Montana State University

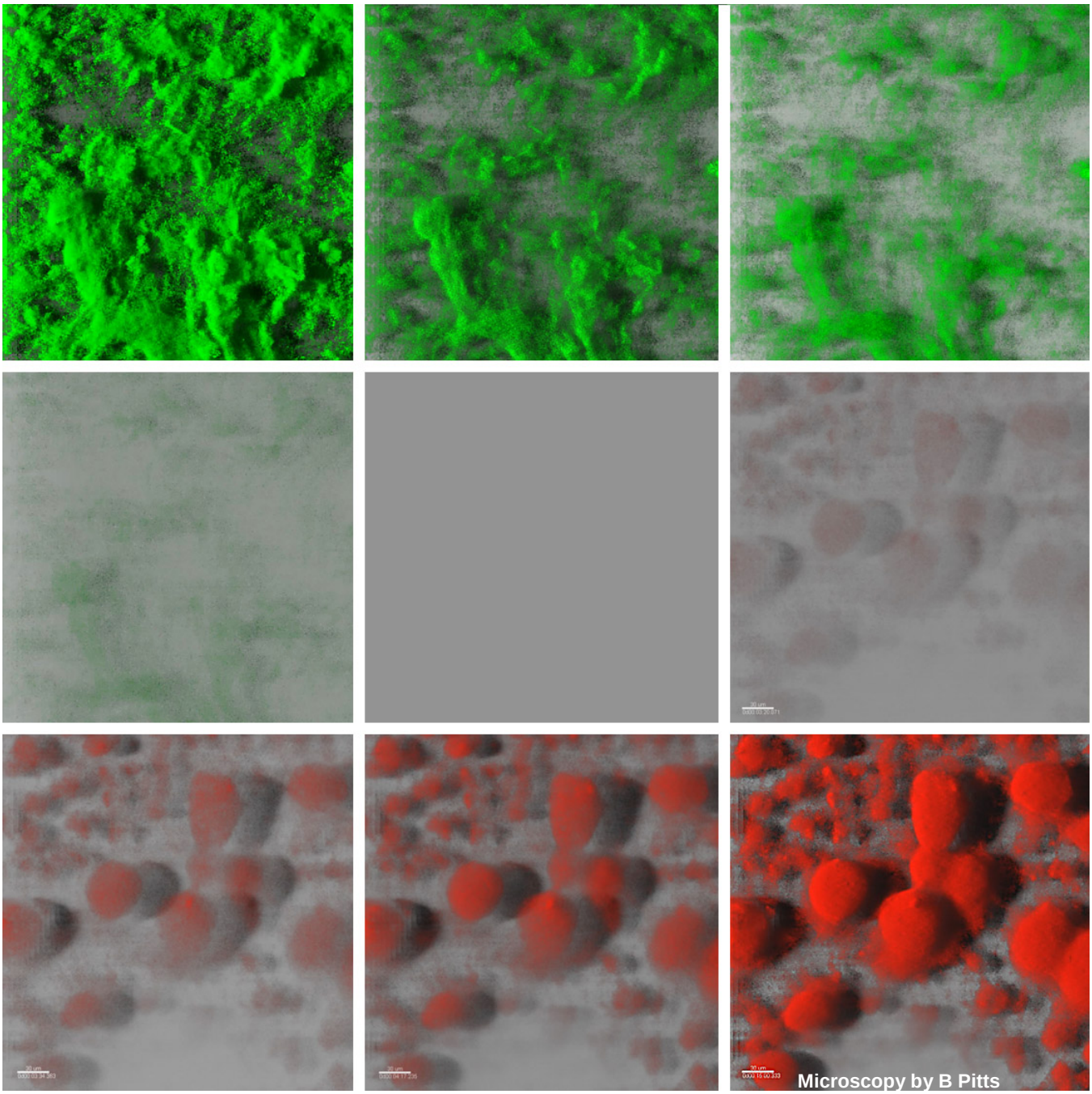
Center for Biofilm Engineering

Bozeman

montana biofilm SCIENCE & TECHNOLOGY meeting

July 17–19, 2012

Proceedings



Montana Biofilm Science & Technology Meeting: July 17–19, 2012
Table of Contents: Speaker Abstracts

Special Presentation

State of the CBE address,
Phil Stewart, MSU-CBE

SESSION 4: Energy & Environmental Biofilms

SESSION 5: Biofilm Methods

SESSION 6: Biofilm Control

Table of Contents: Poster Abstracts

Center for Biofilm Engineering posters

Industry and Agency Posters

Speaker Abstracts

SESSION 1: Anti-Biofilm Coatings

Vascular access product with non-leaching betaine modification reduces microbial attachment

Presenter: Roger Smith, Head of Microbiology,
Co-authors: Vickie Wagner, Heather Lapp, Greg Brotske, Chris Loose
Affiliation: Semprus BioSciences, Cambridge, Massachusetts, USA 1-617-577-7755

Adherence of proteins, eukaryotic cells, and microorganisms to the surface of intravenous devices can lead to complications such as thrombosis and infections. These complications result in lengthened hospital stays, increased patient costs and increased patient morbidity and mortality. Most technologies for inhibiting these complications are limited in duration of efficacy and may lead to toxicity or the generation of drug-resistant organisms. We have developed a non-leaching surface modification based on the polymerization of the zwitterionic sulfo-betaine (polySB). The polySB-modified surface coordinates water molecules to create a water barrier over the device surface, which inhibits protein, eukaryotic cell and microbial adhesion to the surface.

The polySB technology was applied to a polyurethane peripherally inserted central catheter (PICC) and assessed for the reduction of thrombus formation and microbial attachment. To mimic a clinical setting, polySB-modified catheters were exposed to serum and then incubated with microorganisms. Evaluation of microbial adherence on internal and external surfaces showed a statistically significant reduction in microbial adhesion on polySB-modified catheters compared to unmodified samples. To determine if the reduction in microbial adhesion observed with polySB-modified PICCs resulted in a change in host response, polySB-modified and unmodified devices were colonized with *S. aureus* and then implanted into subcutaneous pockets in rabbits. After up to 5 days, catheters were examined to determine the degree of induced inflammation and number of adherent bacteria on catheters. It was observed that there was a statistical decrease in both the amount of inflammation associated with polySB-modified catheters and the number of bacteria recovered from explanted devices.

Catheters were also inserted intravenously and blood was drawn into the catheters to expose lumens to blood cells and proteins. After successive blood draws, lumens were exposed to *S. aureus* for 2 hours. Adherent bacteria were quantified; a 3.8 log reduction in adherent bacteria compared to the initial inoculum was observed with polySB-modified catheters.

Back to page 1

Implant coating that is highly biocompatible, antimicrobial and biofilm resistant in vitro and in vivo

Presenter: Dirk Lange, Assistant Professor
Affiliation: The Stone Centre at Vancouver General Hospital, Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Bacterial colonization on implant surfaces and subsequent infections are common reasons for the failure of many indwelling devices. This accounts for a major portion of nosocomial infections, millions of dollars of extra health care expenses, and significant patient discomfort. Several approaches including antimicrobial and antibiotic-eluting coatings on implants have been attempted; however, these have been plagued by toxicity and biocompatibility problems. Here we report a novel implant coating that is highly biocompatible, antimicrobial and biofilm resistant. This coating consists of covalently grafted hydrophilic polymer chains conjugated with an optimized series of novel antimicrobial peptides (AMPs) on the implant surface. These tethered AMPs maintained excellent antimicrobial activity against both gram-positive and

gram-negative bacteria in vitro and in vivo. We found that this specially structured robust coating was extremely effective in resisting biofilm formation over extended periods of time, and the biofilm resistance depended on the nature of the conjugated AMPs. The coatings were non-toxic as well as hemocompatible. Since such coatings can be applied to most currently used implant surfaces, our approach has significant potential for the development of infection resistant implants.

Evaluation of antimicrobial PICC catheters using an ovine model

Presenter: Elinor deLancey Pulcini, Assistant Research Professor
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

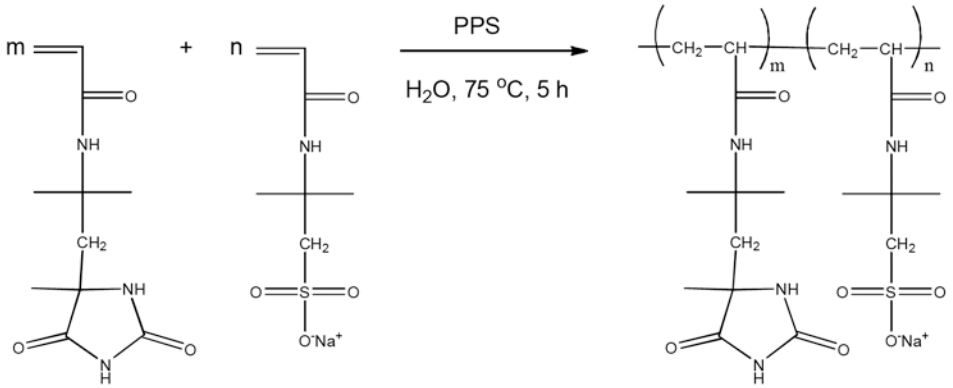
Catheter related bloodstream infections (CRBSI) are a serious problem, resulting in increased hospital costs and increased morbidity and mortality rates. Reduction of bacterial colonization on catheter surfaces may reduce infection, inflammation and thrombus (blood clot) formation. This study, sponsored by Teleflex Medical Inc., examined the effects of chlorhexidine-coated catheters compared with non-coated catheters on surface colonization by *S. aureus* in an ovine (sheep) model of central venous catheter infection. Results indicate a statistically significant reduction in bacterial colonization on both extraluminal and intraluminal surfaces and a reduction in thrombus formation.

Back to page 1

N-halamine biocidal copolymers for coatings and latex paints

Presenter: Idris Cerkez[1]
Co-Authors: Hasan B Kocer[1], SD Worley[1], RM Broughton[2]
Affiliation: [1] Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849
[2] Department of Polymer and Fiber Engineering, Auburn University, Auburn, AL 36849

A series of copolymers containing units of a novel hydantoinyl acrylamide and the sodium salt of 2-acrylamido-2-methylpropane sulfonic acid has been synthesized (Scheme 1). The homopolymer of the hydantoin acrylamide compound was insoluble in water, while the copolymers with the sulfonic acid sodium salt were water dispersible/soluble (Table 1).



Scheme 1. Structure of the synthesized copolymers

The polymers were added into a commercial water-based latex paint, and upon drying, the painted surfaces treated with the water-miscible copolymers were rendered antimicrobial following chlorination with dilute household bleach. The chlorinated homopolymer did not provide an antimicrobial property for the paint because of its tendency to isolate into small islands in the paint, while the copolymers being completely miscible were capable of 6-log inactivations of *Staphylococcus aureus* and *Escherichia coli* O157:H7 within 5 min of contact time.

Table 1. Composition of the synthesized copolymers

Polymer	M_{HA} / (M_{HA}+M_{SA})	Appearance in water
10	1.0	Insoluble
9	0.9	Colloid (opaque)
8	0.8	Colloid (transparent)
7	0.7	Soluble

M_{HA} and M_{SA}: mole fraction of HA and SA in the feed mixture

Back to page 1

SESSION 2: Industrial Biofilms

Online biofilm monitoring in industrial systems

Presenter: Paul Sturman, CBE Industrial Coordinator
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Monitoring biofilms and other surface deposits in real time can offer significant savings to industry by automating the maintenance of correct system-wide antimicrobial concentrations and helping to prevent out-of-spec product loss. While 'on-line' monitoring has been practiced in various forms for decades, advances in sensing technology now allow both improved sensitivity and better differentiation between types of deposits. Despite these advances, online biofilm monitoring has yet to achieve wide acceptance in some industries due to concerns about reliability and cost. Currently available systems use a wide variety of techniques to gather information about sessile deposits, from the historically used heat transfer resistance monitors to FTIR systems capable of assessing the composition of near-surface molecules. This presentation will describe a variety of currently available systems for online monitoring, focusing on the sensitivity of the various methods, what is actually being measured (and its relation to biofilm) and their applicability to various industries.

