm) were partially filled with a cell suspension without hydrogen gas, and the gas portion of the capillary tube was equilibrated in an anaerobic chamber that contained only nitrogen and carbon dioxide. A gas tight syringe with pure hydrogen was attached to the gas side of the capillary and the entire assembly was placed on a microscope stage. A valve installed between the gas tight syringe and the capillary allowed addition of hydrogen in a controlled manner under microscopic observation. High resolution time-lapse images of swimming cells were collected before and after hydrogen introduction and quantified with particle tracking software.

A 1D finite element model was constructed to predict the hydrogen concentration gradient at the point of observation over the duration of the experiment. A Keller-Segel chemotaxis model was also incorporated that allowed for parameter fitting to observed swimming behavior. Biased random walk behavior was observed after hydrogen was allowed to diffuse into the system with population migration towards higher hydrogen concentrations. Biased taxis was not observed when hydrogen was replaced with argon.

To the best of our knowledge this is the first direct observation of taxis towards hydrogen in any domain of life. This represents an important eco-physiological strategy for methanogens, as there are several members of most orders that are either motile or have gas vesicles that can be used to move to a more favorable location. Hydrogen is one of the most important methanogenic substrates, as well as a common source of electrons for other organisms in anaerobic environments, including sulfate-reducing bacteria and acetogens. The ability to move toward higher concentrations of hydrogen could incur an advantage to organisms that are otherwise outcompeted by anaerobes that are able to utilize hydrogen at lower concentrations.

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CBE Poster #599

Date: 02/2013
Title: **NMR technologies for monitoring biological and geochemical processes in the subsurface**
Authors: ***Alexis B. Sanderlin***, Kirkland CM, Vogt SJ, Hiebert R, Grunewald E, and Codd SL
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: U.S. Department of Energy under Grants DEFG02-11ER90025 and 97357 S11-1 81.049

Reducing the negative impact of environmental contamination has become an important issue for society. It is challenging to manage multiple locations where the possibilities of high levels of subsurface contamination exist. A solution to this problem is creating and implementing bioremediation technologies in these areas. Currently, direct sampling of the subsurface requires collecting and sifting through contaminated samples, which poses significant safety, regulatory, and cost issues. Because these issues reach the severity they do, an alternative approach using nuclear magnetic resonance, or NMR, is being investigated as an effective monitoring process.

High-field NMR measurements are capable of detecting biofilm. However, high-field NMR is not suitable for biofilm detection in the subsurface due to the high magnetic field susceptibility effects in these materials. Therefore, it has been proposed to use an in situ NMR machine that will operate at a low field of 275 kHz. A low-field instrument was provided on loan from Vista Clara, Seattle, WA. In the first set of experiments, a 2-in diameter bioreactor was used to grow a *Bacillus mojavensis* biofilm. Samples of clean sand and biofouled sand were compared and the NMR relaxation time clearly differentiated between the two samples. This indicates that low field in situ NMR devices will be able to monitor bioremediation processes. The next phase of this project has begun with the construction of a much larger bioreactor for use with the Javelin tool, also from Vista Clara.

CBE Poster #600

Date: 02/2013
Title: **Diatom biofuels: Optimal nutrient requirements for lipid production**
Authors: ***Karen M. Moll***, Gardner RD, and Peyton BM
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: U.S. Department of Energy, Office of Biomass Programs grant DE-FG36-08G018161

Background: Diatoms offer a unique opportunity for algal biofuel feasibility. Due to the presence of siliceous cell walls, diatoms require less carbon for cellulose cell walls or starch, compared to green algae. Rather, they can store a greater amount of fixed carbon as lipids. Additionally, lipid extraction requires less

energy to disrupt the frustule, thus decreasing the amount of energy required for lipid extraction, and decreasing cost.

Some diatom strains have a naturally high lipid content in the form of triacylglycerol (TAG), especially when stressed. Optimization of lipid production for these strains is critical to improve the feasibility of diatom biofuels. Previous data have shown that diatom growth and lipid accumulation are dependent on silica utilization. The addition of sodium bicarbonate coupled with nitrate limitation significantly increases the rate and extent of lipid accumulation. This study further elucidates conditions to optimize lipid accumulation for a diatom by focusing on combined stresses to induce maximal lipid production.

