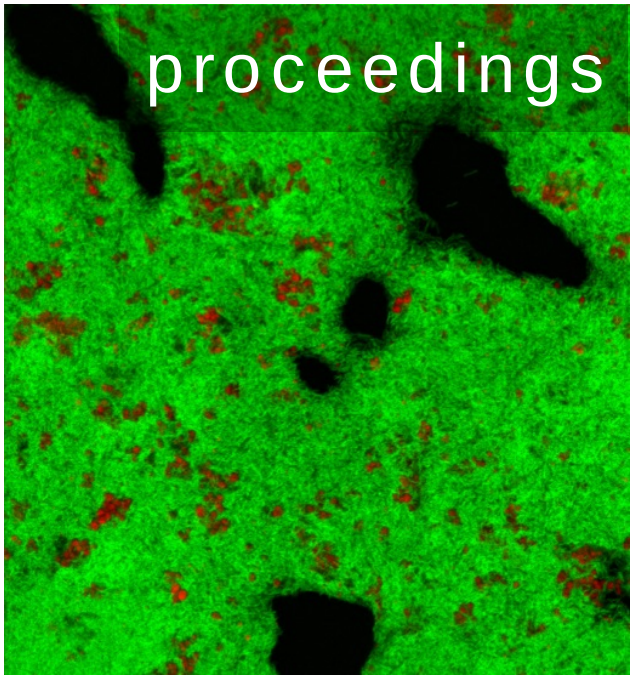
Montana State University

Center for Biofilm Engineering

Bozeman

montana biofilm SCIENCE & TECHNOLOGY meeting

JULY 16–18, 2013



L Camilleri, MSU-CBE 2013

Montana Biofilm Science & Technology Meeting: July 16-18, 2013
Table of Contents: Speaker Abstracts

SESSION 1: Oral Biofilms

SESSION 2: Cyclic-di-GMP

SESSION 3: Biofuels / Algal Biofilms

SESSION 4: Medical Biofilms

Special Presentations

SESSION 5: Biofilm-Mineral Interactions

SESSION 6: Biomimicry

SESSION 7: Biofilm Methods

Table of Contents: Poster Abstracts

Center for Biofilm Engineering posters

Industry and Agency Posters

Speaker Abstracts

SESSION 1: Oral Biofilms

Visualizing the spatial organization of microbes in oral biofilms

Presenter: Jessica L. Mark Welch, Assistant Research Scientist
Co-authors: Alex M. Valm[1], Blair Rossetti[1], Floyd E. Dewhirst[2], and Gary G. Borisy[1]
Affiliation: [1]Marine Biological Laboratory, Woods Hole, MA, USA;
[2]The Forsyth Institute, Cambridge, MA, USA.

Many biofilms, including those in the human mouth, are composed of mixtures of taxonomically distinct microbes that live in spatially and metabolically complex and interactive communities. The spatial organization of the microbes relative to each other and to the substrate is poorly understood, but many cooperative and competitive interactions among microbial cells are critically dependent on distance between the cells. Thus, knowledge of these spatial relationships is critical to understanding the biology of organisms in the biofilm.

Fluorescence in situ hybridization (FISH) targeting ribosomal RNA has been used to identify microbes and to investigate their spatial arrangement, but is generally employed to differentiate only two or three taxa simultaneously. Analysis methods based on DNA sequencing, by contrast, have identified dozens of bacterial genera and several hundred species-level taxa in dental plaque, but the process of nucleic acid extraction for sequencing destroys fine-scale spatial information.

We have developed a method—Combinatorial Labeling and Spectral Imaging–Fluorescence in situ Hybridization (CLASI-FISH)—that can be used to increase dramatically the number of fluorescent labels that can be differentiated simultaneously, and that thus is capable of identifying and visualizing sufficient taxa to begin to describe the structural complexity of oral biofilms. The method entails labeling each taxon with a distinct combination of fluorescent reporter molecules to create a unique spectral signature, which is then recorded using spectral imaging microscopy. Using this method we have differentiated bacterial cells labeled with 28 distinct binary combinations of eight fluorophores in a single microscopic field of view in a proof-of-principle experiment using commercially available microscopes and linear unmixing algorithms.

We have employed CLASI-FISH on dispersed dental plaque, using probes targeting genus-level and family-level groups of oral bacteria, to visualize and identify 15 bacterial taxa simultaneously in a single microscopic field of view. We have also applied the method to semi-thin sections of samples that have been cryosectioned or embedded in paraffin or methacrylate resin so as to preserve the spatial structure of the sample. We employ probes targeting genus-level and family-level groups, followed by application of probes targeting species and groups of species to define more precisely the taxonomy of cells that form structures of interest. These CLASI-FISH data call attention to taxa that may be involved in both cooperative and potentially competitive interactions in dental plaque, and suggest avenues for further genomic, biochemical, and metabolic studies to elucidate the roles and functions of the taxa involved.

Back to page 1

Evaluating oral biofilm treatments using the CDC biofilm reactor and treatment imaging flow cell

Presenter: Garth James, Associate Research Professor, Department of Chemical & Biological Engineering, MSU-CBE
Co-authors: Alessandra Agostinho Hunt, Laura Bickle, Steve Fisher, Kelly Kirker, Elinor deLancey Pulcini
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

The CDC biofilm reactor (CBR) was originally developed by Donlan et al. at the Centers for Disease Control [1]. The Center for Biofilm Engineering used the CBR as the basis of a standard method for growing *Pseudomonas aeruginosa* biofilms that was approved by ASTM International (E 2562-12). Biofilms are grown in the CBR under relatively high shear conditions compared to other biofilm growth systems. This method has been shown to be rugged and repeatable with several different microorganisms [2]. We have used the CBR to grow oral biofilms consisting of either single-species *Streptococcus mutans* biofilms or undefined mixed-species biofilms derived from human saliva. "Supragingival biofilms" were grown using 10%-strength tryptic soy broth amended with sucrose (0.5%) as well as amphotericin B to inhibit fungal growth. "Subgingival biofilms" were grown using modified Bradshaw-Marsh medium. The biofilms were then used to evaluate toothpastes, gels, and mouthwashes. The treatments were applied with fluid shear created by vortexing to simulate the use of oral care products. Using these methods, we were able to distinguish differences in biofilm removal and killing between different toothpaste, gel, and mouthwash formulations, demonstrating that the methods were responsive. Furthermore, treatment effects were visualized in real-time using a newly developed treatment imaging flow cell that was designed to hold CBR coupons. A variety of staining techniques was used to assess bacterial viability as well as biofilm components including nucleic acids, proteins, and extracellular polysaccharides. The combination of these techniques provides new tools for in vitro investigations of oral biofilms.

1. Donlan RM, Murga R, Carpenter J, Brown E, Besser R, Fields B, "Monochloramine disinfection of biofilm-associated *Legionella pneumophila* in a potable water model system," in: *Legionella*, Marre R (and others), Editor, American Society for Microbiology, Washington DC, 2002; 406–410.
2. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM, "Statistical assessment of a laboratory method for growing biofilms," *Microbiology*, 2005; Vol 151: 757–762.

Back to page 1

Survey of emerging approaches for control of oral biofilms

Presenter: Philip S. Stewart, CBE Director and Professor, Department of Chemical & Biological Engineering, MSU-CBE
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

This presentation will review several strategies for the control of dental plaque that are either truly new or are being revisited in the context of biofilm science and theory. The strategies are: antimicrobial peptides, probiotics, anti-biofilm combination therapy, photodynamic therapy, hydrodynamic removal, and natural products. These approaches are illustrated with examples from the recent literature. Antimicrobial peptides specifically engineered to target the cariogenic organism *Streptococcus mutans* demonstrate the potential of peptides to be used in a "surgical strike" capacity. A meta-analysis of clinical trials published last year evaluated use of beneficial probiotic bacteria, mostly *Lactobaccili*, in human subjects and found that these bacteria can suppress the numbers of mutans *Streptococci* and promote the reversal of root caries lesions. A novel anti-biofilm therapy inhibits production of extracellular polysaccharide matrix polymers by *S. mutans*. Photodynamic therapy combines an innocuous chemical photosensitizer with light to achieve an antimicrobial result. New designs for water jets could improve biofilm removal by mechanical means. Finally, there are many recent examples of new plant-derived natural products with potential for combating oral biofilms.

SESSION 2: Cyclic-di-GMP

Cyclic-di-GMP: A universal signaling molecule for bacterial biofilm formation

Presenter: Michael Franklin, Associate Professor, Department of Microbiology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Cyclic-di-GMP, a small molecule synthesized from two guanosine-5'-triphosphate (GTP) molecules, was first discovered as a cofactor essential for bacterial cellulose biosynthesis by *Gluconacetobacter xylinus*. Since then, enzymes for the biosynthesis and degradation of cylic-di-GMP have been found in all bacterial phyla. These enzymes contain unique domains, referred to as the diguanylate cyclase domain (GGDEF domain) and the phosphodiesterase domain (EAL or HD-GYP domain). Cyclic-di-GMP is now considered a universal bacterial secondary messenger molecule, since it is involved in a variety of bacterial cellular functions, including functions associated with the transition from planktonic growth to a biofilm-associated lifestyle. In particular, cyclic-di-GMP is known to be required for the synthesis of various extracellular polysaccharides, including N-acetylglucosamine (PAG) produced by many Gram-positive bacteria, and the PEL, PSL, and alginate extracellular polysaccharides of *Pseudomonas aeruginosa*.

Cyclic-di-GMP is also involved in the production of extracellular adhesive appendages, including the Type IV pili and curli, and is required for surface-associated twitching motility. Cyclic di-GMP is part of the regulatory circuit for the production of the large extracellular adhesive protein, LapA, of *P. fluorescens*. The mechanisms by which cyclic-di-GMP control transition from planktonic to biofilm growth are not fully known, but certain examples have now been characterized. In addition to its role as an allosteric effector of enzyme activity for polysaccharide and appendage production, cyclic-di-GMP has now been shown to interact with certain regulatory proteins or RNAs, controlling gene expression at the transcriptional and post-transcriptional levels. Here, we will review some of the major findings regarding the role of cyclic-di-GMP in bacterial biofilm-associated growth.

Back to page 1

Probing second messenger molecules in biofilm formation: C-di-GMP and pGpG inhibitors and chemical probes

Presenter: William M. Wuest, Assistant Professor, Department of Chemistry
Affiliation: Temple University, 1901 N. 13th St., Philadelphia, PA 19122, USA

Both cyclic diguanylate (c-di-GMP) and pGpG (the breakdown product of c-di-GMP that also serves as a signaling molecule) have been identified as important signaling molecules in many prokaryotic cellular processes. The small molecules play a vital role in signaling pathways that control motility, virulence, resistance, cell-cell communication, photosynthesis, biofilm formation, and other yet unknown functions. These processes are pertinent to the lifestyles of many pathogenic bacteria including; *P. aeruginosa* (cystic fibrosis), *S. aureus* (MRSA), *V. cholerae* (cholera), *B. anthracis* (anthrax), and *S. mutans* (endocarditis, periodontal disease). The enzymes responsible for production and regulation of c-di-GMP are not found in eukaryotic organisms, making them ideal targets for drug design. C-di-GMP is known to interact with a multitude of biological domains ranging from enzymes to riboswitches and presumably others that have yet to be discovered. Most recently, c-di-GMP has also been shown to regulate virulence factors responsible for cholera and anthrax. These approaches will be drastically accelerated by the availability of synthetic probes.