From corrosion to pathogenesis: The role of bacterial nanowires in diverse microbial biofilms

Presenter: Yuri Gorby, Associate Research Professor
Affiliation: Marine and Environmental Biology, University of Southern California, Los Angeles, CA

The relatively recent discovery of electrically conductive protein filaments called bacterial nanowires is fundamentally changing our understanding of components and mechanisms of extracellular electron transfer in microbial biofilms. First described in the metal-reducing bacteria *Geobacter* and *Shewanella*, nanowires were implicated in direct electron transfer to solid phase electron acceptors, including iron and manganese oxides. Further evidence suggested that nanowires from these organisms facilitate charge transfer through thick biofilms to electrode surfaces in electrogenic devices commonly referred to as microbial fuel cells.

Nanowires are not exclusive to metal reducing bacteria, but are, in fact, common to diverse groups of microbes. Those produced by oxygenic, photosynthetic cyanobacteria may be used to access oxygen as an electron acceptor located across redox gradients that form during nighttime respiration, while those produced by sulfate reducing bacteria may help facilitate charge transfer to archaea in methanogenic cocultures. Conductive nanowires have also been detected in a number of opportunistic pathogens,

including *Pseudomonas* biofilms and oral biofilms associated with the condition known as osteonecrosis of the jaw, where they appear to play roles for coordinated respiration and bone dissolution.

This presentation will provide an update on the current status of bacterial nanowire research. Mechanistic descriptions of charge transfer along the length of bacterial nanowires will accompany new insights into the composition and vulnerabilities of these conductive protein filaments. Implications ranging from novel treatment strategies to advanced nanomaterials will be discussed.

The author gratefully acknowledges support from the US Department of Energy, the US Department of Defense, the J. Craig Venter Institute, and the University of Southern California.

This presentation is dedicated to Dr. Bill Costerton: Father of biofilms and Friend to many.

Applications of molecular ecology for industrial purposes

Presenter: Chiachi Hwang, Industrial Research Scientist
Co-Authors: Matthew W. Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

For the past two decades, advancements in molecular techniques have revolutionized the field of microbiological analyses in terms of genomics and molecular ecology. These molecular techniques revealed that microbial populations in the environments are much more diverse than previously thought using traditional cultivation methods. As microbes play crucial roles in many natural and engineered processes, the adoption of molecular techniques has allowed scientists to study microbial consortia relevant to various processes in different environments (*e.g*., thermal springs, industrial processes, the human body), More recently, high throughput technologies (*e.g*., 454 pyrosequencing) have generated massive amounts of data on microbial community diversity and dynamics. With the combined knowledge of ecophysiology, these data offer opportunities to examine interactions among different microbial populations. Novel predictive models are also being developed to aid in comparative analysis, which holds great potential in providing a better understanding of microbial responses to environmental influences. An understanding of the ecology of microbiological processes can help improve quality control and risk assessment in a biotechnological setting.

Back to page 1

SESSION 3: Wound Biofilms

Wound biofilms: Clinical insights attained from animal models

Presenter: Robert Galiano, Assistant Professor, Plastic Surgery
Affiliation: Feinberg School of Medicine, Northwestern University, Evanston, IL, USA

Abstract not available.

Inter-bacterial communication in chronic wounds

Presenter: Alex Rickard
Affiliation: Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA

Chronic wounds are a persistent burden on patients and healthcare practitioners. Greater than five million North Americans suffer from chronic wounds and their care translates to an estimated cost of 20 billion dollars annually. Chronic wounds include diabetic foot ulcers, non-healing surgical wounds, pressure ulcers, and venous ulcers. These non-healing wounds can be caused by the overgrowth of aggregated bacterial communities on damaged/perturbed healthy skin. The aggregated communities possess many of

the hallmark characteristics of biofilms and, in particular, are recalcitrant to many physical and chemical treatment strategies. Molecular studies have shown that chronic wound biofilms contain numerous bacterial species, despite that fact that standard culturing methods only revealed a few. We hypothesize that the numerous chronic wound biofilm species interact with one another and these interactions perpetuate disease or facilitate a transition from disease back to health.

In order to begin to discern possible interactions and the specificity they confer between species, we will present data to demonstrate that chronic wound species produce inter-species and intra-species cell-cell bacterial signal molecules. These are autoinducer-2 (AI-2) and acyl-homoserine lactones (AHLs). Both cell-cell signal molecules can also be detected in wound biopsies. In addition, we will present data to demonstrate that bacteria isolated from a variety of different wound types coaggregate with one another. Coaggregation, the specific recognition and adhesion of bacteria, is known to occur between bacteria from human dental plaque and is hypothesized to contribute toward the development of periodontal disease—a chronic wound of the oral cavity. Here we will show that every identified chronic wound bacterium isolated by our lab can coaggregate with at least one other wound species and many have multiple partners. Coaggregation is often growth-phase dependent and mediated by complex adhesin-receptor interactions. We propose that coaggregation may enhance biofilm retention, act as a targeting mechanism for cell-cell interactions and promote juxtaposition between chronic wound species to enhance cell-cell signaling. Manipulation of coaggregation and/or cell-cell signaling may represent an innovative strategy to prevent the development of chronic wounds and promote healing.

Back to page 1

Biofilm, a micro-revolution:
Molecular diagnostics and personalized medicine in wound care—assessment of outcomes

Presenter: Randy Wolcott, MD, Medical Director
Affiliation: Southwest Regional Wound Clinic, Lubbock, Texas, USA

Wound care providers believe that bacteria play an important role in the nonhealing of wounds, yet, the wound care literature tells a much different story. Large reviews focusing on wound cultures and use of antibiotics in managing chronic wounds suggest that there is no difference in outcomes if we use these tools. So, how do microorganisms impair wound healing if they have any effect at all? Costerton and Stewart, in the *Journal of Science* in 1999, proposed a model for biofilm infection. Although this was based on information focused on cystic fibrosis, the model is applicable to all chronic infections, including the chronic infection of skin which is the chronic wound. Recent advances in technology have allowed us to expand the model by adding the molecular mechanisms by which bacteria recognize a host environment, upregulate adhesions, produce host cell senescence and produce excessive neutrophils and persistent inflammation require for a biofilm infection. This has led to the recognition that bacteria have two different strategies in infecting a human host. One is to breach and destroy host tissue (planktonic) and the second is to colonize and inflame host tissues for necessary nutrients (biofilm).

A clinical culture, a more than 100-year-old technology, is woefully inadequate for diagnosing, not only biofilm phenotype infection, but especially polymicrobial infection. Molecular methods, such as PCR and sequencing, have been utilized by science for years to identify microbes. Now these technologies (and others) are being applied to human infections. Molecular methods have the ability to be much more sensitive than clinical cultures and have the capability of quantitating each microorganism present in the infection. With this more complete characterization of the microorganisms present in a chronic wound, we have come to understand that each wound biofilm is unique in terms of its constituents present and their relative contribution to the biofilm. This requires personalized management. Personalized topical gels have a much better ability to address the entire wound biofilm and to produce a more complete suppression. By evaluating large cohorts, it is clear that by comprehensively identifying the microbes present and then specifically treating this group of microorganisms, wound healing outcomes are dramatically improved.

Antimicrobials vs. polymicrobial biofilms: In vitro studies using wound-colonizing bacteria

Presenter: Garth James, Associate Research Professor
Co-Authors: Kirker KR, Woods J, Boegli L, Durch AM, Avera E, Agostinho AM, deLancey Pulcini E, Carlson RP, and Stewart PS
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Although most wound infections are polymicrobial, traditional development and testing of antimicrobial agents has focused on single species of bacteria. To evaluate the effects of antimicrobials on polymicrobial biofilms, we developed biofilms composed of three species of bacteria, *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Clostridium perfringens* using various model systems including the drip-flow reactor (DFR), colony biofilm model (CBM), and colony drip-flow reactor (cDFR). Treatment of biofilms in the cDFR with antimicrobial wound dressings containing silver led to considerable log reductions in populations of all three species with the greatest effects on *P. aeruginosa* and *C. perfringens.* Antibiotic treatment was evaluated using the DFR. Minimum Inhibitory Concentration (MIC) assays indicated that *P. aeruginosa* and *S. aureus* were sensitive to gentamicin and *Clostridium perfringens* was resistant. Treatment of the polymicrobial biofilms with gentamicin led to slight reductions in the populations of all three species, even though *C. perfringens* was resistant. Combined treatment with gentamicin and two biofilm inhibitor compounds increased the log reduction for *P. aeruginosa* but did not influence the other two species. The CBM was used to compare susceptibilities of single- and mixed-species biofilms to ciprofloxacin and rifampin. Log reductions were similar for all three species in both single species and mixed species biofilms. Overall, the results suggest that disruption of interactions between species (*i.e.*, oxygen consumption by *P.* aeruginosa and *S. aureus* enabling the growth of *C. perfringens*) may expand the efficacy of antimicrobials. Efficacy can also be enhanced through biofilm inhibitors. Growth in a polymicrobial biofilms did not increase tolerance of the three species of bacteria to the antibiotics tested.

Back to page 1

Interfering with quorum sensing: Exploring the potential of combination therapy to treat biofilm infection in chronically infected wounds

Presenter: Gilles Brackman[1], Postdoctoral Researcher
Co-authors: Carmen Alvarez-Lorenzo[2], Morris Srebnik[3], Claes D Enk[4], Angel Concheiro[2], Hans J Nelis[1], Tom Coenye[1]
Affiliation: [1]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium;
[2]Department Farmacia y Tecnologia Farmaceutica, Universidad de Santiago de Compostela, Santiago de Compostela, Spain;
[3]Institute of Drug Research, Hebrew University, Jerusalem, Israel;
[4]Department of Dermatology, Hadassah-Hebrew University Medical School, Jerusalem, Israel.
Contact: Gilles Brackman, Gilles.Brackman@UGent.be

Chronic wounds are a major clinical problem, affecting millions of patients globally. Although several factors can contribute to the failure of healing of these wounds, bacterial infection is suggested as the most important one. The presence of bacterial biofilms contributes to the chronic nature of these wounds. Recently, quorum sensing inhibitors (QSI) have been proposed as potential anti-biofilm agents. In addition, numerous wound dressings are commercially available. However, the effect of wound care products/ dressings on bacterial biofilms is often only moderate or has insufficiently been evaluated. For this reason, we screened several commercially available wound dressings and wound care products for their ability to affect *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms in an in vitro model mimicking chronically infected wounds and soft-tissue. In addition, several experimental dressings were evaluated. These are dressings functionalized with cyclodextrins loaded with antimicrobial compounds and dressings functionalized with QSI. The anti-biofilm properties were evaluated by confocal laser scanning microscopy and culture-dependent (*i.e.*, standard plating) and independent quantification approaches (*i.e.*, viability screening using a fluorescently labeled reporter strain). Further, we investigated whether QSI could enhance the susceptibility of bacterial biofilms to treatment with conventional antimicrobial agents (*i.e.*, vancomycin and clindamycin) and to treatment with commercially available and experimental wound dressings. Finally, we evaluated whether incorporation of the QSI into wound dressings could increase the efficacy of these dressings.

Several thiazolidinedione derivatives and hamamellitannin displayed both biofilm inhibitory as well as biofilm eradicating activities. Our results suggest that these compounds do not interfere with the initial attachment but affect later stages of biofilm formation, matrix production and/or detachment of biofilm cells. Pretreatment during biofilm formation or treatment of mature biofilms with these QSI increased the susceptibility of sessile *S. aureus* and *S. epidermidis* cells towards conventional antibiotics. In addition, the efficacy of commercial wound dressings was enhanced when the QSI were added together with the different dressings and when the QSI were incorporated into the dressings.

