

Montana State University

■ Center for Biofilm Engineering

Bozeman



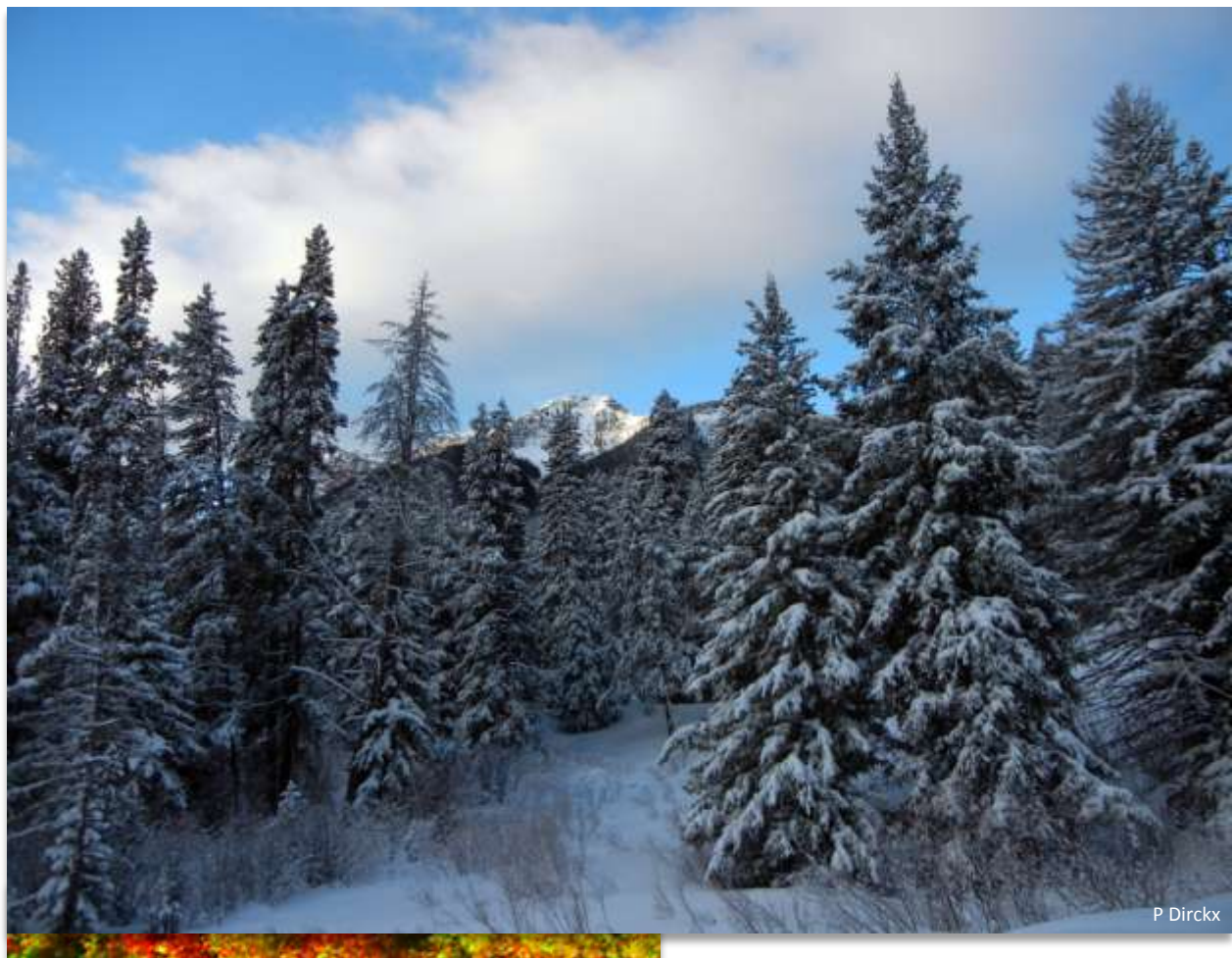
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**montana biofilm**  
SCIENCE & TECHNOLOGY **meeting**