Recently, chemical probes of c-di-GMP have been synthesized to directly interrogate these pathways; however they lack both generality and, more important, cell permeability. Our group has initiated a synthetic endeavor toward the synthesis of a library of unique c-di-GMP and pGpG (the breakdown product of c-di-GMP which also serves as a signaling molecule) analogs with the dual purpose to act as both biofilm

inhibitors and chemical probes. More specifically, our work focuses on the construction of non-hydrolyzable analogs that replace the phosphate linkages with a variety of charge neutral linkers. We have applied this approach toward the synthesis of a pGpG analog to test the feasibility of the proposal. The talk will highlight the conceptualization of the research hypothesis and the synthesis and biological evaluation of our first biologically active analog.

Elucidating and targeting cyclic di-GMP signaling

Presenter: Chris Waters, Assistant Professor, Department of Microbiology & Molecular Genetics
Affiliation: Michigan State University, East Lansing, MI, USA

Cyclic di-GMP (c-di-GMP) is a newly appreciated second messenger signaling molecule that functions in the vast majority of bacteria as the lynchpin to control the switch between a sessile, biofilm lifestyle versus a motile, planktonic state. High levels of c-di-GMP stimulate biofilm formation and inhibit motility. Because this signaling molecule has only been intensively studied for less than a decade, there is a poor understanding of environmental control of c-di-GMP synthesis and degradation, c-di-GMP signal integration, and the signal transduction machinery that translates changes in c-di-GMP to phenotypic modulation. I will discuss my laboratory's explorations of how c-di-GMP controls gene expression in *Vibrio cholerae*—both at the level of transcription initiation by binding to specific transcription factors and at the level of post-transcriptional gene control through interaction with a c-di-GMP-dependent riboswitch. Moreover, I will present our identification of the first small molecules described to antagonize c-di-GMP synthesis enzymes and inhibit biofilm formation in a broad-spectrum fashion. We are currently exploring the potential of these compounds to inhibit problematic biofilm formation in both industrial and medical settings.

Back to page 1

SESSION 3: Biofuels / Algal Biofilms

Biofilm-based sustainable production of wastewater algae for biofuel and other co-products

Presenter: Ronald Sims, Biological Engineering Department Head & Co-Director, SWBEC
Co-Authors: Charles D. Miller, Assistant Professor, Department of Biological Engineering
Affiliation: Sustainable Waste-to-Bioproducts Engineering Center, Utah State University, Logan, UT, USA

Global energy requirements are heavily dependent on fossil fuels such as oil, coal, and natural gas. Our heavy dependence on petroleum based fuels is not sustainable due to diminishing crude oil reserves, increasing fuel costs, and the environmental impact of fossil fuel usage. With the anticipation of fossil fuels being exhausted in the future, novel strategies need to be discovered for alternative energy generation. Microalgae play a vital role in recycling carbon in the biosphere by converting carbon dioxide into organic compounds through photosynthesis. The conversion of algal biomass into bioproducts is not limited to biofuels, but can also include high-value co-products that positively impact the economics of algal biomass production systems. These properties and capabilities of microalgae make them an appealing feedstock for the production of renewable fuels and other bioproducts. Waste stabilization ponds, or lagoons, provide an ideal solution for wastewater treatment in developing countries and rural areas. The ability to couple wastewater treatment and microalgal growth provides a means to reduce microalgae production costs due to the availability of essentially free nutrients, water, sunlight, and atmospheric carbon dioxide. We have been addressing technical and associated economic barriers to large-scale algae biomass production, harvesting, and processing for wastewater treatment, biofuels, and bioproducts at the Logan City, Utah, Lagoons Wastewater Treatment Plant and Algae Test and Evaluation (AT&E) Facility. Our current research demonstrates a novel and scalable (up to 8,000 L) outdoor biofilm system of cultivation and harvesting of

phototrophic biomass with demonstrated downstream conversion to biofuels including acetone, butanol, ethanol, biodiesel, bio-methane and bioplastics. This research is devoted to developing innovative bio-based sustainable bioengineering technologies that recover and recycle nutrients and convert sustainable wastes into renewable bioproducts. A specific theme of this research is that existing infrastructure of wastewater treatment facilities in the US and around the world can be utilized for managed algae production—viewing wastewater not as a waste, but as a resource.

Direct measurement and characterization of active photosynthesis zones inside biofuel producing and wastewater remediating microalgal biofilms

Presenter: Robert Gardner, Postdoctoral Researcher
Co-author: Hans Bernstein, PNNL
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Microalgal biofilm based technologies are of keen interest for engineering processes due to their high biomass concentrations and ability to utilize renewable resources, light, and carbon dioxide. However, the direct measurement and characterization of fundamental parameters required for physiological analyses are challenging to due to the length scale of biofilm thickness and strong chemical and/or light gradients. This study adapted and developed oxygen microsensor-based methodologies for the direct measurement of essential photosynthetic biofilm parameters using a novel rotating algal biofilm reactor (RABR) system and demonstrated their feasibility on a pilot field-scale and laboratory-scale reactor system used for waste-water remediation and biofuel production, respectively. While photoautotrophic biofilms have long been used for wastewater remediation applications, concurrent or strict biofuel production represents a relatively new and under-represented focus area. The specific aims of this study were to: (i) characterize and compare two different RABR biofilms in the context of active photosynthesis zones, (ii) directly measure spatial gradients in steady-state oxygen and photosynthesis microprofiles, (iii) determine rates of photosynthesis and respiration processes, and (iv) characterize and compare the biofuel potential and (neutral lipid) precursor biomolecule composition in these biofilms. Clear differences in photosynthesis, respiration and biofuel-precursor capacities were observed between the two RABR systems and also between different conditions based on biofilm growth orientation in the field and nitrogen availability in the laboratory. Nitrogen depletion was not found to have the same effect on lipid accumulation in the laboratory biofilm cultures as compared to traditional planktonic studies. Physiological characterization of these microalgal biofilms identify potential areas for future process optimization and illustrate on two size scales, the fundamental usefulness of direct measurement of photosynthetic activities for determining ideal biomass areal density and biofilm thickness as it relates to active photo-production and photosynthesis zones.

Key words: Microalgae, Biofilm, Biofuel, Waste-water Remediation, Photosynthesis, Respiration

Back to page 2

Lipid profiling of *Chlamydomonas reinhardtii* cultured under three different inorganic carbon regimes

Presenter: Egan Lohman, PhD candidate, Department of Chemical & Biological Engineering, MSU-CBE
Co-Authors: Robert D. Gardner, Luke Halverson, Brent M. Peyton, Robin Gerlach
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Lipid contents of microalgal cultures are traditionally analyzed at time-of-harvest by quantifying the fatty acid methyl ester (FAME) content of dry biomass after transesterification. This approach, however, is unable to identify the compound each FAME was derived from, and further, does not provide a comprehensive evaluation of lipid profiles over time. By using relatively straightforward methods for quantifying extractable lipid classes (free fatty acids, mono-, di- or tri-acylglycerides) via GC-FID, and total FAME via GC-MS, lipid profiles over time were established for *Chlamydomonas reinhardtii* CC124 when grown under three different inorganic carbon regimes. Extractable lipids were characterized to include

lipid class (FFA, MAG, DAG, TAG) and carbon chain length for comparison against total FAME. Data points were collected each day throughout the growth cycles to provide comprehensive lipid profile analyses for *C. reinhardtii* grown with atmospheric concentrations of CO_2, elevated (5%) levels of CO_2 and for cultures supplemented with 50 mM sodium bicarbonate. All three conditions produced equal concentrations of saturated and unsaturated C16 fatty acids until nitrogen in the growth medium was depleted, after which a shift towards saturated C16 fatty acids was observed. This trend was more significant in cultures grown on elevated CO_2 and cultures supplemented with $NaHCO_3$. Unsaturated C18 fatty acids were the predominant FAME in all three conditions even though C16 triacylglycerides (TAG) were twofold more abundant than C18 TAG, indicating that C18 FAME were derived from compounds other than C18 TAG, such as membrane lipids. Free fatty acids (FFA) were the predominant lipid class prior to nitrogen depletion, but were significantly reduced over time as the organism began synthesizing glycerolipids, suggesting a reallocation of FFA into TAG, DAG and monoacylglycerides (MAG). Most interesting, in all three conditions the total FAME content on a weight-per-weight basis (weight FAME/weight biomass) did not significantly change over time; however lipid production per culture volume increased over time in all three treatments, reaching the highest concentration in cultures grown on 5% CO_2. Our results provide insight into which lipid compounds *C. reinhardtii* synthesizes and how the organism's metabolism changes due to nitrogen depletion and inorganic carbon source availability. The approach presented can be utilized for determining the optimal time-point of harvest during biodiesel production, as well as for screening for high value product accumulation (such as Ω-3 fatty acids).

Back to page 2

SESSION 4: Medical Biofilms

Molecular snapshots of pilus biogenesis, UTI pathogenesis and biofilm formation: Blueprint for therapeutics

Presenter: Scott Hultgren, Professor, Department of Molecular Microbiology; and Director, Center for Women's Infectious Disease Research
Affiliation: Washington University Medical School, St. Louis, MO, USA

Urinary tract infections (UTIs) result in an estimated 8 million outpatient visits yearly in the US alone, with an estimated cost exceeding $2.5 billion. Complicating both treatment and prevention efforts against UTI, there has been a worldwide increase in antimicrobial resistance of bacterial pathogens, including UPEC strains. For example, highly resistant ESBL-producing UPEC strains, once unheard of in the outpatient clinic, are now commonly seen in the urine cultures of persons with recurrent UTI and previous history of multiple courses of antimicrobials. Of concern, there are no evidence-based preventative options for recurrent UTI other than long-term antibiotic prophylaxis. Our studies blend multiple scientific disciplines elucidating bacterial and host mechanisms that determine the onset, course, and outcome of interactions between the host mucosal surface and bacterial pathogens of the urinary tract. Using genetics, genomics, biochemistry, structural biology, high-resolution imaging, animal models, clinical studies, and combinatorial chemistry, we have illuminated how bacterial intracellular lifestyles and community behaviors play critical roles in UTIs and how this further complicates treatment and prevention. We uncovered principles of adhesive pili biogenesis in Gram-negative bacteria via the chaperone/usher pathway; delineating molecular details of donor strand complementation and exchange mechanisms by which subunit folding is coupled with translocation and assembly of pili across the outer membrane. We delineated how uropathogenic *E. coli* (UPEC) use type 1 pili to invade and establish biofilm-like intracellular bacterial communities within bladder cells, subverting extracellular host defenses, and how quiescent intracellular reservoirs can seed recurrent infection. We identified complex networks governing mucosal epithelial responses that determine disease outcome. Further, we elucidated a mechanism by which bacteria form a directed biofilm-associated amyloid fiber called *curli*. Together, our work is changing the way UTIs are evaluated, re-shaping models of bacterial infections in general and spawning new

technologies to design novel vaccines and anti-microbial therapeutics to diagnose, treat, and/or prevent UTIs and their sequelae.