Finally, several experimental wound dressings were observed to have strong biofilm inhibitory and eradicating properties. In conclusion, several potent QSI and experimental wound dressings with strong antibiofilm properties were developed. Our data suggest that QSI may increase the success of treatment of chronically infected wounds by increasing the susceptibility of bacterial wound biofilms towards antibiotics.

Special Presentation

State of the CBE

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

Abstract not included.

Back to page 2

SESSION 4: Energy & Environmental Biofilms

Overview of CBE energy and environmental biofilm research

Presenter: Al Cunningham, Professor, Civil Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

This session features CBE projects designed to develop biotechnology-based concepts and methods that promote environmentally responsible energy development. The first two presentations address the control of greenhouse gas emissions from coal fired power plants and other large point sources for which carbon capture and storage in geologic formations is being explored. CBE research on ureolytic biomineralization is being focused to develop technologies for sealing preferential flow paths near well bores as a way to increase storage security for carbon dioxide and other greenhouse gases sequestered below ground. The second two presentations summarize CBE research focused on developing methods for microbially enhancing coal bed methane production along with optimizing algal biofuel production.

Well leakage mitigation using biomineralization

Presenter: Robin Gerlach, Associate Professor, Chemical and Biological Engineering
Co-Authors: Phillips AJ, Lauchnor E, Ebigbo A, Eldring J, Mitchell AC, Helmig R, Cunningham AB, Stringam J, Morris D
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

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

Two overarching challenges in effectively implementing microbially induced calcium carbonate precipitation (MICP) for geologic carbon sequestration and beyond are (1) controlling the spatial and

temporal distribution of the formed precipitates and (2) controlling the observed inactivation of microbes during the calcium carbonate precipitation process. Failure to control either one of those could result in injection well plugging or the necessity to implement costly cell-reinjection or -resuscitation strategies. A large part of our recent work has focused on optimizing strategies for MICP using 1-in diameter, 2 ft long sand columns at ambient pressure as well as a 1-in diameter, 2-in long, high pressure core test system. These studies form the basis for meso-scale demonstrations using 30-in diameter, 15-in high sandstone cores in a radial flow configuration at ambient and high pressure.

Combinations of experiments and modeling have resulted in improved injection strategies for applying the MICP technology in geologic carbon sequestration and beyond.

Recent Publications:

1. Ebigbo A, Phillips A, Gerlach R, Helmig R, Cunningham AB, Class H, Spangler L. (2012): "Darcy-scale modeling of microbially induced carbonate mineral precipitation in sand columns." *Water Resources Research*. Accepted, June 11, 2012. 2011WR011714.
2. Cunningham AB, Gerlach R, Spangler L, Mitchell AC, Parks S, Phillips A. (2011): "Reducing the risk of well bore leakage using engineered biomineralization barriers." *Energy Procedia*. 4:5178–5185. doi:10.1016/j.egypro.2011.02.495
3. Schultz L, Pitts B, Mitchell AC, Cunningham AB, Gerlach R. (2011): "Imaging biologically induced mineralization in fully hydrated flow systems." *Microscopy Today*. September 2011:10–13. doi:10.1017/S1551929511000848
4. Fridjonsson EO, Seymour JD, Schultz LN, Gerlach R, Cunningham AB, Codd SL. (2011): "NMR measurement of hydrodynamic dispersion in porous media subject to biofilm mediated precipitation reactions." *Journal of Contaminant Hydrology*. 120–121:79-88. doi:10.1016/j.jconhyd.2010.07.009
5. Ebigbo A, Helmig R, Cunningham AB, Class H, Gerlach R. (2010): "Modeling biofilm growth in the presence of carbon dioxide and water flow in the subsurface." *Advances in Water Resources*. 33:762–781. doi: 10.1016/j.advwatres.2010.04.004

Back to page 2

Imaging, microanalysis and modeling of the microbially induced $CaCO_3$ precipitation process

Presenter: James Connolly[1,2], PhD Student, Environmental Engineering
Co-authors: Adam Rothman[1,2], Benjamin Jackson[1,4], Isaac Klapper[1,4], Al Cunningham[1,3] and Robin Gerlach[1,2]
Affiliations: [1] Center for Biofilm Engineering, Montana State University;
[2] Department of Chemical and Biological Engineering, Montana State University;
[3] Department Civil Engineering, Montana State University;
[4] Department of Mathematical Sciences, Montana State University, Bozeman, MT, 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. Two biological processes studied in this work are biofilm formation and microbially induced calcium carbonate precipitation (MICP) driven by ureolysis. These processes are often studied independently, but for accurate characterization of the MICP process, both must be studied together and in the context of pore scale reactive transport.

In a real subsurface environment, where microbiological activity has been stimulated for MICP, it is likely that biofilms will form. The biofilm component of the system is commonly neglected in laboratory experiments because the most common model organism (*Sporosarcina pasteurii*) often does not form a significant biofilm. In practice, a consortium of microorganisms would be stimulated: some microorganisms would be likely to produce biofilm and perform other functions while others would perform the engineered function. The quantitative study of mineral formation linked with biofilm formation and the measurement of aqueous constituents in the system provides a challenge because all require unique analysis techniques. The results of these different techniques must be linked in a useful

way. Computer modeling of the system provides this link where data can be fed to provide experimental validation and the ability to predict system behavior in both laboratory and field settings.

Light microscopy, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) have all been used in this work to provide data for existing and future computer models of the MICP process. The majority of the research presented in this talk will focus on measurement of the rate of urea hydrolysis in a biofilm grown in a small plug flow reactor. The organism used in the kinetic study is an *Escherichia coli* strain capable of thick biofilm formation that has been engineered to constitutively express both the urease enzyme and green fluorescent protein. This model organism provides a new opportunity to image an undisturbed ureolytic biofilm and link biofilm geometry (such as thickness) to bulk chemical analyses in the influent and effluent of flow reactors. The ureolytic *E. coli* biofilm is expected to be more similar to multispecies biofilms likely to be observed in environmental applications of the MICP process. Thus, the data obtained using this system are likely more relevant to field experiments and real applications.

Back to page 2

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

Presenter: Elliott Barnhart, PhD Student, Microbiology
Co-Authors: Elizabeth Meredith (Montana Bureau of Mines and Geology, Billings, MT, USA);
Jennifer McIntosh (University of Arizona, Tuscon, AZ);
Arthur C Clark (US Geological Survey, Denver, CO, USA);
William H Orem (US Geological Survey, Reston, VA, USA);
Alfred B Cunningham, Robin Gerlach, Matthew W Fields (Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA)
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT and US Geological Survey, Helena, MT

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. One promising strategy currently being investigated is the use of algal extracts to stimulate methane production from coal. An increased understanding of enhancement strategies as well as this microbial system, and the biotic and abiotic parameters that control its activity, may expedite development of strategies to stimulate in situ coal bed methane production in an environmentally sustainable fashion.

The ecology and physiology of an 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 pyrotag sequence determination 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 contained coal and was deployed at the bottom of a methane-producing well for approximately 90 days. The composition and structure of this inoculum was also investigated with pyrotag sequence determination and microscopy as well as cultivation techniques (with and without nutrient supplementation) that maximized methane production in batch, bench-scale incubations. These methods showed that the active coal-utilizing microbial consortium was composed of a diverse bacterial community associated with a less diverse methanogenic community. Multivariate statistical methods were used to relate these community features to hydrogeochemical parameters. Fluorescent in situ hybridization analysis of the sampled microbes revealed that microbial populations were associated with coal particles. 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 novel information about in situ and laboratory based microbial methane-producing community composition and physiology provides insight for in situ microbially enhanced coal bed methane production.

Potential role of carbon fixation pathways during bio-oil accumulation in *Phaeodactylum tricornutum*

Presenter: Jake Valenzuela, PhD Candidate, Chemistry and Biochemistry
Co-Authors: Matthew W. Fields
Affiliation: Center for Biofilm Engineering and the Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT

Phaeodactylum tricornutum is a unicellular marine diatom that forms a diverse group of eukaryotic microalgae that account for up to 40% of the total marine primary production each year. In addition to photoautotrophic growth (*i.e.*, carbon fixation via sunlight), diatoms can store carbon and energy in the form of lipids. From an ecological standpoint, it is important to investigate the interplay between carbon, nitrogen, and phosphate levels as it pertains to global primary production as well as lipid production for biofuels. Elucidation of carbon, nitrogen and phosphate metabolism was done with RNA sequencing at three time points throughout growth with changing C:N:P ratios. The time points were selected pre- and post-lipid accumulation while cell number, nitrate, phosphate, chlorophyll, lipids, and dissolved inorganic carbon were monitored. Lipid accumulation increased over time; however, the increase was initiated before external nitrate was completely exhausted. Exogenous phosphate was depleted before nitrate, and these results indicated that the depletion of exogenous phosphate might be an early trigger for lipid accumulation that is magnified upon nitrate depletion. As expected, many of the genes associated with nitrate and phosphate utilization were up-expressed throughout growth. Once lipid accumulation is initiated it can be reversed by the supplementation of nitrate and/or phosphate. The repletion of nitrogen and phosphorous returns the cell to a ratio of C:N:P that does not favor lipid accumulation. This interplay between nitrogen and carbon metabolism has indicated that there is an increased demand for carbon while nitrogen is available but a decrease in demand as nitrogen becomes limited. Transcriptome analysis indicated that *P. tricornutum* utilizes two different mechanisms of carbon fixation based on the levels of carbon and nitrogen in the environment. While many of the genes associated with the C3 pathway for photosynthetic carbon reduction were not significantly altered, genes involved in a putative C4 pathway for photosynthetic carbon assimilation were up-expressed as the cells depleted nitrate, phosphate, and exogenous dissolved inorganic carbon (DIC) levels. *P. tricornutum* has multiple, putative carbonic anhydrases, but only two were significantly up-expressed (2-fold and 4-fold) at the last time point when exogenous DIC levels had increased after the cessation of growth. The results suggest that *P. tricornutum* continued carbon dioxide reduction when population growth was arrested and different carbon-concentrating mechanisms were used dependent upon exogenous DIC levels. In addition, the consumption of carbon dioxide also seems to be more dependent on availability of a nitrogen rather than phosphate. The results shed insight of environmental responses of diatoms to changing levels of dissolved inorganic carbon with respect to nitrogen and phosphorous. The types and timing of cellular responses to inorganic carbon will provide insight into photoautotrophic carbon flow in to biomass and lipid accumulation.

Back to page 2

SESSION 5: Biofilm Methods

In vitro studies of *Legionella pneumophila* grown as mono- and multi-species biofilms

Presenter: Diane Walker, Research Engineer
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Outbreaks of Legionnaire's Disease continue to occur nearly 40 years after identification of its etiologic agent. In nearly all cases, the source is aerosolized *Legionella* species from man-made structures (air conditioning units, cooling towers, dental unit water lines, showers and hot tubs, as examples). Laboratory studies of *Legionella* biofilms are useful for researchers to better understand this pathogen and can also be used to test potential products that might be applied in these structures to help reduce the risk of infection. A literature review was conducted to identify biofilm growth methods employed by researchers to study *Legionella pneumophila* and a compilation of these findings will be presented.

A new flow cell for time-lapse confocal microscopy

Presenter: Betsey Pitts, Senior Research Associate/Facilities Manager, Microscopy
Co-Authors: Lindsey Lorenz, Paul Sturman, Kelli Buckingham-Meyer, Bryan Warwood*, Phil Stewart
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, US
*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, time-lapse confocal microscopy 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 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 in this presentation. Our prototype testing has produced a simple flow cell design which allows for high throughput coupon testing and efficient collection and production of movies of biofilm treatment, as well as quantitative microscopic analysis of biofilm over time.