Methods: A diatom isolated from Yellowstone National Park was grown with varying silica, carbon, and nitrogen concentrations. Growth was monitored using direct cell counts, pH, and chlorophyll. Nitrate and silica utilization were quantified using Ion Chromatography (IC) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), respectively. TAG measurements were monitored by Nile Red fluorescence and confirmed by gas chromatography.

Results: Diatoms grown under higher silica concentrations resulted in an increase in cell yield and dry cell weight, as well as TAG content and biofuel potential. This indicates an optimum silica concentration for growth. Once silica was depleted, lipid accumulation was promoted. The rate of TAG accumulation increased following $NaHCO_3$ addition and nitrate limitation and was approximately double compared to cells that did not receive the two additional stresses.

Conclusions: Following silica depletion, cells appear to redirect carbon into storage molecules (TAGs) that can be converted to biodiesel. The addition of $NaHCO_3$ coupled with nitrate limitation increased the rate of TAG accumulation. Coupling silica utilization with sodium bicarbonate addition and nitrate limitation exceeded TAG concentrations previously obtained and reached those levels at a faster rate. Results have importance on an industrial scale by decreasing the time required to reach maximal lipid accumulation for algal growth systems.

Keywords: triacylglycerol, biodiesel, lipids, diatom, silica, algae

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CBE Poster #601

Date: December 2012

Title: **Isolation and characterization of phototrophs for a renewable organic fertilizer**

Authors: Casey Doney[1], ***Rich Macur***[1,2], Lisa Weeks[2] and Brent Peyton[1,2]

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Sponsored by: American Indian Research and Engineering Initiative - US Department of Energy, American Indian Research Opportunities Program, Montana State University, Bozeman, MT, USA

Native American students from across Montana are working on a project titled "Phototrophs for carbon capture from the coal liquefaction process." This project is designed to aid the development of high quality, clean burning transportation fuels sourced from coal on the Crow Reservation. The Crow Nation contains approximately 3% (10 billion tons) of the U.S. coal reserves, which is one of the largest known coal deposits in the U.S., and is currently exploring multiple avenues to utilize this resource in an environmentally friendly manner. The overall goal of this project is to develop fast-growing strains of nitrogen-fixing cyanobacteria that are adapted to live in south central Montana and to test the use of these strains as an organic fertilizer.

Thus far, the project has yielded 12 nitrogen fixing cyanobacterial strains from south central Montana that could potentially be used to extract waste CO_2 from the coal liquefaction process and provide a fertilizer for

crop production. Greenhouse experiments were conducted using *Anabaena* sp. strain 16 biomass as a substitute for commercial fertilizer. Growth of wheat was significantly greater with cyanobacterial biomass in comparison to controls that received: 1) water, 2) commercial nitrogen fertilizer (35-0-0), or 3) a full suite of macro- and micro-nutrients. The availability of native cyanobacterial populations that are adapted to live in Montana may prove useful when coal-to-liquid fuel technologies are implemented on the Reservation.

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Industry & Agency Posters

Date: 02/2013
Title: **The use of the CDC Biofilm Reactor to test cleaning products**
Authors: ***Brandon Dell'Aringa***, ***Amanda Deal***, Klein D, and Lopolito P
Affiliation: STERIS Corporation, Mentor, OH, USA

Biopharmaceutical, pharmaceutical, medical device, dietary supplement, active pharmaceutical ingredient, cosmetic and personal care product manufacturers face many cleaning issues within a highly regulated and challenging environment. Prior to conducting large-scale field trials to determine the best cleaning products and parameters for use in the facility, performing a laboratory-based feasibility evaluation is often valuable. We used the CDC Biofilm Reactor to generate *Pseudomonas aeruginosa* biofilms on 316L stainless steel coupons. These coupons were then cleaned using various cleaning agents and parameters in an agitated immersion process. The impact of variables such as cleaning product selection, temperature, and coupon hydration were evaluated. Cleanliness of the coupons was evaluated visually and by total organic carbon (TOC) analysis.