Defining biofilms on intravascular catheters as microbial communities

Presenter: Rodney M. Donlan, Research Microbiologist/Biofilm Laboratory
Affiliation: Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA

Microbes may colonize intravascular catheters and develop biofilms on these devices, resulting in catheter-associated bloodstream infections. Biofilms in soil and aquatic systems—and of the human microbiome—exhibit characteristics of microbial communities. Recent studies in our lab and by other groups suggest that microbial biofilms on intravascular catheters and connectors also display attributes of microbial communities. Catheter biofilms may contain multiple populations, interact with the substratum and the host environment, and exhibit resilience and stability. Work in our lab on needleless connectors of central venous catheters using culture-independent methods suggests that a highly diverse community of organisms originating from the skin and gastrointestinal microbiome as well as from the healthcare environment can colonize these devices. Approximately 94% of needleless connectors collected from patients in an intensive care facility contained viable organisms, as determined by solid phase cytometry. Amplification of the 16S ribosomal RNA gene from biofilm DNA samples from needleless connectors demonstrated that biofilm communities on these devices were diverse; culture-dependent methods will grossly underestimate the diversity of microbial communities on these devices. Biofilm communities are also known to interact with the substratum of the catheter or connector and exhibit resilience and stability, withstanding high shear forces, an immune response, and systemic antimicrobial agents. However, key questions remain. Evidence for interactions between organisms in the community (such as quorum sensing and horizontal gene transfer), metabolic interactions between different populations, and temporal progression of the community is still lacking. Answers to these questions can provide a basis for understanding pathogen survival and dispersal and result in central line maintenance practices that can reduce the incidence of central line-associated bloodstream infections.

Back to page 2

Catheter-associated urinary tract infection: How pathogenesis affects prevention and management

Presenter: Rabih O. Darouiche, MD; VA Distinguished Service Professor, Department of Medicine, Surgery and PM&R; Director, Center for Prostheses Infection
Affiliation: Baylor College of Medicine, Baylor University, Baylor, TX, USA

Healthcare-acquired infections are associated with major morbidity, often are lethal, can be difficult to manage, and are expensive to manage. Catheter-associated urinary tract infection is the most common healthcare-acquired infection. This explains the pressing need for investigation and implementation of multidisciplinary clinically protective approaches. A clear delineation of the pathogenesis of catheter-associated urinary tract infection could help analyze the reasons for clinical efficacy, or lack thereof, of potentially preventive approaches. Although adherence to traditional infection control measures has somewhat reduced the incidence of catheter-associated urinary tract infection, such infectious urinary complications continue to occur at an unacceptably high rate. Since a sizable portion of catheter-associated urinary tract infections is thought to be potentially preventable, it is essential to explore the potential benefit of novel strategies that could further enhance protection against infection.

Efficacy of disinfection devices and protocols to remove bacterial spore contamination of needle-free connectors

Presenter: Elinor deLancey Pulcini[1], Assistant Research Professor, Department of Chemical & Biological Engineering, MSU-CBE
Co-Authors: Matthew R. Trebella[2], Garth A James[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA; [2]Bard Access Systems, Salt Lake City, UT, USA.

A variety of disinfection devices have recently become available to replace standard alcohol preparatory pads for the disinfection of needle-free 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) of *Bacillus cereus* spores. The connectors were then cleaned with the Site-Scrub® IPA Device (Bard), Curos® Port Protector (Ivera Medical) or an IPA Preparatory Pad (ApliCare) according to the manufacturer's instructions for use. Then 5 ml of sterile PBS was flushed through each connector. The number of spores (Log_{10} CFU/connector) was determined in the flush solution by plate count. All devices significantly reduced the number of spores in the flush relative to the untreated control (p=0.0000–0.0003). In order of the most to least effective spore decontamination, the ranking was: Site-Scrub® IPA Device, IPA pad, and Curos® Port Protector. Flush counts from connectors cleaned with the Site-Scrub® device were significantly lower than the other devices tested (p=0.0000–0.0079). This analysis of spore ingress through needle-free 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 needle-free devices, particularly for microorganisms resistant to the chemical disinfectant applied.

Back to page 2

Special Presentation

State of the CBE

Presenter: Phil Stewart, CBE Director
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

SESSION 5: Biofilm-Mineral Interactions

Session introduction: Minerals and biofilms at the CBE

Presenter: Robin Gerlach, Associate Professor, Department of Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

An overview will be given of the biofilm and mineral formation-related work at the CBE. The session introduction will touch on ureolysis-induced calcium carbonate precipitation, mining-related activities, sulfate-reducing biofilms, microbially enhanced coal-bed methane production as well as the role of mineral dissolution and precipitation in algal biotechnology.

Controlling permeability reduction in the subsurface through biofilm-induced mineral precipitation: A multi-scale approach

Presenter: Adrienne Phillips[1,2], PhD student, Department of Chemical & Biological Engineering
Co-authors: Ellen Lauchnor[1], Joe Eldring[1], Anozie Ebigbo[4], Richard Esposito[5], Robin Gerlach[1,2], Alfred Cunnginham[1,3], Lee Spangler[6]
Affiliations: [1] Center for Biofilm Engineering,
[2] Department of Chemical & Biological Engineering, and
[3] Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[4] Department of Hydromechanics & Modeling of Hydrosystems, University of Stuttgart, Stuttgart, Germany.
[5] Southern Company Generation, Birmingham, AL, USA.
[6] Energy Research Institute, Montana State University, Bozeman, MT, USA.

Subsurface leakage mitigation strategies using ureolytic biofilm- or microbially induced calcium carbonate precipitation (MICP) have been investigated for sealing high permeability regions such as fractures. This technology may help in the deep subsurface to improve security of geologically stored carbon dioxide, to seal subsurface hydraulic fractures, or to enhance oil recovery. Sealing technologies using low-viscosity fluids—such as those used to promote MICP—are advantageous, since they may penetrate small aperture fractures not reachable by cement-based sealing technologies.

First, injection strategies to control region-specific precipitation were developed in two-foot long sand-filled columns. *Sporosarcina pasteurii* biofilms were established, and calcium and urea solutions were injected to promote mineralization. These injection strategies resulted in: 1) promoting homogeneous distribution of calcium carbonate along the flow path, and 2) minimizing near-injection point plugging. A Darcy-scale model was developed and calibrated against the first two column experiments and then used to predict the results of two more column experiments.

Second, the developed injection strategies were used to seal a hydraulically fractured, 74 cm diameter (meso-scale) Boyles Sandstone core under ambient pressures.

Finally, a novel high pressure test vessel (Figure 1) was developed to house the core at pressures up to 96 bar (1400 psi). The fractured core's permeability was reduced by promoting MICP at 44 bar (650 psi) of confining pressure, corresponding to depths approximately 1,500 feet below ground surface. After MICP treatment, the sandstone core withstood three times higher well bore pressures before re-fracturing compared to the initial fracturing event.

These studies suggest biofilm-induced $CaCO_3$ precipitation technologies may potentially seal and strengthen high permeability regions or fractures in the subsurface. MICP has been researched by others for applications such as: consolidating porous materials, improving or repairing construction materials, and remediating environmental concerns. Continued efforts to model and understand micro-scale processes that influence meso- and macro-scale distribution of calcium carbonate will help develop the use of MICP for field application.

Figure 1. High pressure test vessel for the examination of biogeochemical processes under radial flow and subsurface relevant pressures and scale conditions. The vessel is rated to 96.5 bar (1400 psi) and can house samples 74 cm in diameter and 38 cm high.

Back to page 2

Image-based modeling of biofilm-induced calcium carbonate precipitation

Presenter: James Connolly[1,2], PhD student, Department of Chemical & Biological Engineering
Co-Authors: Adam Rothman[1,2], Benjamin Jackson[1,4], Isaac Klapper[5], Al Cunningham[1,3], Robin Gerlach[1,2]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Chemical & Biological Engineering,
[3] Department of Civil Engineering, and
[4] Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA.
[5] Department of Mathematics, Temple University, Philadelphia, PA, USA.

Pore scale biological processes in the subsurface environment are important to understand in relation to many engineering applications including environmental contaminant remediation, geologic carbon sequestration, and petroleum production. Specifically, biofilm induced calcium carbonate precipitation has been identified as an attractive option to reduce permeability in a lasting way in the subsurface. This technology may be able to replace typical cement-based grouting in some circumstances; however, pore-scale processes must be better understood for this technology to be applied in a controlled manner.

There are many metabolic processes capable of inducing carbonate mineral precipitation; this work concentrates on urea hydrolysis. Urea is hydrolysed to form ammonia and carbon dioxide. The end effect of this is a pH increase—and in the presence of calcium (or other divalent cations), an increase in the saturation index. Once the solution reaches a supersaturated state, calcium carbonate precipitation is possible. In heterogeneous environments it is possible to have a wide range of saturation states within close spatial proximity. Furthermore, it is well known that biofilm also causes heterogeneous environments, so it is challenging to predict the spatial orientation of mineral precipitation with respect to a porous support structure and biological activity.

The work presented will focus on efforts to observe biofilm growth and mineral precipitation in micro-fabricated flow cells combined with finite element modeling as a tool to predict local chemical gradients of interest (See Figure 1). We have been able to observe this phenomenon over time using a novel model organism that is able to hydrolyse urea and express a fluorescent protein that allows for non-invasive observation over time with confocal microscopy. The results of this study show the likely existence of a wide range of local saturation indices even in a small (1 cm length scale) experimental system. Interestingly, the locations where the highest saturating index is predicted do not correspond to the locations of highest precipitation density, highlighting the need for further research.

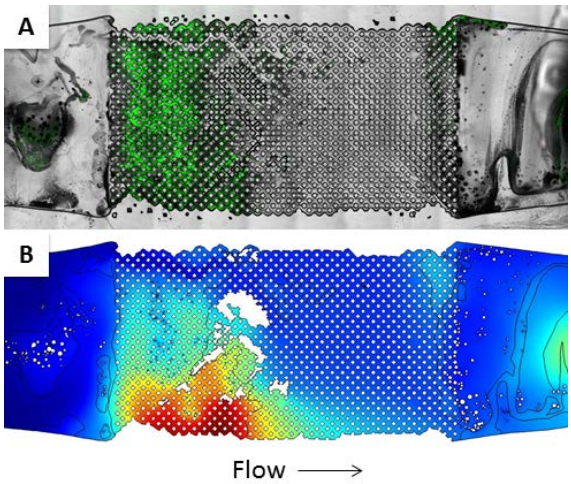


Figure 1. A micro-fabricated flow cell containing biofilm-induced calcium carbonate precipitation. (A) Experimental results: Active biofilm is in green and dark circles are calcium carbonate crystals. Note the channeling behavior in the top of the image, leaving a large, hydraulically inactive area in the biofilm mass. (B) Finite element model: The prediction of relative saturation of calcium carbonate (as calcite). Fluid enters the system at a low saturation state (blue) but areas of high supersaturation (red) are predicted within the hydraulically inactive area in the biofilm. If only effluent saturation was measured, precipitation may not even be predicted, but we see local, pore-scale behavior dictating system behavior in this case. The flow cell is 1 cm in length and the porous media elements are 100 μm.

Back to page 3

Critical occlusion via biofilm induced calcite precipitation in porous media

Presenter: Tianyu Zhang, Assistant Professor, Department of Mathematical Sciences
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

A model for biofilm induced calcite precipitation with constant head driven flow is presented in the context of a single pore within a porous medium. The model is based on a mixture model including biomaterial, mineral material, and water with dissolved components. Computational results suggest the possibility of critical clogging in the sense that there is a critical pressure head such that, for pressure drops below this critical level, pore clogging occurs relatively quickly while for pressure drops above, clogging occurs after much longer times, if at all.