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**February 7–8, 2012**

**proceedings**



## **abstracts**

Montana Biofilm Science & Technology Meeting: February 7–8, 2012

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### **Speaker Abstracts**

#### **SESSION 1: Oral Biofilms**

##### **Antimicrobial penetration and efficacy in an in vitro oral biofilm model**

*Presenter:* Phil Stewart, CBE Director

*Co-authors:* Corbin A, Pitts B, Parker A, Stewart PS

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

The penetration and overall efficacy of six mouthrinse actives were evaluated using an in vitro flow cell oral biofilm model. The technique involved pre-loading biofilm cells with a green fluorescent dye that leaked out as the cells were permeabilized by a treatment. The loss of green color, and of biomass, was observed by time-lapse microscopy during 60 minutes of treatment under continuous flow conditions. The six actives analyzed were: ethanol, sodium lauryl sulfate (SLS), triclosan (TRN), chlorhexidine digluconate (CHX), cetyl pyridinium chloride (CPC), and nisin. Each of these agents effected loss of green fluorescence throughout biofilm cell clusters, with faster action at the edge of a cell cluster and slower action in the cluster center. The time to reach half of the initial fluorescent intensity at the center of a cell cluster, which can be viewed as a combined penetration and biological action time, ranged from 0.6 min to 19 min for the various agents. These times are much longer than the predicted penetration time based on diffusion alone, suggesting that anti-biofilm action was controlled more by the biological action time than by the penetration time of the active. None of the agents tested caused any removal of the biofilm. The extent of fluorescence loss after 1 hour of exposure to an active ranged from 87% to 99.5% with CHX being the most effective. Extent of fluorescence loss in vitro, but not penetration and action time, correlated well with relative efficacy data from published clinical trials.

##### **Biofilms in dental unit waterlines**

*Presenter:* Alessandra Marçal Agostinho, DDS, PhD, Research Scientist

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

Bacterial contamination of dental unit water has become a growing concern for clinical dentistry, especially with the increasing number of immunocompromised patients and aging of the population. Although the problem has been recognized for many years, no definitive solution has been found.

During the first few years after the discovery of microbial contamination in dental unit waterlines, researchers assumed the systems were retracting bacteria from patients' mouths. However, many studies have since confirmed that biofilms formed by waterborne microorganisms in the dental unit tubing were the source of the contamination.

The potential adverse consequences of contaminated dental water unit lines include not only disease, but also economic risks (such as adverse publicity and litigation) to dental professionals. In 1996, the American Dental Association Council on Scientific Affairs recommended "an ambitious and aggressive course to encourage industry and the research community to improve the design of dental equipment so that by the year 2000, water delivered to patients during nonsurgical dental procedures consistently contains no more than 200 colony forming units per milliliter of aerobic mesophilic heterotrophic bacteria at any time in the unfiltered output of the dental unit."

The continuous use of biocidal agents, although sometimes effective, may have negative side effects, such as interference with the bonding properties of dental materials and potential health hazards to both health care providers and patients. While intermittent treatment regimens seem to be more realistic, dentists still struggle to select appropriate agents in order to achieve the water quality standard established for dental unit waterlines. Efficient, low cost solutions are needed to improve dental unit water quality.

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At the Center for Biofilm Engineering we have developed a laboratory model dental unit waterline system for the growth of biofilms; in combination with our state-of-the-art imaging capabilities, it allows the testing of new technologies and promising biocides.

### **The drip-flow reactor as a test system for oral care products**

*Presenter:* Garth James, CBE Medical Projects Manager; Associate Research Professor, Chemical & Biological Engineering, MSU

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

In vitro models are useful for pre-clinical testing of novel ingredients and formulations of oral care products such as mouthrinses and toothpastes. While it is impossible to accurately model the complexities of the oral environment with an in-vitro system, it is important to mimic this environment as closely as possible when testing oral care products. We have used the drip-flow biofilm reactor (DFR) as a model system for growing both supragingival and subgingival plaque-like biofilms. In the DFR, biofilms grow at a gas-liquid-solid interface, which is similar to the oral cavity. The use of hydroxyapatite-coated glass slides as the substratum for biofilm growth mimics the tooth surface. These slides can also be treated with filter-sterilized saliva to mimic the acquired pellicle. Although the model can be inoculated with a single species of oral bacteria, inoculation with whole saliva creates a more robust polymicrobial biofilm that more closely represents complex oral flora. The community structure of the biofilms can be manipulated through the composition of the growth medium. Media with low buffering capacities select for acid producing streptococci typical of supragingival plaque and caries formation. In contrast, media with high buffering capacities favor the growth of gram-negative bacteria associated with subgingival plaque, gingivitis, and oral malodor. The overall strength of the medium also has a large impact on biofilm thickness and tolerance to treatment. Biofilms grown using high-strength medium for relatively long growth times often do not respond to common agents such as chlorhexidine that have been shown clinically to be effective anti-plaque agents. Thus, testing experiments using the DFR can be tailored to most accurately mimic the oral environment.