Back to page 2

Correlation of the single tube method and the Treatment Flow Cell: Log reductions and confocal microscopy

Presenter: Kelli Buckingham-Meyer, Research Scientist
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Recent development of a new flow cell used for time-lapse confocal microscopy provides a useful tool for visualizing effects of treatments on biofilm under flow or no flow conditions in real time. The flow cell, which was designed to hold CDC Biofilm Reactor coupons, marries nicely with a newly approved ASTM standard method (E2871) "The Standard Test Method for Evaluating Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm Grown in the CDC Biofilm Reactor Using the Single Tube Method." Method E2871 uses a closed system approach to measure a log reduction in viable cell density. A coupon is placed

in a single tube for the treatment, neutralization and sampling steps to prevent the loss of cells. An untreated control is run in parallel to the treated coupons. Treatment parameters such as disinfectant concentration, contact time (usually minutes) and temperature (22–50°C) used in the "Single Tube Method" can be applied to a biofilm coupon treated in the flow cell. Coupons are placed in the flow cell, the flow cell is placed on the stage of the confocal microscope, the biofilm is visualized and then disinfectant or control water is pumped through the flow cell. The effects of the treatment are visualized over the course of the desired contact time. The resulting stacks of images are then constructed into movies using Imaris (Bitplane) image analysis software. The movies provide information on disinfectant modes of action such as biofilm removal and length of time to see an effect on intact biofilm under flow or no flow conditions in real time.

During the development of the "Single Tube Method," log reduction values were obtained for three classes of disinfectants at two concentrations each. These same disinfectants were applied to biofilm coupons in the treatment flow cell. Movies shown will demonstrate visual effects of treatments and controls on the biofilm in real time.

SESSION 6: Biofilm Control

Back to page 2

Antimicrobials vs. biofilms: A review

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

This review examines selected practical features of biofilm control with antimicrobial agents with an eye open for strategies to improve the efficacy of existing regimens and discover new chemistries. Interpretations are illustrated with data from the literature along with some unpublished results from CBE laboratories. Examples discussed range across the spectrum of actives from industrial biocides to medical antibiotics. An overarching theme of the talk is the necessity of using biofilm methods to arrive at the right solutions to biofilm problems. As no single method uniquely predicts real-world phenomena, it is advisable to use a method that sets a high performance standard and to use more than one method to ensure a robust conclusion. Biofilm cell density (*e.g.*, cfu per square cm) at the time of treatment is an important determinant of antimicrobial efficacy for treatments as different as chlorine bleach and aminoglycoside antibiotics. Dense biofilms harbor inactive or dormant cells. There is potential to discover new agents that target non-growing cells and are thereby more effective against biofilms. Videos of biofilm during antimicrobial treatment highlight the failure of most treatments to remove biofilm. There is a huge need, and commensurate opportunity, to discover agents that promote detachment. It is important to measure removal in addition to killing. Measuring removal routinely will be the first step towards devising ways to optimize the biofilm removal process. The rationale for formulating combinations of antimicrobials for controlling biofilms will be outlined and illustrated with case studies.

Ignorance, bliss, and antibiotic tolerance of nutrient-limited bacteria

Presenter: Pradeep Singh, Associate Professor, Microbiology
Affiliation: University of Washington, Seattle, WA, USA

Antibiotic failure is a major public health problem. While genetic mechanisms can produce antibiotic resistance, treatment also fails when genetically sensitive bacteria develop phenotypic (or non-inherited) antibiotic tolerance. Research with many bacterial species indicates that a prime cause of antibiotic tolerance is the presence of starved or nutrient-limited cells that resist killing no matter how much drug is applied. The biofilm mode of bacterial growth, which is responsible for many chronic infections, is a significant cause of bacterial starvation in vivo. Nutrient limitation within biofilm clusters is due to reduced diffusion and nutrient consumption by bacteria with first access to growth substrates. Starvation produced by these mechanisms causes biofilms to be extremely tolerant to killing by most all antibiotics. How does

nutrient limitation produce such pronounced antibiotic tolerance? Previous studies point to two general mechanisms. One idea suggests that growth arrest produced by starvation is itself protective. An alternative hypothesis is that starved cells induce active mechanisms producing tolerance, and that interfering with these responses could reverse tolerance. We tested this hypothesis by manipulating the stringent response (SR), a regulatory mechanism induced when bacteria sense stress and starvation. The SR is mediated by the alarmone (p)ppGpp, synthesized by the *relA* and *spoT* gene products.

We compared the antimicrobial tolerance of the *relA spoT* mutant (unable to induce the SR) to the wild type strain. In log-phase planktonic cells, serine starvation caused growth arrest in both strains but only conferred ofloxacin tolerance to the wild type. This indicates that starvation alone (without induction of the SR) was not sufficient to confer tolerance. Inactivation of the SR also increased the killing of biofilm bacteria by a broad range of antimicrobials (ofloxacin, meropenem, colistin, gentamicin) by 1,000- to 10,000-fold, and oxidants by 100- to 10,000-fold.

We tested the in vivo relevance of our findings using animal infection models that measured the ability of antimicrobials to rescue animals from lethal infection, and the efficacy of biofilm killing. Mice infected with intra-peritoneal stationary phase wild type bacteria died despite ofloxacin treatment. In contrast, mice infected with the *relA spoT* mutant were rescued by treatment. Ofloxacin also produced 60-fold greater killing of *relA spoT* mutant bacteria (compared to wild type) in a subcutaneous biofilm implant model. These data show that the stringent response mediates the antimicrobial tolerance of *P. aeruginosa* in biofilms, stationary growth bacteria, and in vivo infection models. Thus, antimicrobial tolerance in biofilms and other nutrient limited conditions may depend upon orderly and regulated response to starvation that could be targeted using new therapeutic approaches.

Back to page 2

Non-biological approach to eradication of biofilm bacteria and the EPS biomass structure

Presenter: Chris Samuel, Vice President of Sales & Marketing
Author: Matthew F Myntti, President and Head of Research & Development
Affiliation: Microbial Defense Systems, LLC, Jacksonville, FL, USA

Current approaches to eliminating bacteria in biofilms form limitations in their effectiveness and toxicity. In general, these technologies do not address the removal of the EPS structure and do not treat the persister cells within the biofilm because they cannot penetrate through the EPS. Those treatment solutions which do treat the resistant bacteria within the biofilm are of high toxicity and pose threats to anyone coming into contact with them or to the environment. By combating biofilm with a structural rather than biological approach, MDS has been able to combine existing chemical technologies to produce a synergistic effect that offers a unique mix of properties. This technology attacks the EPS structure while simultaneously killing the bacteria within. The results are a chemistry that has significant efficacy across a broad range of microbial species, no resistance mechanism, low toxicity, is inexpensive, non-corrosive, and non-caustic.

In vitro testing at the Center for Biofilm Engineering (CBE) against a 7-day biofilm in the drip flow reactor has demonstrated greater than a 5 log reduction of *Staphylococcus aureus* and greater than 7 log reduction of *Pseudomonas aeruginosa* with a 5 minute static application of a 6.5 pH solution. Additionally, this technology has been proven effective against mixed-species oral biofilms in the drip flow reactor, yielding greater than a 2 log reduction as compared to less than a 0.5 log reduction from chlorhexidine gluconate at 0.12%. Further testing at CBE against a *Candida albicans* biofilm demonstrated a multi-log reduction, as compared to no reduction from flucanozole.

In addition, MDS solutions are effective at removing the biomass of the EPS structure, providing for prolonged intervals for bacterial regrowth. In vitro testing at the CBE against a 7 day biofilm in the drip flow reactor has demonstrated greater than 60% mass reduction with a 15 second moderate flow of the 6.5 pH solution.

Poster Abstracts

Center for Biofilm Engineering posters

CBE Poster #560

Date: 11/2011
Title: **Potential of microbes to increase CO_2 storage security**
Authors: ***Robin Gerlach***[1,2], Mitchell AC[2,3], Ebigbo A[4], ***Adrienne Phillips***[1,2], Spangler L[5], Cunningham AB[2]
Affiliation: [1] Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[3] Institute of Geography and Earth Sciences, Aberystwyth University, UK
[4] Dept. of Hydromechanics and Modeling of Hydrosystems, University of Stuttgart, DE
[5] Energy Research Institute, Montana State University, Bozeman, MT, USA
Sponsored by: United States Department of Energy's EPSCoR program and the DOE ZERT program

Geologic Carbon Capture and Storage (CCS) involves the injection of supercritical CO_2 into underground formations, such as brine aquifers, where microbe-rock-fluid interactions will occur. These interactions may be important for the long-term fate of the injected CO_2, particularly near well bores and potential leakage pathways. This poster presents concepts and results from bench- to meso-scale experiments focusing on the utility of attached microorganisms and biofilms to enhance storage security of injected CO_2, via mineral trapping, solubility trapping, formation trapping, and leakage reduction. Batch and flow experiments at atmospheric and geologic CO_2 storage-relevant pressures have demonstrated the ability of microbial biofilms to decrease the permeability of natural and artificial porous media, to survive the exposure to $scCO_2$, and to facilitate the conversion of CO_2 into long-term stable carbonate phases as well as to increase the solubility of CO_2 in brines. Recent work has focused on large scale (75 cm diameter, 38 cm high sandstone) radial flow systems, as well as the molecular characterization and isolation of microbes from geologic carbon sequestration-relevant environments. Methods for microscopic and macroscopic visualization of relevant processes from the pore to the bulk scale are being developed and have been proven to be essential tools in establishing the necessary understanding to increase CO_2 storage security. As a result, reactive transport models describing the influence of biological processes on CO_2 storage security have been developed and are continuously being modified to include relevant processes.

Back to page 3

CBE Poster #562

Date: 02/2012
Title: **Quality-score refinement of SSU rRNA gene pyrosequencing differs across gene region for environmental samples**
Authors: ***Kara Bowen De León***, Ramsay BD, and Fields MW
Affiliation: Department of Microbiology, Thermal Biology Institute, and Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: ENIGMA (http://enigma.lbl.gov/)

Due to potential sequencing errors during pyrosequencing, species richness and diversity indices of microbial systems can be miscalculated. The 'traditional' sequence refinement method of removing sequences that are short, contain Ns, or have primer errors is not sufficient to account for overestimations. Recent in silico and single organism studies have revealed the importance of sequence quality scores in the estimation of diversity; however, this is the first study to compare quality-score stringencies across four regions of the SSU rRNA gene sequence (V1V2, V3, V4, and V6) with real environmental samples compared directly to corresponding clone libraries produced from the same primer sets. The pyrosequences were

subjected to varying quality-score cutoffs that ranged from 25 to 32, and at each quality-score cutoff either 10% or 15% of the nucleotides were allowed to be below the cutoff. With the tested samples we observed that the quality scores that followed the trajectory similar to that of the clone libraries were the V1V2, V4, and V6 regions—$Q27_{15\%}$, $Q30_{10\%}$, and $Q32_{15\%}$, respectively—and the most stringent Q tested ($Q32_{10\%}$) was not enough to account for species richness inflation of the V3 region pyrosequencing data. Results indicated that quality-score assessment greatly improved estimates of ecological indices for real environmental samples (species richness and α-diversity) and that the effect of quality-score filtering was region-dependent.