Back to page 3

SESSION 6: Biomimicry

The art and science of biomimicry

Presenter: Dayna Baumeister, Co-founder
Affiliation: Biomimicry 3.8, Missoula, MT, USA

Biomimicry is an emerging discipline of an ancient practice. As the conscious emulation of nature's "genius," the practice of biomimicry has emerged as both a science (with specific methodologies) and an art (requiring creative thinking and interdisciplinary communications). This presentation will provide an overview based on our 15+ years of experience working with scientists, designers, engineers, and business-oriented colleagues in the field of biomimicry. The audience will gain an understanding of where insights from biology can inform design, how those ideas can be incorporated and the value in doing so. Focusing more on the practice, and less on the outcomes, the presentation seeks to provide listeners with a framework to explore how biomimicry thinking might inform their own processes.

Natural product mimetics that inhibit and disperse bacterial biofilms

Presenter: Christian Melander, Co-founder and Chief Research Officer, Agile Sciences; and Associate Professor, Department of Chemistry
Affiliation: Agile Sciences and North Carolina State University, Raleigh, NC, USA

Marine natural products have served as a rich source of biologically active small molecules. We have exploited the inherent anti-biofilm activities of the 2-aminoimidazole-containing secondary metabolites oroidin and bromoageliferin to generate simple, synthetically accessible small molecules that inhibit and disperse both Gram-positive and Gram-negative biofilms as well as mixed species biofilms. The historic development of these compounds, in addition to unpublished work highlighting their efficacy in vivo against *Pseudomonas aeruginosa* biofilms and in vitro against mycobacterium biofilms will be presented.

Back to page 3

Activity of antimicrobial peptide mimetics against fungal biofilms in vivo

Presenter: Gill Diamond, PhD, Professor, Department of Oral Biology
Co-Authors: Jorge Masso, Lisa K. Ryan, Klaudia Tokarz, Richard W. Scott, Katie Freeman
Affiliation: UMDNJ-New Jersey Dental School, Newark, NJ, USA

Oral candidiasis is a severe problem in immune compromised individuals, causing painful infections of the mouth due to a complex biofilm of Candidal species, consisting of hyphae and blastoconidia. While antimicrobial peptides exhibit strong antifungal activity against Candida in vitro, their use as therapeutic agents has been hampered by a number of factors, including cost of synthesis and inactivation of the peptides in vivo. Here, we showed the anti-Candida activity and mechanism of action of three small molecules (PMX 519, PMX1502 and PMX1570) that mimic the structure and properties of defensins, which were obtained by large-scale screening. These mimetics have shown promising in vitro results in killing *C. albicans* and are inactive against two species of oral commensal bacteria. Our results showed potent antifungal activity against planktonic cells and biofilms, and the killing kinetics showed rapid fungicidal activity within 6 hours. To demonstrate efficacy against an infection, we tested the three mimetics in two different mouse models of oral Candidiasis. Infected mice were treated with 10mg/ml of the compounds in a neutral hydrogel, applied three days after infection. Treatment with all three compounds led to a significant decrease in overall infection, better than the standard agent, Nystatin. PMX1502 showed the best performance, with an almost complete ablation of the infection by 24 hours after treatment. To examine the mechanism of action, we demonstrated that exposure to the mimetic caused a rapid influx of propidium iodide and efflux of ATP. Examination of the morphology using fluorescence and transmission electron microscopy (TEM) suggests that these mimetics bind to the cell membrane and can disrupt or affect integrity in the membrane. In conclusion, antimicrobial peptide mimetics are potential therapeutic agents against oral candidiasis, with the ability to kill the microbes while in a biofilm state.

Back to page 3

Packing them in: Using self-assembled protein cages to direct synthesis and packaging of polymers, minerals, and proteins

Presenter: Trevor Douglas, Professor, Department of Chemistry & Biochemistry
Affiliation: Center for Bio-Inspired Nanomaterials, Montana State University, Bozeman, MT, USA

Protein cages have emerged as useful platforms for synthetic manipulation with a range of applications from materials to medicine. Synthetic manipulation can impart new function, combining the best of evolution and directed synthetic design. We have developed a library of protein cage architectures—which differ in size, porosity, and stability—for synthetic manipulation. This library of cages includes ferritins, ferritin-like proteins, heat shock proteins, and virus capsids. The use of virus capsids has resulted in a paradigm shift from the study of viruses as disease causing agents to their realization as highly useful supramolecular assemblies, which can be chemically and genetically modified. In particular, the unique scaffold-templated self-assembly of the bacteriophage P22 capsid has been utilized for the directed synthesis and packaging of a range of gene products as well as organic, inorganic, and polymeric materials.

The packaging of material on the inside of the protein cages can dramatically change the physical properties of both the cage and the encapsulated cargo. We are investigating the effects of molecular crowding on encapsulated enzymes and polymers, the effects of the protein cage on the surface properties of encapsulated magnetic materials, and the influence of the encapsulated cargo on the physical properties of these composite materials when assembled into higher order structures. We are developing a wide range of bio-inspired composite materials that integrate protein architecture with organic and inorganic synthetic components as controlled nano-reactors and for targeted diagnostic agents. In addition, the use of protein cage architectures for antigen display, to direct specific immune responses for therapeutic applications, will also be discussed.

SESSION 7: Biofilm Methods

Results of a multi-laboratory evaluation of the Single Tube Method (ASTM Method E2871)

Presenter: Darla Goeres, Assistant Research Professor, Department of Chemical & Biological Engineering, MSU-CBE
Co-Authors: Al Parker, Danielle Orr, Blaine Fritz, Kelli Buckingham-Meyer, Lindsey Lorenz, Diane Walker
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

In 2012 ASTM Committee E35 approved Method E2871 titled "Standard Test Method for Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor Using the Single Tube Method." Method E2871 is the fifth biofilm method approved by E35. The Single Tube Method was originally developed in the EPA's Microbiology Laboratory Branch (MLB) for the purpose of determining the efficacy of liquid disinfectants against biofilm bacteria. Although E2871 was developed and validated using biofilm grown in the CDC biofilm reactor according to ASTM Method E2562, the method is flexible enough that biofilm grown in the other standardized reactors may be used. Under a contract with the EPA, the Standardized Biofilm Methods Laboratory (SBML) collaborated with the MLB to refine and validate the method in a two-laboratory mini-collaborative study. Based upon the favorable results, the SBML edited the method to be consistent with ASTM Form & Style Guidelines and submitted it to ASTM E35.15 for review and approval. The method was approved in April 2012. Once again working within ASTM Guidelines, the SBML conducted an Inter-laboratory Study (ILS) during the fall of 2012. This presentation will report the results of an ILS conducted in each of nine laboratories using three chemistries (sodium hypochlorite, phenol, and a quaternary/alcohol blend) applied at presumed high and low efficacy levels in triplicate experiments using three coupons per experiment.

Back to page 3

Putting proteomics and metabolomics to work for you

Presenter: Brian Bothner, Associate Professor, Department of Chemistry & Biochemistry
Affiliation: Montana State University, Bozeman, MT, USA

Cellular response to stress (metals, reactive oxygen species, viral infection) involves numerous networks and signaling pathways. The integration of signals leads to changes in gene and protein regulation which are ultimately "read-out" as changes in metabolism. We are applying mass spectrometry-based proteomics and metabolomics methods to elucidate cellular response to stress. In addition to standard proteomics approaches, activity based protein profiling (ABPP) is being used to address the activity of proteins on a global scale by tagging species with chemically reactive small molecules that specifically target active site residues. Our approach is motivated by the idea that biological mechanisms and states are best understood by combining information from different classes of biomolecules. Example applications will include Norovirus infection in a model system, redox biology of extremophiles, and looking for biomarkers in urine.

Biofilms on orbit and on Earth: Current methods, future needs

Presenter: Leticia Vega, Scientist, Water Recovery Systems Team
Affiliation: NASA-Johnson Space Center (Jacobs Engineering), Houston, TX, USA

Biofilms have played a significant role on the effectiveness of life support hardware on the Space Shuttle and International Space Station (ISS). This presentation will discuss how biofilms impact flight hardware, how on orbit biofilms are analyzed from an engineering and research perspective, and future needs to analyze and utilize biofilms for long duration, deep space missions.

Back to page 3

Poster Abstracts

Center for Biofilm Engineering posters

CBE Poster #563

Date: July 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 & Environmental Sciences, and
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3] Desert Research Institute, Reno, NV, USA.
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.

Back to page 4

CBE Poster #569

Date: February 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
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA; *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.

Back to page 4

CBE Poster #585

Date: February 2012
Title: **Field Emission Microscopy and growth modeling of a *Desulfovibrio alaskansis* G20 biofilm**
Authors: ***Gregory Krantz,*** Fields MW, and 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).

Back to page 4

CBE Poster #589

Date: November 2012
Title: **Genetic basis of *Pseudomonas aeruginosa* biofilm antibiotic tolerance**
Authors: ***Phil Stewart,*** Folsom JP, Williamson KS, Franklin MJ, Boegli L, and 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.

Back to page 4

CBE Poster #590

Date: December 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, and Spangler L
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: US Department of Energy

We have investigated mitigation strategies for sealing high permeability regions (such as 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.

Back to page 4

CBE Poster #593

Date: February 2013
Title: **Laboratory-scale column studies to evaluate ureolytically driven $CaCO_3$ mineralization**
Authors: ***Ellen Lauchnor***, Phillips A, Cunningham AB, and Gerlach R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: US 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.

Back to page 4

CBE Poster #594

Date: February 2013
Title: **In situ and 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]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Microbiology, and
[3] Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[4] US Geological Survey, Reston, VA, USA.
Sponsored by: US Geological Survey, US 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.

CBE Poster #595

Date: February 2013
Title: **Microscopic evidence of difference in *Pseudomonas aeruginosa* biofilm architecture between the front and back surfaces of a CDC coupon**
Authors: ***Lindsey Lorenz***, Buckingham-Meyer K, and Goeres D
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

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 sides of the CDC biofilm reactor coupons and qualitatively demonstrates the importance of fluid dynamics in influencing biofilm architecture.

Back to page 4

CBE Poster #598

Date: January 2013
Title: **Taxis toward hydrogen in *Methanococcus maripaludis***
Authors: Kristen Brileya[1,2,3], ***James Connolly***[1,4], Gerlach R[1,4], and Fields M[1,2,3]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[2] Department of Microbiology, Montana State University, Bozeman, MT, USA
[3] ENIGMA (http://enigma.lbl.gov)
[4] Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: US DOE Office of Biological & 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.

Back to page 4

CBE Poster #599

Date: January 2013
Title: **NMR technologies for monitoring biological and geochemical processes in the subsurface**
Authors: Sanderlin AB, ***Catherine M. Kirkland***, Vogt SJ, Hiebert R, Grunewald E, and Codd SL
Affiliation: Center for Biofilm Engineering and Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: US DOE

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 are severe, 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, an in situ NMR machine has been proposed 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-inch diameter bioreactor was used to grow a *Bacillus mojavensis* biofilm. Samples of clean sand and biofouled sand were compared and the NMR relaxation time was 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.

This material is based upon work supported, in part, by the Department of Energy under Grants DEFG02-11ER90025 and 97357 S11-1 81.049.

Back to page 4

CBE Poster #600

Date: February 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: US 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 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

Back to page 4

CBE Poster #602

Date: February 2013

Title: **Spatial analysis of the microbial community in mining waste rock: Activities and signatures**

Authors: ***Dana Skorupa*** and Kirk LB
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Teck Coal

Baseline microbial community structure and richness were investigated in a mine waste rock dump, which served as a source of constituents of interest (CI's) including selenium (Se) and nitrate (NO_3). Pyrosequencing of 16S rRNA gene amplicons from drill core samples enabled tracking of spatial changes within a 60 meter depth profile. Analyses showed communities shifting as a function of depth, which is likely due to changes in the geochemistry. Ordination analyses revealed the clustering of samples based on important bacterial communities. Microbes with the metabolic potential to reduce CI's such as Se, NO_3, and Iron (Fe), and those able to break down hydrocarbons were present in the waste rock, and occur in association with low oxygen zones, supporting the hypothesis that sub-oxic regions within the waste dump were present. Contrastingly, few sequences associated with sulphate-reducing populations were detected across the majority of transect positions. The possible presence of nitrate and selenate reducing bacterial populations provide important insights into whether microbial communities in waste rock can be managed through dump design and dump management to help influence the biogeochemical cycling and release of CI's.

CBE Poster #604

Date: May 2013
Title: **Microbial diversity and ecophysiology of cryoconite sediments from the McMurdo Dry Valleys, Antarctica**
Authors: Amber Schmit[1], ***Heidi Smith***[1], Pitts B[1], Foster R[2], and Foreman C[1]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[2] Max-Planck Institute for Marine Microbiology, The Biogeochemistry Group
Sponsored by: National Science Foundation

Cryoconites are formed by windblown sediments that settle on a glacial surface to an equilibrium depth within the ice. Cryoconite holes provide an aqueous environment in the ice and contain biologically active aggregations of microbes associated with sediment granules. A detailed investigation of the microbial diversity, activity, and granule structure of an Antarctic cryoconite from the Canada Glacier in the McMurdo Dry Valleys showed that there are metabolically active microorganisms associated with sediment particles.

Cryoconite granules were analyzed using scanning electron microscopy and powder x-ray diffraction, which showed that granule composition is relatively homogenous and primarily composed of silica oxides. Bulk organic matter content and composition were determined using step-wise thermogravimetric analysis. The organic matter was found to be 6.5% of the dry weight, with the greatest mass loss occurring between 200°C and 350°C, indicating that the majority of organic matter present is thermolabile. Confocal microscopy of individual sediment grains confirmed the association of microbial populations with sediment surfaces. Using fluorescent in situ hybridization (FISH), the total bacterial cell abundance is 7.26×10^5 cells ml^{-1} of sediment slurry. *Bacteroidetes* comprise 78.2% of the bacterial population followed by *Alphaproteobacteria* 2.6%, and *Gammaproteobacteria* 0.3%. Carbon fixation and ammonia assimilation rates were determined at the single-cell level using Halogen In Situ Hybridization-Secondary Ion Mass Spectroscopy (HISH-nanoSIMS). Uptake rates of ^{13}C using HISH-nanoSIMS were determined for the dominant Cyanobacteria, an *Oscillatoriales* sp., which showed high enrichment of ^{13}C, indicative of carbon fixation. There was no evidence of carbon transfer and successive uptake of released exudates by heterotrophic bacteria. Nitrogen uptake rates of ^{15}N labeled ammonium were calculated in both the *Oscillatoriales* and *Bacteroidetes* populations. Cryoconite sediments are important reservoirs of organic carbon, nutrients and microbial activity on glacial surfaces, where resources are sparse. Cryoconites accumulate windblown sediment, which promotes the aggregation of microorganisms to granule surfaces providing a refuge for microbial life in these harsh environments.

Back to page 4

CBE Poster #605

Date: April 2013
Title: **Effects of culturing conditions on hydrocarbon production by *Ascocoryne sarcoides***
Authors: ***Natasha Mallette***[1,2], Peyton BM[1,2], Carlson R[1,2], and Strobel G[3]
Affiliation: [1] Center for Biofilm Engineering,
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[3] Department of Plant Science and Plant Pathology, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation

Beyond characterization of cellulolytic fungal enzymes, very little research has examined the potential role of fungi in renewable fuel production. *Ascocoryne sarcoides* (NRRL 50072) is an endophytic fungus that can utilize cellulose as its sole carbon source. This fungus excretes "myco-diesel," a mixture of straight and branched chain hydrocarbons of C5-C10 chain length, in the range of gasoline and aviation fuel, including heptane, 2-pentene, octane, 1-methyl-cyclohexene, 3,5-octadiene, and cyclodecene. Experimental results from shake flask and 5 liter reactor runs have verified hydrocarbon compound (HC) production under a

wide array of growth conditions. The identifiable HC production by *A. sarcoides* is distributed over many types of organic compounds including acids, aromatics and alkanes. Growth on cellulose produced a higher number of fuel-related HC including alkanes and aromatics than growth on glucose. Oxygen level in the headspace also had a distinct impact on HC diversity; oxygen levels below 21% produced greater diversity. In late stationary growth on cellulose, there were more oxygenated compounds compared with aromatic and alkane production in the earlier stages of growth. A possible metabolism change in stationary phase is the oxidation of non-oxygenated HC to gain electrons. Future research will focus on combining knowledge of genetics with metabolic modeling to develop a deeper understanding of how *A. sarcoides* and other fungi are able to produce these fuel hydrocarbon compounds from cellulosic substrates.

CBE Poster #606

Date: July 2013

Title: **Changes in groundwater quality resulting from closure of an abandoned mine adit in the New World District, Cooke City, Montana**

Authors: Bozeman LR[1] and ***Lisa B. Kirk***[2,3]

Affiliation: [1] Department of Earth Sciences, and
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3] Enviromin, Inc., Bozeman, MT, USA.

Sponsored by: USDA Gallatin National Forest and Enviromin, Inc.

The historic New World district near Cooke City hosts abandoned Au-Ag-Cu mine workings from the early 1900s. The Glengary adit was driven beneath the Como basin to access Au and Cu, where it subsequently produced acid rock drainage (ARD) until it was closed using a hydraulic adit plug method in 2005. The adit produced ARD that has been monitored since 1989. Significant changes in the geochemistry of the water during the 8 years following closure of the adit include an increase in pH from approximately 3 to almost 6, with an associated increase in alkalinity and decreased Cu and Zn concentrations. Total dissolved solids concentration (TDS) has increased, due to increased concentrations of Fe, Ca, Mn, and SO_4. To evaluate these hydrogeochemical changes, data collected from the Glengary adit pre- and post-closure were downloaded from the USDA USFS New World Mining District Response & Restoration Project database (http://www.maximtechnologies.com/newworld/documents.html) for further geochemical analysis. Five pairs of pre-closure samples collected under low and high flow conditions were chosen for comparison with six additional years of data from the adit post closure using Stiff and Durov diagrams to evaluate changes in major ion chemistry. The Geochemist Workbench SpecE8® software was used to calculate changes in the relative equilibrium of the adit water with Fe, Mn, Al, SO_4, and CO_3 minerals resulting from the adit closure and subsequent changes in groundwater elevation and chemistry. Monitoring results show reduced acidity and metal concentrations associated with modeled precipitation of the minerals ferrihydrite, alunite, and gibbsite, but increased TDS due to dissolution of jarosite under increasingly alkaline conditions post-closure. Future work is planned to evaluate the iron redox chemistry and microbial community of the closed adit.

Back to page 4

CBE Poster #607

Date: June 2013
Title: **Biomineralization using biofilms: Estimating kinetic parameters using a simple flow channel model**
Authors: ***Ben Jackson***[1,2], Connolly, J[1,3], Rothman, A[1,3], Klapper, I[1,2,4], and Gerlach, R[1,3]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Mathematical Sciences, and
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[4] Department of Mathematics, Temple University, Philadelphia, PA, USA.
Sponsored by: National Science Foundation (NSF 0934696)

Microbially induced calcite precipitation (MICP) via urea hydrolyzing bacteria may be a useful mechanism in the formation of bio-cement, which has applications in soil stabilization, carbon sequestration, and concrete remediation. MICP is implemented in complex systems containing biofilms where the kinetic rates of ureolysis are not well known. We use a standard diffusion-advection-reaction equation coupled with a 1D Fickian biofilm model to describe the transport and kinetics of urea in a flow channel containing biofilm. Biofilm height profiles and urea effluent concentration values from tube reactor experiments conducted at Montana State University's Center for Biofilm Engineering are used to empirically solve for the Michaelis-Menten kinetic parameters of urea in the biofilm.

Back to page 4

CBE Poster #608

Date: June 2013
Title: **Rate of photosynthesis measurement for algal biofilms**
Authors: ***Muneeb S. Rathore***[1,2], Gardner R[1,2], Bernstein H[1,2], Moll K[1,3], Kesaano M[4], Sims RC[4], Carlson RP[1,2], Walker DK[1,2], Miller CM[4], and Peyton BM[1,2]*
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Chemical & Biological Engineering, and
[3] Department of Microbiology, Montana State University, Bozeman, MT, USA.
[4] Biological Engineering Department, Utah State University, Logan, UT, USA.
Sponsored by: NSF-IGERT, Church & Dwight Co, DOE, USTAR

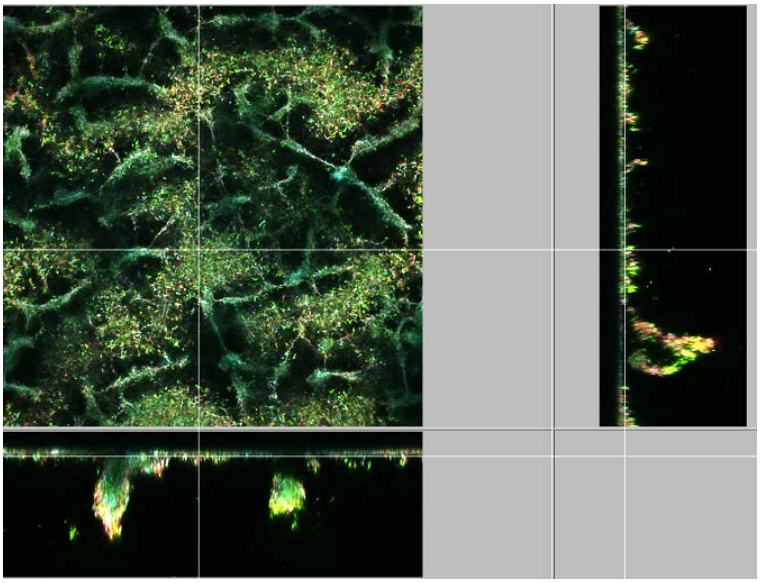
Concentrated biomass in the form of biofilms has gained attention because of the potential ease of harvesting the biomass for biofuel and bioproduct applications. In fact, it may be an ideal biotechnological platform for waste-water remediation or specialty chemical development. Algal biofilms were grown on lab-scale Rotating Algal Biofilm Reactors (RABRs) to characterize the biofilm growth through the rate of photosynthesis occurring within a biofilm. Oxygen microsensors were used for the measurement of oxygen concentration in the biofilm, and the rate of photosynthesis was then determined through flux calculations. Photosynthetic activity was observed to be greatest near the biofilm surface, possibly due to the greater photon flux at this location, and photoactivity was found to decrease to a negligible level at the bottom of the biofilm, where respiration became more pronounced as compared to photosynthesis. It was thus observed that the difference in physiology of the algal biofilms for photosynthesis and respiration can be established through microsensor oxygen measurement at steady state. Lipid accumulation, stimulated from nutrient-deplete stress, was analyzed using gas chromatography – flame ionization detection and mass spectroscopy for the lab-scale RABR systems. It was found that nutrient-deplete stress caused a minimal lipid accumulation in the algal biofilm. Additionally, confocal microscopy was used to image chlorophyll autofluorescence, triacylglycerol accumulation with Bodipy 505/515, and attached biomass. The results of this study indicate that additional research and development is needed for algal biofilms to be used as major source of algal biomass for biofuel and bioproduct applications.