CBE Poster #563

Date: 02/2012
Title: **Dissolved organic matter in the WAIS Divide ice core**
Authors: D'Andrilli J[1,2] , **Christine Foreman**[1,2], McConnell J[3] and Priscu J[1]
Affiliation: [1] Department of Land Resources and Environmental Sciences, and
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[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 3

CBE Poster #564

Date: 01/2012
Title: **Structure impacts function for a syntrophic biofilm of *Methanococcus maripaludis* and *Desulfovibrio vulgaris***
Authors: ***Kristen A. Brileya***[1], Sabalowsky A[1,2], Ramsay B[1], Zane G[3], Wall JD[3], Fields MW[1,2]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[2] Department of Microbiology, Montana State University, Bozeman, MT, USA
[3] Biochemistry Division, University of Missouri; ENIGMA (http://enigma.lbl.gov)
Sponsored by: US DOE Office of Biological and Environmental Research Division

Transfer of reduced carbon and electrons between microbial community members is of interest in anoxic systems, and methanogenesis represents a crucial trophic level that can include sulfate-reducing bacteria and methanogenic archaea. The current work uses a dual-culture approach to examine the structure of a syntrophic biofilm formed by the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough and the methanogenic archaeon *Methanococcus maripaludis.* Biofilm was grown in a continuously stirred reactor where cells could attach to a silica surface or remain suspended. Under the tested conditions, *D. vulgaris* formed monoculture biofilm, but *M. maripaludis* did not. However, *M. maripaludis* did form pellicles in static batch cultures while *D. vulgaris* did not form a pellicle. Under syntrophic conditions, a methanogenic biofilm formed and reached steady-state in approximately 14 days based upon protein and methane levels. Biofilm establishment was dependent upon initial colonization by *D. vulgaris* that was followed by recruitment of *M. maripaludis* into the biofilm matrix. Steady-state biofilm was fixed for Fluorescence in situ Hybridization (FISH) and confocal laser scanning microscopy (CLSM). FISH revealed a framework of *D. vulgaris* with both single cells and large micro-colonies of *M. maripaludis* interspersed throughout the biofilm. 3D-FISH and CLSM of hydrated intact biofilm confirmed steady-state biofilm irregularity, with ridge, valley and spire macro-architecture. Colorimetric assays indicated cell-associated carbohydrate was composed of .035 µg hexose/µg protein, .017 µg pentose/µg protein and .011 µg uronic acid/µg protein, similar to *D. vulgaris* mono-culture biofilm and approximately 5 times less than *M. maripaludis* pellicles. Filaments presumed to be protein have been observed in dual-culture biofilm matrix with electron and atomic force microscopy, and matrix was sensitive to proteinase K treatment during preliminary work with Catalyzed Reporter Deposition FISH. Compared to wild-type planktonic cells, a Δ*fla*G mutant was deficient in biofilm formation, with only sparse colonization, as determined by Field Emission Scanning Electron Microscopy (FE-SEM) and quantification of protein and carbohydrate. Interestingly, the Δ*fla*G mutant was not affected in motility, and intact flagella were observed via TEM. The gene, DVU1442, had closely related sequences in other *Desulfovibrio* species, including *Desulfovibrio* DP4, Miyazaki, G20, and *D. magneticus*. These data suggested that *Desulfovibrio* species have a specialized flagellum filament used for biofilm formation and maintenance as opposed to motility. In addition, Δ*fla*G did grow syntrophically with *M. maripaludis* in the planktonic state, but did not form coculture biofilm. Syntrophic biofilm 3-D structure appears to be initialized by *D. vulgaris* that provides an advantageous environment for *M. maripaludis* to establish micro-colonies throughout the *D. vulgaris* scaffold, and that *M. maripaludis* might use hydrogenotaxis for incorporation into the biofilm.

Back to page 3

CBE Poster #565

Date: 01/2012
Title: **In vitro efficacy of bismuth thiols against biofilms formed by bacteria isolated from human chronic wounds**
Authors: ***James P. Folsom***, Baker B, Stewart PS
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Montana Board of Research and Commercialization Technology

Aim: The purpose of this study was to evaluate the antimicrobial efficacy of thirteen bismuth thiol preparations for bactericidal activity against established biofilms formed by two bacteria isolated from human chronic wounds.

Methods: Single species biofilms of a *Pseudomonas aeruginosa* or a methicillin-resistant *Staphylococcus aureus* were grown in either colony biofilm or drip-flow reactor systems. Biofilms were challenged with bismuth thiols, antibiotics or silver sulfadiazine, and log reductions were determined by plating for colony formation.

Conclusions: Antibiotics were ineffective or inconsistent against biofilms of both bacterial species tested. None of the antibiotics tested was able to achieve >2 log reductions in both biofilm models. The 13 different bismuth thiols tested in this investigation achieved widely varying degrees of killing, even against the same microorganism in the same biofilm model. For each microorganism, the best bismuth thiol easily outperformed the best conventional antibiotic. Against *P. aeruginosa* biofilms, bismuth-2,3-dimercaptopropanol (BisBAL) at 40–80μg ml^{-1} achieved >7.7 mean log reduction for the two biofilm models. Against MRSA biofilms, bismuth-1,3-propanedithiol/bismuth-2-mercaptopyridine *N*-oxide (BisBDT/PYR) achieved a 4.9 log reduction.

Significance and Impact of the Study: Bismuth thiols are effective antimicrobial agents against biofilms formed by wound bacteria and merit further development as topical antiseptics for the suppression of biofilms in chronic wounds.

Back to page 3

CBE Poster #566

Date: 01/2012

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

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

Affiliation: [1] Center for Biofilm Engineering, [2] Dept. of Chemical and Biological Engineering, and [3] Dept. of Civil Engineering, Montana State University, Bozeman, MT, USA
[4] Department of Chemical, Biological & Environmental Engineering, Oregon State University, Corvallis, OR, USA

Sponsored by: National Science Foundation and the United States Department of Energy

Biological processes in the subsurface environment are important to understand in relation to many engineering applications including, but not limited to: groundwater remediation, geologic carbon sequestration, and petroleum production. Two biological processes studied here are biofilm formation and microbially induced calcium carbonate precipitation. Many analytical tools are available to researchers for the study of these processes, but microscopic imaging provides additional information and validation to these data sets. For example, visualization of biofilm geometry in the pore space is important for the characterization of hydrodynamic changes in a porous medium affected by biofilm growth.

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

CLSM in order to validate the use of these contrast agents in 3D systems. Images from this comparative study will be presented.

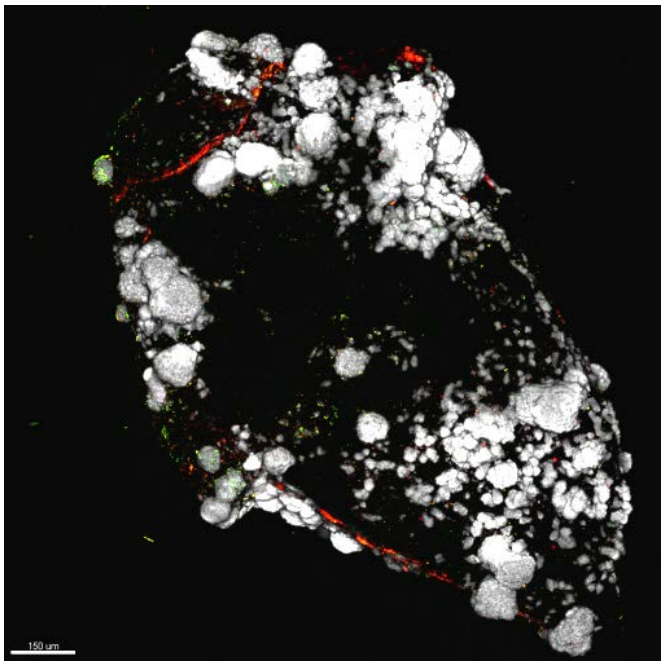


Figure 1. A CLSM reconstruction of a sand grain colonized by *Sporosarcina pasteurii* under ureolytic conditions where calcium carbonate (shown in white) has been precipitated. The sample was stained with Invitrogen LIVE/DEAD so areas with healthy cells are shown in green. Regions with cells that have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

Back to page 3

CBE Poster #567

Date: 04/2012

Title: **Evaluation of 3M™ Petrifilm™ AC plates as an equivalent alternative to drop plating on R2A agar plates in a biofilm system**

Authors: ***Blaine Fritz***, Goeres D, Walker DK, Parker A, Orr D

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Sponsored by: MSU University Scholars Program and Michael J. Svarovsky, 3M

This project evaluated 3M™ Petrifilm™ Aerobic Count (AC) plates as an alternative, more efficient method for bacterial enumeration. Using Petrifilm™ allows the researcher to avoid preparing agar plates for bacterial enumeration. Currently, the majority of scientific literature concerning enumeration of bacteria on Petrifilm™ is from the food industry. There are no published studies examining the use of Petrifilm™ for enumeration of biofilm bacteria. A *Pseudomonas aeruginosa* biofilm was grown in a CDC reactor according to ASTM Method E2562. The mature biofilm was exposed to chlorine (buffered water for controls) and neutralized. The biofilm was removed from the surface, disaggregated, and serially diluted. Samples from the dilution tubes were plated in duplicate on Petrifilm™ AC plates, drop plated on R2A plates, and incubated at 36°C for 48 hours. The colonies were enumerated after 24 and 48 hours. The experiment was replicated three times by one technician and four by the other. Statistical analysis revealed no statistically significant difference between the results from the different plating methods. Equivalence testing demonstrated equivalence, assuming that $\log_{10}[CFU/cm^2]$ differences as large as 0.37 can be considered negligible. The results from this study demonstrate that Petrifilm™ AC plates could replace drop plating on R2A agar as an alternative method for bacterial enumeration.

CBE Poster #568

Date: 01/2012

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

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

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

Sponsored by: Department of Defense, Department of Energy, Molecular Bioscience Program

Back to page 3

Phaeodactylum tricornutum is a unicellular diatom that belongs to the class *Bacillariophyceae*. The full genome has been sequenced (<30 Mb), and approximately 25 to 30% TAG accumulation has been reported under different growth conditions. In order to elucidate gene expression profiles of *P. tricornutum* during nutrient-deprivation and lipid-accumulation, cell cultures were grown with nitrate and phosphate at a ratio of 20:1 (N:P) and whole-genome transcripts were monitored over time. The specific NR fluorescence (NR fluorescence per cell) increased over time; however, the increase in NR fluorescence was initiated before external nitrate was completely exhausted. Phosphate was depleted before nitrate, and *P. tricornutum* appears to accumulate and store external phosphate under the tested growth conditions. Three transcriptomic time points were selected based upon different growth phases with dynamic NR fluorescence. The first sample (Q1) represented exponential growth with high external nitrate, phosphate, and DIC levels and low NR fluorescence. The second sample (Q2) represented the transition between exponential and stationary phases—with depleted nitrate and phosphate levels and low DIC, but increasing NR fluorescence. The third sample (Q3) represented extended stationary phase induced by depleted nitrate and phosphate, rebounding DIC but high NR fluorescence. RNA-seq analyses assembled 30,373 transcripts to 10,124 mapped loci and 1,812 genes were differentially expressed at statistically significant levels between phases. Of all significant genes, approximately 180 genes were differentially expressed between all three time points, 546 genes between any two time points, and 177 genes between only two time points. With a focus on nitrogen and carbon metabolism, the expression trends for key genes were determined. The up-expression of both putative nitrate (469- and 808-fold) and phosphate (199- and 507-fold transporters were observed during exponential growth as nitrate and phosphate were depleted. Both nitrate (NADH-dependent) and nitrite reductase (Fd-dependent) were up-expressed (over 200-fold) as nitrate levels were depleted. In conjunction with the nitrate assimilation, glutamine synthetase, glutamate synthase, asparagine synthetase, glutamate dehydrogenase, and carbamoyl-phosphate synthetase were up-expressed (3-fold to 175-fold). The highest overall up-expression was observed in the cytosolic glutamate dehydrogenase, but the largest increase from basal levels was observed in the chloroplastic glutamine synthetase. All of these genes displayed a down-expression in prolonged stationary-phase during sustained increases in NR fluorescence. Many of the genes associated with the C3 pathway for photosynthetic carbon reduction (PCR) were not significantly altered; however, genes involved in the C4 pathway for photosynthetic carbon assimilation (PCA) were up-expressed as the cells depleted nitrate, phosphate, and DIC levels. Gene products involved in C4-PCA were up-expressed and included PEP carboxylase, PEP carboxykinase, and pyruvate carboxylase; however, PEP carboxykinase and one form of the pyruvate carboxylase displayed the highest up-expression during DIC depletion. The malate dehydrogenase, malic enzyme, and pyruvate-P dikinase were up-expressed 6-fold, 8-fold, and 3-fold respectively, and could be responsible in recycling oxaloacetate, malate, and pyruvate for delivery of CO_2 for PCR. *P. tricornutum* has multiple, putative carbonic anhydrases, but only two were significantly up-expressed (2-fold and 4-fold) at the last time point when DIC levels had increased. The results indicated that during nitrate and phosphate depletion, *P. tricornutum* depleted external DIC levels and initiated lipid accumulation. Based upon

transcript levels, C4 based carbon assimilation was used in response to depleted DIC during presumptive lipid accumulation.

CBE Poster #569

Date: 01/2012
Title: **Design and testing of a flow cell for microscopy of biofilm during treatment**
Authors: ***Betsey Pitts, Lindsey Lorenz***, Sturman P, Buckingham-Meyer K, Warwood B*, 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 3

CBE Poster #570

Date: 04/2012
Title: **Modeling kinetics of ureolytic bacteria in flow systems**
Authors: ***Adam Rothman***, Connolly J, Jackson B, Gerlach R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Microbially induced calcite precipitation (MICP) is a promising technology that can be used in a wide range of applications from carbon sequestration to bioremediation of heavy metals. MICP can be performed by ureolytic soil bacteria, like *Sporosarcina pasteurii* through manipulation of the local and bulk saturation index. The purpose of this research is to develop kinetic parameters for ureolytic bacteria in biofilm flow systems analogous to what could be found in the subsurface and to apply these parameters to generate models for how urea is utilized by the biofilm. Both *S. pasteurii* and a recombinant *Escherichia coli* strain were grown in flow systems to generate kinetic data. The biofilm systems were maintained until steady state was reached. Steady state aqueous measurements along with biofilm geometry data were used to generate a basic COMSOL model of the process.

CBE Poster #571

Date: 01/2012
Title: **Impact of biofouling on porous media transport dynamics measured by magnetic resonance displacement relaxation correlation**
Authors: ***Alexis B. Sanderlin*** [1, 2], Vogt SJ [1, 2], Codd SL [1, 3], Seymour JD [1, 2]
Affiliation: [1] Center for Biofilm Engineering,
[2] Dept. of Chemical and Biological Engineering,
and [3] Dept. of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: U.S. DOE Grants EPSCoR DE-FG02-08ER46527 and US DOE OS BER DE-FG02-07-ER-64416, U.S. NSF CAREER AWARD 0642328 to SLC

Biofilms permeate our everyday lives, particularly in biofouling of porous media used for biomedical and industrial filtration, and geological materials relevant to environmental processes. Understanding how these biofilms impact transport processes in porous media is critical to eradicating them in unfavorable situations and promoting their growth in beneficial ones, such as carbon sequestration. Most methods of studying biofilms require the sample to be destroyed for examination. With Magnetic Resonance (MR), the biofilm can be observed during its life cycle without destroying the sample under study.

Bacillus mojavensis was grown in an MR magnet at 21°C in a 50-mm long, 10-mm I.D. liquid chromatography column filled with 240-µm, monodispersed polystyrene beads and analyzed using MR images and relaxation time measurements. Employing different observation times for both T_2-T_2 and propagator-T_2 measurements, the growth and decay of the biofilm is clearly seen as the zero-flow peak increases with biofilm development and decreases with the biofilm sloughing process. This quantifies the amount of biomass present. An outstanding question in the modeling of transport in biofouled porous media is the presence or absence of flow within the biomass. The unique data obtained indicates clearly for the first time that flow does not occur within the biomass.

Back to page 3

CBE Poster #572

Date: 01/2012
Title: **Magnetic resonance relaxation of alginate solutions and gels**
Authors: ***Sarah J. Vogt***[1], Fabich HT[1], Sherick ML[1], Brown JR, Seymour JD[1,2], and Codd SL[1,3]
Affiliation: [1] Center for Biofilm Engineering,
[2] Dept. of Chemical and Biological Engineering, and
[3] Department of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: MLS and HTF are funded by INBRE Grant Number P20RR016455 from the National Center for Research Resources (NCRR), NIH. DOE EPSCOR DE-FG02-08ER46527

Alginate—a biopolymer produced both by algae and by certain types of bacteria—has a variety of industrial uses, including use as a food additive. A primary focus of this work is on alginate biopolymer systems that form physical gels with cations. These gels are candidates for tissue growth constructs due to their ability to spontaneously form structures such as capillaries during diffusive reaction and their role in chronic *P. aeruginosa* infections in cystic fibrosis (CF) patients. The differences in gel structure between algal- and microbial-produced alginates are investigated. Of particular interest is the difference in gel structure and formation between acetylated alginate formed by a *P. aeruginosa* isolate FRD1 from CF patients and de-acetylated alginate from a genetic mutant FRD1153. One- and two-dimensional magnetic resonance (MR) relaxation and diffusion correlation experiments have been performed on alginate systems and the effects of hydrogen exchange and polymer mobility have been studied.

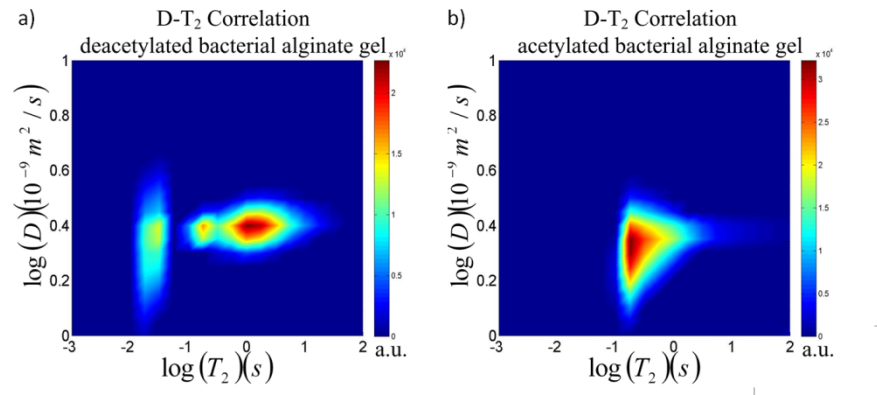


Fig. 1: Diffusion-T_2 spectra of heterogeneous alginate gels formed with 1M $CaCl_2$ from 2%wt. alginate solutions from two different genetic variants of *P. aeruginosa*: a) FRD1153, which produces de-acetylated alginate, and b) FRD1, which produces acetylated alginate.

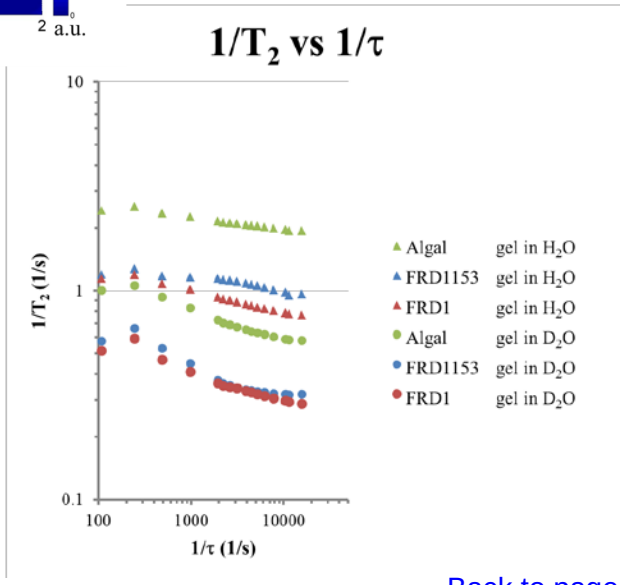


Fig. 2: T_2 dispersion curves of homogeneous alginate gels formed with gluconic acid δ-lactone and $CaCO_3$ from 1%wt. alginate solutions for three different genetic variants of *P. aeruginosa.* Gels were formed in both distilled water (H_2O) and 99.9% D_2O to investigate the effect of proton exchange between the polymer matrix and the bulk water.

Back to page 3

CBE Poster #573

Date: 01/2012

Title: **Analysis of homogeneous and inhomogeneous gelation of alginate derived from *Pseudomonas aeruginosa***

Authors: ***Matthew L. Sherick***, Fabich HT, Vogt SJ, Seymour JD, Franklin MJ, and Codd SL

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Sponsored by: Equipment funding was provided by the US NSF MRI Program and the MJ Murdock Charitable Trust. MLS and HTF are funded by INBRE Grant Number P20RR016455 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). DOE EPSCOR DE-FG02-08ER46527.

Mucoid strains of *Pseudomonas aeruginosa* bacteria produce the extracellular polysaccharide alginate, which forms a physical biopolymer gel upon introduction of a divalent cation [1, 2]. Both acetylated and de-acetylated bacterial alginate have been extracted by refining isolation procedures found in publications [3], and their gels analyzed using Nuclear Magnetic Resonance (NMR) techniques. Alginate gels have potential applications in the field of artificial tissue engineering [4] due to their ability to form mesoscale structures. This system is also of interest in cystic fibrosis (CF), as patients are vulnerable to chronic *P. aeruginosa* infections [1]. Studying bacterial alginate formation and gelation provides a greater insight into the role of water molecular dynamics in gels produced by these infections.

Gelation of algal alginate has previously been thoroughly examined using NMR [5], and a point of interest is to compare the properties of bacterial alginate gels with those of algal alginate gels. In addition, acetylated bacterial alginate isolated from *P. aeruginosa* FRD1 is shown to have different gel properties than de-acetylated alginate isolated from *P. aeruginosa* FRD1153, with the latter forming a more inhomogeneous

gel using a diffusion reaction front process. Homogenous gels were prepared and analyzed with the same NMR techniques.

1. Nivens DE, et al, "Role of alginate and its O acetylation in formation of *Pseudomonas aeruginosa* microcolonies and biofilms," *J Bacteriol*, 2001; 183(3): 1047–1057.
2. Skjakbraek G, Grasdalen H, and Smidsrod O, "Inhomogeneous polysaccharide ionic gels," *Carbohydrate Polymers*, 1989; 10(1): 31–54.
3. Franklin MJ and Ohman DE, "Identification of Algf in the alginate biosynthetic gene-cluster of *Pseudomonas aeruginosa* which is required for alginate acetylation," *J Bacteriol*, 1993; 175(16): 5057–5065.
4. Khademhosseini A, Vacanti JP, and Langer R, "Progress in tissue engineering," *Scientific American*, 2009; 300(5): p. 64-+.
5. Maneval JE, et al, "Magnetic resonance analysis of capillary formation reaction front dynamics in alginate gels," in press, *Magnetic Resonance in Chemistry*, 2011.