Back to page 5

CBE Poster #609

Date: April 2013
Title: **Fluorescent imaging of *Pseudomonas aeruginosa* biofilms**
Authors: ***Amanda Richards,*** Pitts B, Stewart PS, and Franklin M
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: National Institute of General Medical Sciences of the National Institutes of Health under the Montana IDeA Network of Biomedical Research Excellence

Biofilms are surface-associated microbial communities, where the cells are attached to surfaces by extracellular matrix materials. The extracellular matrix is not well defined, but is thought to include secreted extracellular polysaccharides. The primary goal of this research was to characterize extracellular matrix of biofilms by using fluorescent probes and microscopic imaging. *Pseudomonas aeruginosa*, an opportunistic pathogenic bacterium, was used as the test organism, since it has the ability to produce three different extracellular polysaccharides, termed Psl, Pel, and alginate. *P. aeruginosa* PAO1, which produces Psl, was the main strain used. Three commercial fluorescent stains, Cell Mask Orange (CMO), Bodipy 630/650 X-SE (BOD), and *Griffonia (Bandeiraea) simplicifolia* lectin I (GSL-I) were found to stain the matrix of this strain. One stain from the MSU fluorescent probe core facility was also found to stain PAO1. Interestingly, the matrix material of PAO1 was found to form a fibrous structure, with the cells attached to the matrix fibers. Two stains, CMO and BOD, appear to bind different components of the matrix, suggesting that the matrix may contain material other than polysaccharide.



Recently, we analyzed *P. aeruginosa* PA14, which produces the Pel polysaccharide. Thus far, no stains have been found that effectively bind Pel, but CMO had some small sections of sporadic staining of the bottom layer of the matrix. These results demonstrate that the matrix material of *P. aeruginosa* biofilms is structured and forms adhesive material for the bacterial cells. Future work will examine the developmental process associated with biofilm extracellular matrix formation.

Figure 1. Confocal scanning laser micrograph of PAO1 GFP PMF230 after 70 hours of growth at 37° centigrade and stained with both Cell Mask Orange and Bodipy 630/650 X-SE. Cells appear green, CMO appears red, and BOD appears cyan. From the cross sections it can be seen that BOD is staining within the colonies, whereas the CMO stains on top of BOD and seems to be evenly mixed with cells.

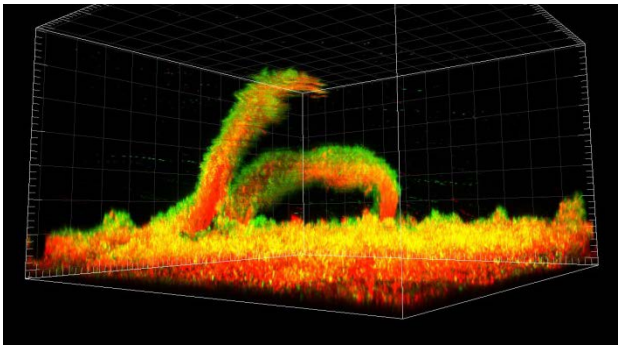


Figure 2. Confocal scanning laser micrograph of PAO1 pilA GFP PMF230 after 47 hours of growth at 37° centigrade and stained with Cell Mask Orange. The matrix appears red and the cells appear green.

Back to page 5

CBE Poster #610

Date: July 2013
Title: **Lipid profiling of *Chlamydomonas reinhardtii* grown under three different inorganic carbon regimes**
Authors: ***Egan Lohman*** [1,2], Gardner RD, Halverson L, Peyton BM, and Gerlach R
Affiliation: [1] Center for Biofilm Engineering, and
[2] Department of Civil Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: NSF-IGERT, NSF-SEP, DOE

Lipid contents of microalgal cultures are traditionally analyzed at time-of-harvest by quantifying the fatty acid methyl ester (FAME) content of dry biomass after transesterification. This approach, however, is unable to identify the compound each FAME was derived from, and further, does not provide a comprehensive evaluation of lipid profiles over time. By using relatively straightforward methods for quantifying extractable lipid classes (free fatty acids, mono-, di- or tri-acylglycerides) via GC-FID, and total FAME via GC-MS, lipid profiles over time were established for *Chlamydomonas reinhardtii* CC124 when grown under three different inorganic carbon regimes. Extractable lipids were characterized to include lipid class (FFA, MAG, DAG, TAG) and carbon chain length for comparison against total FAME. Data points were collected each day throughout the growth cycles to provide comprehensive lipid profile analyses for *C. reinhardtii* grown with atmospheric concentrations of CO_2, elevated (5%) levels of CO_2 and for cultures supplemented with 50 mM sodium bicarbonate. All three conditions produced equal concentrations of saturated and unsaturated C16 fatty acids until nitrogen in the growth medium was depleted, after which a shift towards saturated C16 fatty acids was observed. This trend was more significant in cultures grown on elevated CO_2 and cultures supplemented with $NaHCO_3$. Unsaturated C18 fatty acids were the predominant FAME in all three conditions even though C16 triacylglycerides (TAG) were twofold more abundant than C18 TAG, indicating that C18 FAME were derived from compounds other than C18 TAG, such as membrane lipids. Free fatty acids (FFA) were the predominant lipid class prior to nitrogen depletion, but were significantly reduced over time as the organism began synthesizing glycerolipids, suggesting a reallocation of FFA into TAG, DAG and monoacylglycerides (MAG). Most interesting, in all three conditions the total FAME content on a weight-per-weight basis (weight FAME/weight biomass) did not significantly change over time; however lipid production per culture volume increased over time in all three treatments, reaching the highest concentration in cultures grown on 5% CO_2. Our results provide insight into which lipid compounds *C. reinhardtii* synthesizes and how the organism's metabolism changes due to nitrogen depletion and inorganic carbon source availability. The approach presented can be utilized for determining the optimal time-point of harvest during biodiesel production, as well as for screening for high value product accumulation (such as Ω-3 fatty acids).

Back to page 5

CBE Poster #611

Date: June 2013
Title: **Implementation of a two-stage vertical flow treatment wetlands at a ski area**
Authors: **Chris R. Allen**[1,2], Stein OR[1,2], Davis KJ[1,2], Moss JJ[1,2], Burr MD[1], and Jones WL[1,2]
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Sponsored by: MT DEQ and Bridger Bowl Inc.

A pilot scale, two-stage vertical flow treatment wetland was constructed at the Bridger Bowl Ski Area outside of Bozeman, MT. (49°45' N 110°53' W elevation 1,900 m). The pilot treatment system was designed to treat 3.8 m^3 d^{-1}—approximately 1/3 of the ski area's average daily flow—and to act as a research station allowing experimentation with organic and hydraulic loading rates. The ski area operates annually from November to April with peak loading corresponding with low ambient temperatures and high snowfalls. The ski area receives an annual average of 8.9 m snowfall, with monthly average temperatures that range from -6.9° to 6.3°C during the operating season. After primary treatment, the average concentrations of

COD, total nitrogen, phosphorous, and alkalinity are approximately 900, 170, 18, and 900 mg l^{-1} respectively. Upon arrival at the treatment system, average influent water temperature is less than 6°C.

The pilot vertical flow treatment wetland system consists of two parallel trains of two vertical wetland cells in series (four cells total, each with a surface area of 4.9 x 4.9 m and approximately 1.2 m deep). The treatment layer of the first cells in series feature a coarser media (gravel, $d_{50} \approx 5$ mm), while the treatment layer in second series is a medium sand ($d_{50} \approx 0.6$ mm). Coarser drainage layers underlie the treatment layers and the treatment layer in the cells is overlain by a gravel cover for frost protection.

The basic operational scheme features a pump station that can deliver septic tank effluent to the first cells in series. Different hydraulic loading rates can be applied to each parallel cell. Effluent from the first cells is combined in a second pump station, which applies flow to the second cells in series. Effluent from the second cell is combined in a third pump station, which can recycle water back to the first cells in series. Different recycle rates to each cell in parallel are possible. This scheme offers maximum flexibility to vary hydraulic and organic loads and recycle rates at several points within the system. Flow rates for each wetland cell can be measured from the influent (pump calibration) and effluent (V notch weirs). Influent chemical composition can be measured from each pump station and from the effluent of each cell (grab samples or by utilizing time-averaged auto samplers). In addition, sample ports have been incorporated within each cell to allow for vertical profiling of performance. Systematically varying hydraulic loading and/or recycle rates allows for performance evaluation over a wide range of operational possibilities.

Construction of the wetland was completed in October of 2012, too late in the season for planting to begin. The system was run as a two-stage gravel filter over the 2012/2013 ski season. After preliminary testing of the hydraulic components of the system in November and December, wastewater was directed to the wetland in January 2013. In June of 2013 the wetland was planted with two species: *Schoenoplectus acutus*, and *Carex utriculata*. Both species will be tested for their climate suitability and performance enhancing nutrient removal. Optimization and monitoring of the system will continue for the next three years of operation. Data from the first year of operation as a gravel bed system will be presented in the poster.

ACKNOWLEDGEMENTS
The authors wish to thank Carlos Arias and Günter Langergraber for helpful advice for the system design criteria, Ray Center at Rocky Mountain Engineers for further engineering services, the Montana Department of Environmental Quality for construction and monitoring funds, and the great folks at Bridger Bowl Inc. for providing the site and construction of the system.

Back to page 5

CBE Poster #612

Date: July 2013
Title: **Pore-scale modeling of biofilm-induced calcium carbonate precipitation**
Authors: ***James Connolly***[1,2], Radu A[5], Jackson B[1,4], Picioreanu C[5], Klapper I[6], Cunningham AB[1,3] and Gerlach R[1,2]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Chemical & Biological Engineering,
[3] Department Civil Engineering, and
[4] Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA;
[5] Delft University of Technology, Faculty of Applied Sciences, Department of Biotechnology, The Netherlands;
[6] Department of Mathematics, Temple University, Philadelphia, PA, USA.
Sponsored by: US National Science Foundation, US Department of Energy

Pore-scale biological processes in the subsurface environment are important to understand in relation to many engineering applications including environmental contaminant remediation, geologic carbon

sequestration, and petroleum production. Specifically, biofilm induced calcium carbonate precipitation has been identified as an attractive option to reduce permeability in a lasting way in the subsurface. This technology may be able to replace typical cement-based grouting in some circumstances; however, pore-scale processes must be better understood for it to be applied in a controlled manner.