Back to page 3

CBE Poster # 575

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

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

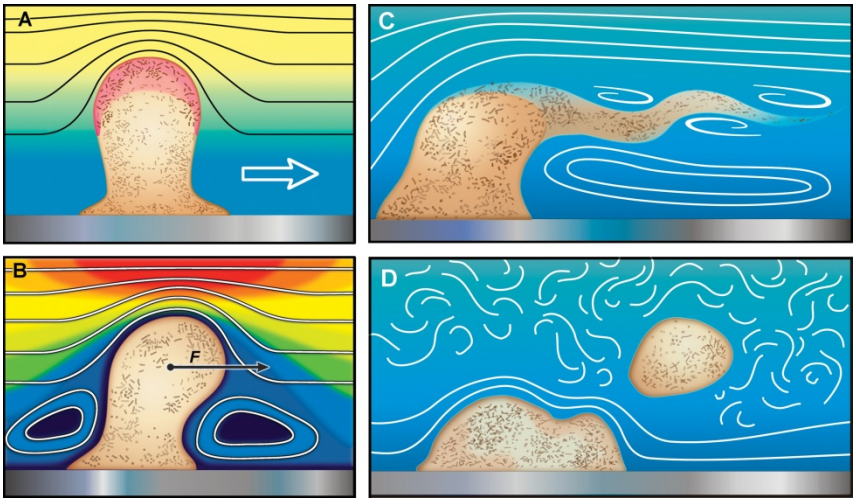


Figure 1. Effects of fluid flow on microbial biofilm. The four panels illustrate different phenomena, and correspond to increasing fluid velocity from A to D. The direction of fluid flow (block arrow) is from left to right. Solid lines are pathlines, the trajectory of an individual fluid particle. (A)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

CBE Poster #576

Date: 07/2012
Title: **Chromium responses and biofilm formation in *Desulfovibrio vulgaris* RCH-1, a sulfate-reducing bacterium isolated from 100H chromium-contaminated groundwater, are temperature-dependent**
Authors: ***Lauren Franco***[1,2], Gorby YA[3], and Fields MW[1,2]
Affiliation: [1]Department of Microbiology, and
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;
[3]Department of Geology, University of Southern California, USA
Sponsored by: US Department of Energy

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

Back to page 3

CBE Poster #577

Date: 07/2012
Title: **Microbial community dynamics in groundwater and surrogate sediments during HRC® biostimulation of Cr(VI)-reduction**
Authors: ***Kara De León***[1,2], Ramsay BD[2], Newcomer DR[3], Faybishenko B[4], Hazen TC[5,6], and Fields MW[1,2]
Affiliation: [1]Department of Microbiology, [2]Center for Biofilm Engineering, Montana State University;
[3]Pacific Northwest National Laboratory; [4]Lawrence Berkeley National Laboratory;
[5]University of Tennessee; [6]Oak Ridge National Laboratory
Sponsored by: This research was conducted by the Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) Scientific Focus Area and was funded by the U.S. Department of Energy Office of Biological and Environmental Research Division under Contract No. DE-AC02-05CH11231 to Lawrence Berkeley National Laboratory.

The Hanford 100-H site is a chromium-contaminated site that has been designated by the Department of Energy Environmental Management as a field study site for in situ chromium reduction. In August 2004, the first injection of hydrogen release compound (HRC®) resulted in an increase of microorganisms and a reduction of soluble chromium(VI) to insoluble chromium(III). Little is understood about the microbial community composition and dynamics during stimulation. The aim of this study is to compare microbial

communities of groundwater and soil samples across time and space during a second injection of HRC®. A second injection occurred November 2008 and geochemical data collected throughout the study showed an overall decrease in nitrate, sulfate, and chromium(VI). Spatial and temporal water and soil samples (n=34) were collected pre-and post-injection from four wells at the field site. Soil columns constructed from stainless steel mesh were lined with nylon mesh and filled with Hanford soils from the 100-H site. The soil columns were used to represent not only the microbes flowing through the soil via groundwater, but the microbes that require a matrix in order to grow. DNA was extracted from each of the samples and the V1V2 region of the 16S rRNA gene was sequenced via multiplex pyrosequencing. Soil sample populations differed from the corresponding groundwater (even at the phyla level) and were more diverse (p=0.001). While many of the populations were observed in both groundwater and surrogate sediments, the respective matrices appeared to enrich for particular OTUs. Of 667 total genera, 141 and 69 were unique to groundwater and soil, respectively. Genera observed only in the sediment included Marinomonas while genera observed only in the groundwater included Desulfonauticus, Desulfomicrobium, and Syntrophobacter. Pseudomonas, Acidovorax, Clostridium, and Herbaspirillum were dominant regardless of sample type. Results do not indicate a large shift in dominant organisms in soil from pre- to post- injection, and this may be due to the organisms remaining dominant from the first stimulation. Correlation analyses of genera were done for each sample type using SparCC. Metal-reducing organisms such as Geobacter, Desulfovibrio, and Geothrix were correlated in soil while possible fermenting bacteria such as Clostridium, Pelotomaculum, and Pelosinus were correlated in groundwater. For each well, HRC® injection resulted in increased diversity, but the greatest changes during stimulation occurred in the populations of mid-dominance either between wells or across time. These organisms could be important to consider as possible indicator species in future work that includes targeted isolations to better understand the mechanisms of microbial interactions.

Back to page 3

CBE Poster #578

Date: 01/2012

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

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

Affiliation: [1]Department of Chemical and Biological Engineering,
[2]Department of Earth Sciences,
[3]Department of Microbiology,
[4]Department of Land Resources and Environmental Sciences,
[5]Department of Chemistry and Biochemistry,
[6]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

Sponsored by: National Science Foundation, IGERT

Microbial community interactions represent a research area of growing interest to many scientific and engineering disciplines, including ecologists, geobiologists and bioprocess engineers. Community-level behavior is a complex result of both system members and their interactions, which can complicate the development of testable hypotheses for *ex vivo* microbial systems even when individual microbial community components are well understood. In the absence of suitable natural analogs, computer (in silico) models of interactions between distinct microbial metabolic groups can assist the characterization of complex microbial ecosystem behavior. Due to increases in sequencing speed and decreases in sequencing costs, relevant genomic data for many microbial systems are readily available. This genomic information can be translated into collections of biochemical reactions that capture the organism's metabolic potential, permitting a "systems based" analysis of relationships between environmental parameters, genome/metagenome content and community functioning. By assessing metabolite flux between individual community members and their effects on community-level behavior, in silico methods can be used to generate hypotheses related to environmental conditions and community structures that achieve useful outcomes. These outcomes include understanding and optimizing both natural and industrial processes of pressing societal importance, such as increased biofuel production or more efficient waste remediation. This presentation discusses in silico techniques used to build models representing six fully sequenced

anaerobic organisms, and their combined community potential for predicting syntrophic methane and hydrogen production.

CBE Poster #579

Date: 07/2012
Title: **Fungal bioconversion of cellulose to hydrocarbons**
Authors: Peyton BM[1,2], Carlson R[1,2], Strobel G[3], Mitchell Smooke M[4], Strobel S[5], ***Natasha Mallette***[1,2], Hunt K[1,2], Pankratz E[1,2], and Tosatto L[4]
Affiliation: [1]Center for Biofilm Engineering, [2]Chemical & Biological Engineering, [3]Plant Sciences, Montana State University, Bozeman, MT, USA;
[4]Mechanical Engineering & Materials Science, [5]Molecular Biophysics & Biochemistry, Yale University, New Haven, CT, USA
Sponsored by: Sponsored by NSF EFRI (Emerging Frontiers in Research and Innovation)—0937613
NSF Chemical, Bioengineering, Environmental, and Transport Systems (CBET)—0802666

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

Back to page 3

CBE Poster #580

Date: 07/2012
Title: **Characterization of new siderophores produced by a Soda Lake isolate**
Authors: ***Luis O. Serrano Figueroa****, M. S.*[1, 2, 4], Shwartz B[1], Richards AM[2 & 3]
Affiliation: [1]Molecular Biosciences Program,
[2]Center for Biofilm Engineering,
[3]Department of Chemical and Biological Engineering,
[4]Department of Microbiology, Montana State University, Bozeman, MT, USA
Sponsored by: National Science Foundation

Soap Lake, located in Washington State, was the subject of an NSF-funded Microbial Observatory and is a naturally occurring saline and alkaline lake. Several organisms inhabiting this lake have been identified as producers of siderophores that are unique in structure. An isolate most closely related to *Halomonas variablis* was found to produce a unique suite of amphiphilic siderophores. Bacterial isolates, enriched from Soap Lake sediment and water samples, were screened for siderophore production. Siderophore production was confirmed through the chrome azurol S agar plate method. Bacterial isolate SL01 was found to produce relatively high concentrations of siderophores in liquid medium. The Csaky and Arnow assays classified the siderophores as possessing hydroxamate moieties and lacking catecholate moieties, respectively. Siderophores from SL01 were separated from the culture supernatant using solid phase extraction and purified by HPLC. Siderophore structure was determined using LC/MS/MS. Partial sequences, approximately 900 base pairs, of the 16s rDNA genes of this isolate was compared to those in the NCBI database using the BLAST search to determine its closest phylogenetic neighbors. A distinct, new family of amphiphilic siderophores was produced by isolate SL01, a microbe that was found to be most closely related to *Halomonas variablis*. The siderophores comprising this suite ranged in size from 1050 to

1100 amu and consist of a conserved peptidic head group, which coordinates iron, coupled to fatty acid moieties. These siderophores resemble the amphiphilic aquachelin siderophores produced by *Halomonas aquamarina* strain DS40M3, a marine bacterium as well as siderophores from another Soap Lake isolate that was found to produce amphiphilic siderophores. Bacteria thriving under saline and alkaline conditions are capable of producing unique siderophores resembling those produced by microbes inhabiting marine environments.

CBE Poster #581

Date: 04/2012
Title: **Optimization and kinetics of ureolysis by *Sporosarcina pasteurii***
Authors: ***Dayla Morris***, Gerlach R, Lauchnor E, Phillips A
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: Research sponsored by the MSU Undergraduate Scholars Program and the US Department of Energy

Urea hydrolysis is one bacterially mediated reaction used in microbially initiated calcium precipitation. The hydrolysis of urea by the bacterium *Sporoscarcina pasteurii* was investigated using different media sources. Optimization experiments were designed to determine whether low-cost sources of nutrients could be used in growth media in place of expensive lab-grade nutrient sources. Kinetic analysis was performed to determine rates, and fit these rates to a first order kinetic model. The first order model was then used to predict how these bacteria would grow using different nutrient sources and to determine optimized media for growth, ureolysis, and calcium precipitation. The optimized media were extensively studied using batch experiments to prove their effectiveness at promoting growth of the bacteria (*S. pasteurii*), urea hydrolysis and calcium deposition. The definitive goal of these studies was to help make ureolytic bacteria a cost effective option for biomineralization projects in the field. The first order results showed that cheaper commercial grade urea in the form of fertilizer can be used in place of lab-grade urea, as well as calcium chloride ice melt in place of lab-grade calcium chloride. It also showed that the amount of ammonium chloride initially supplied in the media may be reduced without inhibiting growth or ureolyis. All of these results contribute to achieving a lower cost method for microbially induced calcium precipitation.

Back to page 4

CBE Poster #582

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

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

CBE Poster #583

Date: 06/2012
Title: **Expression of the molecular chaperone, ibpA in *Pseudomonas aeruginosa* biofilms**
Authors: Compton KD, Williamson KS, ***Michael Franklin***
Affiliation: Department of Microbiology and Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: This work was supported by Public Health Service grant AI-094268 from the National Institute of Allergy and Infectious Diseases.

Bacteria growing in biofilms are heterogeneous in gene expression and cell physiology. Heterogeneity influences infectious disease processes, since certain biofilm subpopulation have increased tolerance to antimicrobial agents. In previous studies, we characterized gene expression heterogeneity of *P. aeruginosa* biofilms by microarray analysis of subpopulations, isolated using laser capture microdissection (LCM). We found that thick *P. aeruginosa* biofilms contain at least two distinct subpopulations of cells—an actively growing cell fraction and a population of slowly metabolizing and possibly dormant cells. While the dormant cells had below detectable mRNA levels of most genes, they contained high mRNA abundances of the molecular chaperone, IbpA. In *E. coli*, IbpA is part of the heat shock response, delivering misfolded proteins to refolding or degradation complexes. To characterize *ibpA* expression in *P. aeruginosa* biofilms, we mapped the transcriptional start site using 5'RACE, and show that it is eight base pairs downstream of a putative RpoH promoter sequence. We constructed single-copy YFP fusions to the *ibpA* promoter and translational fusions to the IbpA protein. Similar to observations of *E. coli* planktonic cells by Lindner et al. (PNAS 105:3076-81), we observed that in *P. aeruginosa* biofilms only a fraction of the cells contain the IbpA-YFP fusion protein, and that IbpA localizes to one cell pole. The 5'UTR of the *ibpA* mRNA is predicted to form two hairpin loops. To determine the effect of the 5' UTR hairpin loops on *ibpA* mRNA abundances in cells at the bottom of the biofilm, we constructed single copy deletions of one or both hairpins. LCM and qRT-PCR show that for cells at the bottom of the biofilms, deletion of the second hairpin has no effect on *ibpA* mRNA abundance. However, when both hairpins are deleted, *ibpA* mRNA is reduced in cells at the bottom of the biofilms. The results indicate that *ibpA* mRNA is abundant in the dormant biofilm subpopulation, and that polar IbpA is produced in a subset of these cells. The abundance of the *ibpA* mRNA may be due to increased half-life of the transcript, due to RNA folding and stability.