There are many metabolic processes capable of inducing carbonate mineral precipitation; however, urea hydrolysis is the focus of this work. Urea is hydrolysed to form ammonia and carbon dioxide. The end effect of this is a pH increase, and in the presence of calcium (or other divalent cations), an increase in the saturation index. Once the solution reaches a supersaturated state, calcium carbonate precipitation is possible. In heterogeneous environments it is possible to have a wide range of saturations states within close spatial proximity. Furthermore, it is well known that biofilm also causes heterogeneous environments, so it is challenging to predict the spatial orientation of mineral precipitation with respect to a porous support structure and biological activity.

The work presented will focus on efforts to observe biofilm growth and mineral precipitation in micro-fabricated flow cells combined with finite element modeling as a tool to predict local chemical gradients of interest (See Figure 1). We have been able to observe this phenomenon over time using a novel model organism able to hydrolyse urea and express a fluorescent protein that allows for non-invasive observation over time with confocal microscopy. The results of this study show the likely existence of a wide range of local saturation indices even in a small (1 cm length scale) experimental system. Interestingly, the locations where the highest saturating index is predicted do not correspond to the locations of highest precipitation density, highlighting the need for further research. Predictive biofilm/mineral models will also be presented, where biofilm and mineral growth occur entirely in silico. This entirely computer-based model allows for us to change parameters and predict how to optimize and characterize pore scale processes more completely.

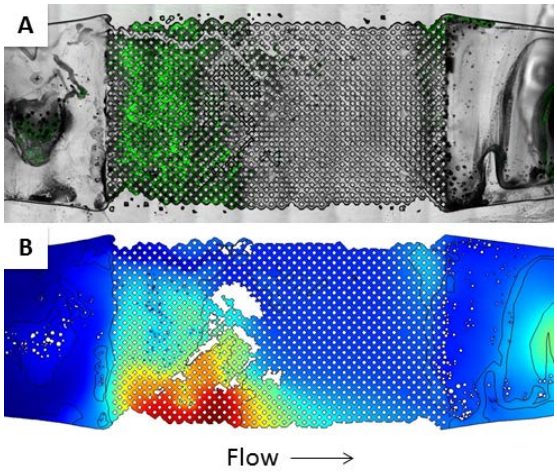

Figure 1. A micro-fabricated flow cell containing biofilm-induced calcium carbonate precipitation. ***(A)*** Experimental results: Active biofilm is in green and dark circles are calcium carbonate crystals. Note the channeling behavior in the top of the image, leaving a large hydraulically inactive area in the biofilm mass. ***(B)*** Finite element model: The prediction of relative saturation of calcium carbonate (as calcite). Fluid enters the system at a low saturation state (blue) but areas of high supersaturation (red) are predicted within the hydraulically inactive area in the biofilm. If only effluent saturation was measured, precipitation may not even be predicted, but we see local, pore-scale behavior dictating system behavior in this case. The flow cell is 1 cm in length and the porous media elements are 100 μm.

Back to page 5

CBE Poster #613

Date: June 2013
Title: **Analysis of TAG accumulation extent with the use of alternative grades of sodium bicarbonate and alternative bicarbonate salts**
Authors: ***Todd Pedersen***, Gardner R, and Peyton BM
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Church and Dwight Inc.

Culturing of microalgae for triacylglycerol (TAG) accumulation is a rapidly expanding focus of research due to TAG's potential for use as a biofuel precursor. Optimizing accumulation of TAGs has the potential to enhance the cost effectiveness of industrial scale-up. Most often, cultures are grown utilizing CO_2 as the inorganic carbon source. However, bicarbonate (HCO_3^-), another inorganic carbon source, has also been shown to function as a lipid accumulation trigger in various strains of microalgae (Gardner, et al. 2012; Gardner, et al. 2013; Mus, et al. 2013). Here, we amend cultures with varying bicarbonate salt compositions, as a lipid accumulating trigger, and detail the results using quantitative measurements of neutral lipids (*i.e.,* TAGs). Algal physiological effects from sodium bicarbonate, potassium bicarbonate, ammonium bicarbonate, sodium carbonate, and sodium sesquicarbonate were studied at 50 mM C initial concentrations. Studying these effects serves to provide insight into cheaper alternatives that could be used to obtain equivalent results to their expensive counterparts. Lipid accumulation was monitored with an optimized procedure for Nile Red fluorescence, which has been shown to correlate to culture TAG properties (Cooksey, 1987), with 20% dimethyl sulfoxide (DMSO) and incubated 10 minutes prior to fluorescence measurement. Cultures amended with sodium bicarbonate displayed the most lipid accumulation with negligible differences between the analytical grade and industrial grade. Potassium bicarbonate and sesquicarbonate also demonstrated increased lipid accumulation but with less extent. Sodium carbonate demonstrated less than one third the extent of lipid accumulation than sodium bicarbonate triggers, and the 0 mM HCO_3^- control displayed minimal lipid accumulation. The ammonium bicarbonate treatments became toxic to the cultures by producing ammonia in concentrations inhibitory to photosynthesis and growth.

Back to page 5

CBE Poster #614

Date: June 2012
Title: **Service learning to address drinking water quality through community-based participatory research on the Crow Reservation**
Authors: ***Eric Dietrich***[1,5], Rao V[2], Allen C[1,5], Doyle J[3], Old Coyote TJ[3,4], Eggers MJ[3,4,5], Kuennen R[6], and Camper, AK[3,5]
Affiliation: [1] Department of Civil Engineering, Montana State University, Bozeman, MT, USA;
[2] Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA;
[3] Crow Environmental Health Steering Committee;
[4] Little Big Horn College;
[5] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;
[6] Amway Corporation.
Sponsored by: Amway Corporation, Center for Native Health Partnerships, National Institutes of Minority Health and Health Disparities, Environmental Protection Agency

This investigation tested prototype biofilm filtration units as a source of safe drinking water on the Crow Reservation through a collaboration between the Crow Environmental Health Steering Committee, Amway Corporation, and MSU's Engineers Without Borders (EWB) chapter. In response to long-standing community concerns about the poor quality of well water available to homeowners in rural areas on the reservation, Amway-designed and donated units were tested as a potential means to treat surface water as a substitute.

Working with community members who operated prototype units in their homes, EWB student members conducted sampling trips on a monthly basis for eight months, testing source and product water for microbial contamination and collecting source water samples for chemical analysis. Qualitative data were also collected regarding operators' experiences with the units, indicating in combination with quantitative data that they represent a viable water treatment technology for Crow homeowners.

The project, which ultimately provided 15–20 students with a hands-on research opportunity and meaningful cross-cultural experience, represents a potential model for future efforts to foster engagement experiences at MSU and in other higher education settings.

Figure 1. Student participants (from left) Varsha Rao (CBE), Ryanne Daily, and Eric Dietrich (CBE) collect source water samples at the Crow Agency swimming hole during Crow Fair in August 2012.

Back to page 5

CBE Poster #615

Date: June 2013
Title: **Differences in peripheral IV blood control catheter design and biofilm formation**
Authors: ***Marcia Ryder***[1], ***Elinor Pulcini***[2], Parker A[2], and James G[2]
Affiliation: [1] Ryder Science, Escondido, CA
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Smiths Medical Inc.

Background/Objectives: The insertion of peripheral intravenous catheters (PIVC) is the most common invasive procedure performed by nurses. The new generation of PIVCs developed to reduce blood exposure during insertion utilizes additional internal components within the catheter hub. These components increase the internal surface area or dead space that is thought to increase biofilm formation and subsequent transfer of bacteria into the bloodstream. This raises concern for increased risk of bloodstream infection. The purpose of the this study was to compare biofilm formation on the various internal components of the catheter hub and bacterial transfer rate between 3 valved blood control PIVCs in a clinically simulated in vitro model.

Methods: Three PIVCs were tested: Smiths Medical ViaValve™ Safety I.V. catheter (C1), BD Insyte™ Autoguard™ BC Shielded I.V. Catheter (C2) and the B. Braun Introcan Safety® 3 catheter (C3). Six experiments were run with three time points measured within each run: 0, 72 and 96 hours. A needleless connector was attached to each catheter, inoculated by flushing with 0.5 ml of a 10^4 colony forming units per ml (CFU/ml) of *S. aureus,* and incubated at room temperature for 2 hours. The connectors were then replaced with new sterile connectors and unattached bacteria were rinsed from the fluid path using sterile Phosphate Buffered Saline (PBS). Catheters were then either sampled or subjected to simulated clinical use by flushing 17 times daily with 0.5 ml sterile nutrient and 1 flush at the end of the day with normal saline for 72 and 96 hours. Catheters were sampled with a two-step procedure. First, each catheter was flushed to recover planktonic bacteria and plated to determine CFU/ml (Flush 1). The connector surface was disinfected, sonicated in PBS to remove firmly attached bacteria, and flushed a second time and plated (Flush 2). At Time 96 hours, one of each catheter type was destructively sampled (including the hub, spike,

septum, and tip). Each part was vortexed, sonicated, and vortexed again to detach and disaggregate the biofilm and form a bacterial suspension for viable plate counts (CFU/ml). One of each assembly type was formalin fixed for scanning electron microscopy (SEM), disassembled and imaged.

Results: The log sum CFU for biofilm formed on all internal fluid pathway surfaces within C1 was 3.84, C2 was 4.02, and C3 was 4.90. Biofilm was observed by SEM on internal component surfaces with higher CFU counts. When pooled across time points and all experiments, there were statistically significantly smaller bacterial mean log densities for C1 compared to C2 (p-value = 0.003 and 0.001 for Flush 1 and Flush 2, respectively) and for C1 compared to C3 catheter (p-value=0.014 and 0.010 for Flush 1 and Flush 2, respectively).

Conclusions: There were differences in biofilm formation and bacterial transfer among the devices. This was likely due to differences in internal surface areas, volumes and dead spaces. These differences may increase the potential risk for transfer of bacteria into the bloodstream for certain blood control valved PIVC designs.

Back to page 5

CBE Poster #616

Date: April 2013
Title: **Biofilm induced biomineralization in a radial flow reactor**
Authors: Neerja Zambare[1,2], ***Robin Gerlach***[1,2], and ***Ellen Lauchnor***[1]
Affiliation: [1] Center for Biofilm Engineering, and
[2] Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: US Department of Energy (FE0004478, DE-FE0009599)
Zero Emissions Research and Technology (ZERT) (DE-FC26-04NT42262)
Subsurface Biogeochemical Research Program (SBR) (DE-FG02-09ER64758)
National Science Foundation (DMS-0934696)

Biomineralization is the formation of minerals by living organisms, which can be performed actively or passively—as in the case of microbially induced mineralization. Microbially induced calcium carbonate precipitation, or MICP, occurs when bacteria create conditions favorable for carbonate precipitation in an environment. MICP is being looked at as a reinforcement technique. Some potential applications of MICP are sealing fractures around wells to prevent leaks, sequestering heavy-metal contaminants in groundwater by co-precipitation with carbonate minerals, or making subsurface barriers to prevent flow by plugging subsurface pores. These applications all involve injecting MICP treatments into wells, where radial flow of fluids can impact the transport and reactions involved in MICP. Our research deals with ureolytic micro-organisms that facilitate calcium carbonate precipitation under engineered conditions. We specifically deal with *Sporosarcina pasteurii*, which contains an enzyme that catalyzes the hydrolysis of urea (aka ureolysis).