Back to page 4

CBE Poster #584

Date: 06/2012
Title: **Characterization of physiological heterogeneity in *Pseudomonas aeruginosa* biofilms**
Authors: ***Kerry Williamson***[1], Richards L[1], Perez-Osorio AC[2], McInnerney K[1], Pitts B[1], Stewart PS[1], and Franklin MJ[1]
Affiliation: [1]Department of Microbiology and Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;
[2]Washington State Dept. of Health, Shoreline, WA
Sponsored by: This work was supported by Public Health Service grant AI-094268 from the National Institute of Allergy and Infectious Diseases.

Bacteria growing in biofilms are physiologically heterogeneous, due in part to their adaptation to local microenvironments. Chemical diffusion and bacterial metabolism result in varying levels of oxygen, nutrients, waste products, and signaling compounds throughout the biofilms. In this study, the physiology of two distinct *Pseudomonas aeruginosa* biofilm subpopulations was characterized by using a combination of differential GFP-labeling, antibiotic susceptibility assays, and transcriptomics. Selective GFP-labeling and cell sorting (FACS) demonstrated that cells near the air interface of the biofilms were actively dividing, while those near the substratum were in inactive, yet viable. The cells at the substratum were not

susceptible to killing by the antibiotics tobramycin and ciprofloxacin. To explore cellular adaptation within biofilms, we used laser capture microdissection (LCM) and microarrays to obtain complete transcriptomic profiles of cells from the upper and lower strata of the biofilms. Surprisingly, cells near the top of the biofilms had high mRNA levels for genes regulated by the hypoxia induced regulator Anr. These cells also exhibited increased expression of stress response genes regulated by RpoH and RpoS. In contrast, cells in the lower portion of the biofilms had very little overall mRNA expression, including little expression of Anr-regulated genes, indicating low metabolic activity and perhaps prolonged exposure to anoxic conditions. The most abundant mRNA transcripts isolated from cells near the substratum include those for the protein IbpA, a chaperone of misfolded proteins, and for the ribosome hibernation factors Rmf and PA4463. The products of these genes may play a role in maintaining viability of the dormant cells at the bottom of the biofilms. To test this, we cultured *P. aeruginosa* Δ*rmf* and ΔPA4463 mutant strains in biofilms and analyzed cellular uptake of the membrane integrity stain propidium iodide (PI). The Δ*rmf* mutant showed greater PI uptake at the bottom of the biofilms than did the wild-type strain. The results demonstrate that in thick *P. aeruginosa* biofilms, cells are physiologically distinct spatially, with the actively dividing cells under hypoxia-stressed condition, and cells deep in the biofilm in a viable but antibiotic tolerant slow-growth state.

CBE Poster #585

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

Microbially Induced Corrosion (MIC) is a major concern for industrial ferrous metal pipelines and can result in pipeline failure. Sulfate Reducing Bacteria (SRB) have been implicated in contributing to MIC due to their production of corrosive H_2S gas. *Desulfovibrio alaskansis* G20 (G20) is a SRB isolated from a producing oil well in Ventura, California. This study evaluates whether G20 pure culture can form a biofilm on steel substrate, and attempts to characterize the G20 biofilm with the Biological Accumulation Model (BAM).

Back to page 4

CBE Poster #586

Date: 07/2012
Title: **In vitro comparison of biofilm formation in blood control (BC) and non BC-PIV catheters**
Authors: ***Elinor deLancey Pulcini***, Ryder M*, Parker A, Fisher S, Bickle L, James G
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
* Ryder Science
Sponsored by: Smith Medical, Inc.

The prevention of bloodborne pathogen exposure for healthcare workers has been an ongoing challenge. Valve technology within peripheral IV catheters was developed to reduce the use of needles and has addressed the problem. However, questions have been raised about increased infection risk with increased intraluminal surface area in the catheter for biofilm formation. A clinically simulated in vitro model was used to compare bacterial transfer and biofilm formation over a 96-hour period in a novel valved peripheral IV catheter to two non-valved catheters. There were statistically significantly fewer bacteria in flush samples from the ViaValve® Safety I.V. catheters compared to BD Insyte™ Autoguard™ BC Shielded I.V. catheters in a clinically simulated model. However, in general valved PIV catheters did not demonstrate a greater tendency for biofilm formation than non-valved catheters under the conditions of these tests.

CBE Poster #587

Date: 07/2012
Title: **Diatom biofuel viability: An investigation on SI:C:N required for optimal lipid accumulation**
Authors: ***Karen Moll***
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: U.S. Department of Energy, Office of Biomass Programs grant DE-FG36-08G018161

Background: Some algal strains naturally possess high lipid content in the form of triacylglycerol (TAG) when stressed. Optimization of lipid production for these strains is critical to improve the feasibility of algal biofuels. Previous data have shown that diatom growth and lipid accumulation are dependent on silica utilization and the addition of sodium bicarbonate significantly increases the rate and extent of lipid accumulation. This study further elucidates conditions to optimize lipid accumulation for a diatom by focusing on the relationship between silica, carbon and nitrogen and their combined potential to cause physiological stress for increased lipid production.

Methods: Silica, carbon and nitrogen concentrations were varied to determine optimal lipid production. 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 lipid content, but gave decreased diatom specific growth rate above 2mM silica. 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.

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 inorganic carbon 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.

Back to page 4

CBE Poster #588

Date: 07/2012
Title: **Lipid-derived biofuels metabolic analysis of lipid accumulation in a microalga**
Authors: ***Jean-Paul Toussaint,*** Ramos L, Mus F, Carlson R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

As concern grows about the supply of fossil fuels, new alternative energy sources are being investigated including renewable biofuels. Microalgae represent a competitive biofuel strategy when compare with "traditional" agricultural crops. Green algae and diatoms are of considerable interest as a biodiesel source because they accumulate significant amounts of energy-rich compounds, such as triacylglycerol (TAG) that can be used to synthesize biodiesel. The following research project investigates factors that control TAG accumulation in the marine diatom *Phaeodactylum tricornutum* using physiological and molecular approaches. The first phase of the project identified optimal growth conditions that promote TAG accumulation in *P. tricornutum*. It has been found that nitrogen limitation, pH stress and the addition of bicarbonate or acetate stimulate lipids accumulation in *P. tricornutum* cells by 5- to 10-fold as compared to controls. Fundamental physiological data including photosynthetic pigment content, protein levels and carbohydrate content have been collected and correlated to TAG synthesis. A transcriptomic analysis is currently in progress to identify and characterize essential genes involved in TAG accumulation.

Information on the abundance of specific transcripts under lipids accumulation conditions will permit description of bioenergetic and metabolic processes involved in TAG accumulation and to identify associated regulatory factors. This project advances algal biofuels research by elucidating both the physiological and transcriptomic basis of TAG accumulation in the marine diatom *Phaeodactylum tricornutum* providing a rational basis for TAG synthesis control.

Back to page 4

Industry & Agency Posters

Date: 07 / 2012
Title: **Metagenomic and metaproteomic analyses of marine biofilms**
Authors: Dagmar Hajkova Leary†, W. Judson Hervey, IV†, Robert W. Li‡, Nikolai Lebedev§, Jeffrey R. Deschamps§, Anne W. Kusterbeck§, and Gary J. Vora*§
Affiliation: † National Academy of Sciences, National Research Council, Postdoctoral Research Associate, US Naval Research Laboratory, 4555 Overlook Ave.-SW, Washington, D.C. 20375, ‡ Bovine Functional Genomics Laboratory, Animal and Natural Resources Institute, United States Department of Agriculture, Beltsville, Maryland, § Center for Bio/Molecular Science and Engineering, US Naval Research Laboratory, 4555 Overlook Ave.-SW, Washington, D.C. 20375

Marine biofilms are complex mixtures of eukaryotic and prokaryotic microorganisms which adhere to any surface submerged in seawater. Their formation is a problem for all industries operating in a marine environment. Despite decades of research that has focused on understanding the formation and prevention of biofilms, relatively little is known about the microbial consortia and activity that are responsible for biofilm formation in different marine environments. This gap in our understanding is due in large part to the current inability to cultivate the vast majority of marine microbes in the laboratory and overall lack of genomic information. New techniques, *i.e.*, metagenomics and metaproteomics, can overcome this problem. Metagenomics studies genetic material recovered directly from environmental samples. It can provide a snapshot of all microorganisms present in the samples; however it is not capable of determining which organisms are biologically active. In order to do that, metaproteomics—the study of expressed proteins in an environmental sample—can be employed. Thus when metagenomics and metaproteomics are used on the same sample, they enable us to gain unprecedented insight into the microbial composition as well as the biomolecular activity of true environmental samples. In this study, PhyloChip microbial profiling, fluorescence spectroscopy, elemental analyses, metagenomic sequencing, and qualitative/quantitative metaproteomic analyses were used to begin to investigate the role differing environments play on the composition and function of complex biofilms sampled at the air-water interface from hulls of two U.S. Navy vessels that were deployed in different marine environments. Prokaryotic community analyses of both biofilms using PhyloChip-based 16S rDNA metagenomic profiling revealed two markedly different and taxonomically diverse communities with the majority of OTUs assigned to members of the Gammaproteobacteria, Alphaproteobacteria and Bacteroidetes, 4,101 taxa with significant inter-ship abundance differences and ship-1 demonstrating greater archaeal and bacterial subfamily richness than ship-2. Subsequent metagenomic sequencing resulted in the identification of 243,146 (ship-1) and 183,173 (ship-2) ORFs of which 89,504 and 76,123 could be annotated by SwissProt, respectively. Analyses of the metagenomes suggested that while both communities were dominated by prokaryotic organisms (92.9% prokaryotic versus 7.1% eukaryotic sequence reads), ship-1 contained 5.3X more light-harvesting Cyanobacteria reads and was significantly enriched in Bacteroidetes sequences whereas ship-2 was enriched with Proteobacteria sequences and light-harvesting genes from photosynthetic eukaryotes. Qualitative LC-MS/MS metaproteomic analyses using the same biofilm samples identified 678 unique proteins from both biofilms (356 from ship-1, 389 from ship-2, 67 proteins in common) and revealed little overlap in species and protein composition between the two biofilms. Interestingly, the metaproteomic

results contrasted with the metagenomic results in that the majority of identified and quantified annotated proteins were of eukaryotic origin (47.7% eukaryotic versus 13.7% prokaryotic), assigned to Stramenopiles (ship-1/ship-2 = 8.3%/21.4%), Amoebozoa (0.6%/5.8%), Fungi (3.9%/11.0%), Cnidaria (5.5%/0.6%), Opisthokonta (73.5%/30.1%) and Viridiplantae (6.1%/19.7%). Furthermore, ship-1 contained an abundance of cytoskeleton and cell adhesion proteins whereas ship-2 appeared to be enriched for eukaryotic photosynthesis proteins suggesting that while both biofilms were phototrophic, the underlying phototrophic nature of the biofilms differed and was imparted primarily by photosynthetic cyanobacteria (ship-1) or diatoms (ship-2). These findings were further supported by photosynthetic pigment analyses and the organic carbon isotope values in both biofilms that resulted from phototrophic processes. Combined, our findings begin to provide a baseline for understanding the organismal and biomolecular composition of marine biofilm communities, bring attention to caveats for the interpretation of standalone environmental 'omics' datasets, and introduce the use of ship hull biofilms as natural sampling tools for the study of marine microbial communities from different geographic environments.

Back to page 4