The main objective of this project was to study the effects of calcium carbonate precipitation formed by a bacterial biofilm on fluid flow through porous media. The radial flow reactor is made of two circular parallel plates with 1 mm diameter glass beads packed between the plates, which comprise a porous medium. The effects of varying parameters on calcium carbonate precipitation are being investigated through this project. The parameters that are being varied are the calcium media flow rate and the urea-to-calcium concentration ratios. Three experiments with flow rates of 2.5 mL/min, 5 mL/min and 10 mL/min have been performed. Plugging in certain regions by the precipitated calcium carbonate caused flow channels to form. Tracer study photos showed how preferential flow had developed because of the plugging. Current experiments at a flow rate of 5 mL/min have shown an average carbon precipitation efficiency of 12%. In comparison, at the slower flow rate (2.5 mL/min), 14% of the influent calcium was precipitated in the reactor. Concentration varying experiments are currently in progress. The results of

these studies will provide us with data to quantify the effects of fluid velocity on biofilm induced biomineralization. Relationships for fluid velocity, calcium carbonate formation, ammonium formation, plugging of the pore space, etc., can be formed based on the data from this project. The Damköhler number for flow in this radial system is being studied based on the calcium precipitation rates and the spatial fluid velocity.

Back to page 5

CBE Poster #617

Date: April 2013
Title: **Application of a Michaelis-Menten based kinetics model on ureolysis by *Sporosarcina pasteurii***
Authors: ***Dayla Topp***, Lauchnor E, and Gerlach R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Montana State University Undergraduate Scholars Program

Understanding microbial kinetics is a major part of predicting rates of microbially driven processes in natural and engineered systems. Urea hydrolysis is one reaction that occurs in environments such as soils, wastewater treatment and the human urinary system, where it can induce kidney stone formation (Mobley et al., 1989). It is also a key reaction that can promote microbially induced mineral formation, which is being studied for engineering applications such as cementing porous media or to seal fractures in underground confining rock layers (Cunningham et al., 2011).

Hydrolysis of urea is the chemical reaction catalyzed by the enzyme urease, in which urea is broken down into CO_2 and ammonium, thereby increasing the pH and favoring the precipitation of calcium carbonate. This can be written as the following equation:

$$(NH_2)_2CO + H_2O \rightarrow NH_3 + H_2NCOOH \rightarrow 2NH_4^+ + H_2CO_3 + 2OH^-$$

However, one of the biggest challenges to making this technology field-relevant is understanding the dependence of urea hydrolysis on factors such as cell density, pH, concentration of urea, and potential product inhibition by ammonium. The aim of this research was to develop a Michaelis-Menten enzyme kinetic model of urea hydrolysis by the ureolytic bacterium *Sporosarcina pasteurii*.

Previous kinetic approaches for this process only apply a zero order or first order kinetic approach, even though Michaelis-Menten makes more sense based on the saturation of the enzyme at high substrate concentrations. Previous analyses of ureolysis rates have also ignored any effects of changing cell concentration (or growth) on the reaction rates. So experiments were designed around kinetic approaches in order to calculate parameters and find the best fitting expression incorporating bacterial growth.

Results showed that the data fit a Michaelis-Menten model best with an R^2 value of 0.95. The K_m and V_{max} were found to be 181 $\frac{mmol}{L}$ and 280 $\frac{mmol}{Lhr}$, respectively. Within the cell density range that was tested, a linear correlation between rate and optical density was observed, indicating that the rate correlates with cell density. This knowledge could be used in model simulations of processes that include ureolysis, such as biomineralization for applications like carbon sequestration, in the near future.

References:

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Mobley HL and Hausinger RP, "Microbial ureases: Significance, regulation, and molecular characterization," *Microbiology*, 1989: 53–85.
Parks SL, "Kinetics of calcite precipitation by ureolytic bacteria under aerobic and anaerobic conditions," MS thesis, Department of Chemical & Biological Engineering, Montana State University, 2009.

Back to page 5

CBE Poster #618

Date: July 2013
Title: **Chemical structure characterization of peptidic head groups from new siderophores produced by a Soda Lake Isolate**
Authors: ***Luis O. Serrano Figueroa***[1,2 & 5], Schwartz B[2, 4 & 5], and Richards AM, PhD[3]
Affiliation: [1] Center for Biofilm Engineering,
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Sponsored by: National Science Foundation

Background: 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.

Methods: Bacterial isolates enriched from Soap Lake sediment and water samples were screened for siderophore production. Siderophore production was confirmed through the chrome azurol S (CAS) agar plate and liquid methods. Bacterial isolate SL01 was found to produce relatively high concentrations of siderophores in liquid medium. 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 were compared to those in the NCBI database using the BLAST search to determine the closest phylogenetic neighbors.

Results: Two distinct, new families of amphiphilic siderophores were 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 1080 to 1135 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 (SL28) that was found to produce amphiphilic siderophores.

Conclusions: Bacteria thriving under saline and alkaline conditions are capable of producing unique siderophores resembling those produced by microbes inhabiting marine environments. Currently, there is on-going work to determine the "R" group, or aliphatic group, structure via fatty acid methyl ester (FAME), and more mass spectrometry for detailed peptide group structure.

Back to page 5

Industry & Agency Posters

Date: July 2013
Title: **Evaluation of disinfection efficacy of ozone and chlorinated cleaner against the biofilm of *Klebsiella michiganensis* and *Pseudomonas aeruginosa***
Authors: ***Ratul Saha***[1], Atwain A[1], Saha N[2], and Donofrio RS[1]
Affiliation: [1]Applied Research Center, NSF International, Ann Arbor, MI
[2] University of Calcutta, Kolkata, India

Biofilm is a complex community of microorganisms growing on a biotic or abiotic surface in an aqueous environment. Household microorganisms mostly occur in the form of biofilm on wet surfaces in the bathroom and kitchen area. Microorganisms constituting biofilm communities are less susceptible to antimicrobial agents and are thus difficult to control. Recently, incorporation of ozone as a control agent in

the consumer product industry has been initiated. In this study, disinfection efficacy of ozone and a commercial chlorinated cleaner were evaluated against the biofilm of a commonly occurring *Pseudomonas aeruginosa* and *Klebsiella michiganensis*, a newly described species recovered from a toothbrush holder. Single-species biofilm was grown on borosilicate glass and polycarbonate coupons using the CDC bioreactor. Mature biofilms were exposed to ozonated water (ranging from 0.2–0.37 ppm) for a contact time of 2 min and 4 min, and chlorinated cleaner (1.94%) for 2 min and 10 min. Similarly, planktonic cells of both the target bacteria were also tested. Log reduction of each treatment was calculated against control coupons. A univariate ANOVA was conducted to test if there was any significant difference in log reduction with a) coupon types, organisms and exposure times within biofilm treated with ozone; and b) treatment type in biofilms. Another set of one-way ANOVA was performed to determine differences in killing effect with ozone a) between planktonic and biofilm cells, b) between target organisms in planktonic forms, and c) between target organisms in biofilms. Ozone demonstrated an average log reduction of 0.88 (±0.13) and 0.12 (±0.01) for *K. michiganensis* and *P. aeruginosa*, respectively, for 2 min; whereas, for 4 min an average log reduction of 1.15 (±0.16) (*K. michiganensis*) and 0.29 (±0.03) (*P. aeruginosa*) were observed in biofilms. In planktonic cells of *P. aeruginosa,* the log reduction was 2.61 and 3.31 for 2 and 4 min, respectively, and it was > 4.0 log reduction for *K. michiganensis*. Chlorinated cleaner demonstrated >4.0 log reduction for both the bacteria in biofilm and planktonic form. Results indicated that the disinfection efficacy of ozone varied between the two species of bacteria ($F=110.5$, $p<0.001$, $n=8$) and the two exposure times tested ($F=8.152$, $p<0.04$, $n=8$), but there were no differences in log reduction between the types of coupons tested ($F=0.004$, $p>0.95$, $n=8$). Within the biofilms, the log reduction in bacterial counts varied significantly between ozone and chorine treatments ($F=48.26$, $P<0.001$, $n=8$). Significant differences in log reduction were observed for all three factors (between planktonic and biofilms $F=60.85$, $p<0.001$, $n=12$; between target organisms: in planktonic form $F=22.22$, $p<0.04$, $n=4$; in biofilms $F=54.54$, $p<0.001$, $n=8$). Based on the results it can be concluded that *K. michiganensis* was more susceptible to both the disinfectants compared to *P. aeruginosa*. Also, commercial chlorinated cleaner was highly effective against both biofilms and planktonic cells compared to ozonated water within a shorter contact time.

Back to page 5

Date: July 2013
Title: **In vitro biofilm model, drip-flow reactor, using pigskin**
Authors: ***Saurab Sainju***, Schmidt C, and Butler M
Affiliation: BioScience Laboratories, Bozeman, MT

Chronic wounds are a major health concern to a patient's well-being as well as a financial burden to the patient and the healthcare system. The presence of biofilms in chronic wounds has been implicated in hindering the healing process and reducing the effectiveness of topical antimicrobials (James et al, 2008). New products are continually being made to disrupt activity of biofilms in wounds, leading to more efficient care.

The drip-flow reactor provides a good in vitro biofilm model of a chronic wound (Goeres et al, 2009; Agostinho et al, 2011). This model delivers a constant flow rate of medium and simultaneously presents a liquid-air interface to the growing biofilm, consistent with the environment of a chronic wound. A glass slide or coupon within the reactor usually represents the growing surface for the biofilm in this model. A recent review described several in vivo models of biofilm-infected wounds (Seth et al, 2012), in which methods used mice, rodents, and porcine for growing biofilms. We have adapted the drip-flow biofilm reactor by replacing the glass slide with a strip of sterilized pigskin, in hopes of better simulating a real wound, in vitro. This work uses a *Pseudomonas aeruginosa* biofilm growing on pigskin and subsequent testing of various wound care products.

Date: July 2013
Title: **Bioorganic approaches to better understand biofilm processes in bacteria**
Authors: ***William M. Wuest***, Wu B, Sreenilayam G, Brzozowski R, Jennings M, Fletcher M, Harris R, Kinzie C, Carson C, and Hauseman Z
Affiliation: Department of Chemistry, Temple University, Philadelphia, PA

The overarching goal of the Wuest research group is to utilize chemical biology to understand biofilm processes. Bacteria possess signaling systems that detect environmental threats (antibiotics, pH imbalance), which then go on to trigger biofilm formation. Bacterial biofilms are increasingly resistant to antibiotic treatment and develop persistent infections within the human body. Our group is implementing a variety of methods to interrogate the signaling process, with the overarching goal of identifying novel treatments and new targets for pharmaceutical development.

Currently, we are actively pursuing a number of synthetic projects targeting known biofilm inhibitors, signaling molecules, and protein-protein interactions. In conjunction, we are also using microbiological and biochemical approaches to discover new therapeutics, identify unprecedented biological targets, and test the efficacy of compounds developed in-house. The goal of each of these projects is to make chemical analogs that mimic the natural compounds but are more "drug-like" thus possessing better pharmacokinetic properties and maximizing the likely success of therapeutic development. The group is highly interdisciplinary and has key collaborations throughout the Northeast (H. Sondermann, M. Filiatrault–Cornell University, G. O'Toole–Dartmouth Medical School, H. Sintim–University of Maryland) and also at Temple (Bettina Buttaro–Medical School, Vince Voelz–Chemistry Department), permitting the expedited testing of compounds and evaluation of their mechanisms of action.

Back to page 5