### **Investigations into mobile genetic elements and horizontal gene transfer within oral biofilms**

*Presenter:* Adam P. Roberts, Lecturer, Department of Microbial Diseases

*Affiliation:* UCL Eastman Dental Institute, 256 Gray's Inn Road, London, WC1X8LD, UK

**Introduction:** Oral biofilms are among the most heterogeneous bacterial communities found in multicellular animals. This heterogeneity is due in part to the many different habitats and nutrients available in this environment. Many oral bacteria have evolved to be able to exchange genetic information, allowing them to respond rapidly to the many different environmental stresses encountered in the oral cavity. Biofilms are a good environment for the transfer of DNA between different bacteria due to the stable architecture and close proximity of the resident cells. In addition, the role of extracellular DNA (eDNA) in biofilm growth is beginning to be appreciated. I have been investigating the transferability of broad host range, mobile genetic elements (MGEs), called conjugative transposons, within oral biofilms. Conjugative transposons are capable of inter-generic transfer and usually encode antibiotic resistance genes. I have also been investigating the potential of eDNA to transform resident bacteria within oral biofilms. The ability of DNA to transfer in the oral biofilm environment therefore has clinical implications for humans due to antibiotic resistance transfer but, paradoxically, may also be the Achilles' heel of resident bacteria that we wish to control.

**Methods:** Constant depth film fermenters were used for transfer studies. A known sensitive consortia inoculum (up to 20 species) and a single donor strain containing a characterized conjugative transposon (Tn916) were included. Tn916 confers tetracycline resistance; therefore successful transfer can be monitored by selection of resistance. To study the transfer potential of eDNA, purified genomic DNA of donor strains was used to seed the growing tetracycline-sensitive biofilm consortia. Furthermore, the ability of various oral, biofilm-growing bacteria to release eDNA is also being examined.

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**Results:** I have shown that DNA can be transferred from transient bacteria (*Bacillus subtilis*) to members of a model oral biofilm community, demonstrating that it is likely that oral bacteria can acquire genetic information from environmental and/or transient bacteria. Additionally, members of the most common oral genera (*Streptococcus* spp. and *Veillonella* sp.) have been shown to act as donors of conjugative transposons to other members of the oral community growing as a multispecies biofilm. I also have data demonstrating that the addition of purified DNA from a tetracycline resistant *Veillonella* sp. donor is sufficient to transform oral streptococci to antibiotic resistance. Finally, recent work has examined the quantity and source of eDNA produced by a range of oral bacteria: *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus sanguis*, *Enterococcus faecalis* and the gram-negative *Porphyromonas gingivalis*; eDNA is detectable in varying amounts and I have shown that the eDNA released by oral streptococci is of random chromosomal origin.

**Discussion:** These data provide more evidence that gene transfer is likely to be occurring among the oral microbial community, at least by transformation and conjugation. Furthermore, the multiple roles of eDNA are only just beginning to be understood. Extracellular DNA has been shown to be essential in biofilm formation (structural), it can be used as a food source (nutritional) and I have demonstrated that it can be inherited and expressed (informational). This opens up the possibility of molecular control strategies. By seeding mature, problematic biofilms with DNA containing MGEs, it is likely that this DNA will be taken up by resident, competent bacteria and subsequently transferred within the biofilm to other suitable recipients. If this MGE contains a gene encoding, for example, an antimicrobial or a toxin that is under the control of an inducible promoter, then genetic control of bacteria could conceivably be achieved with very little invasive activity. This methodology would also be applicable for bioremediation strategies where metabolic capabilities rather than antimicrobial properties are encoded on MGEs.

## **SESSION 2: Thermal Biofilms**

### **Transport and microbial consumption of oxygen in acidic geothermal iron-oxide mats**

*Presenter:* Hans C. Bernstein, PhD student in Chemical Engineering

*Co-Authors:* Beam JP, Inskeep WP, and Carlson RP

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

The role of dissolved oxygen as the principal electron acceptor for microbially mediated iron oxidation was investigated within the primary flow path of acidic geothermal springs in Norris Geyser Basin, Yellowstone National Park. Previous data has suggested that Fe(II)-oxidizing microbial populations (e.g., *Metallosphaera yellowstonensis* and potentially other novel members of the domain *Archaea*) represent the primary-producers within these biofilm communities. Consequently, the availability of oxygen is hypothesized to limit microbial Fe(II)-oxidation and primary-productivity in this system. In situ measurements of oxygen profiles were obtained perpendicular to the direction of convective flow across the aqueous phase-Fe(III)-oxide mat interface using oxygen microsensors. Dissolved oxygen concentrations drop below detection near the surface of the Fe(III)-oxide mat, indicating reactive oxygen consumption and defined spatial gradients. Evaluation of the oxygen flux across the liquid/mat boundary showed that convection was negligible compared to diffusive transport in the mat. Reaction-diffusion models were evaluated assuming both zero- and first-order reaction kinetics. The in situ measurements and models suggest that the rate of oxygen consumption exceeds the rate of diffusion. Thus, microbially mediated Fe(II)-oxidation in this system is likely restricted by mass transfer limitations, resulting in an active surface layer of Fe(III)-oxide biomineralization. Microbial Fe(II)-oxidation activity requires dissolved O<sub>2</sub>; however the consequential biomineralization resulting in the extracellular mat formation restricts the availability of molecular O<sub>2</sub>. The qualitative observation of this phenomenon aids the understanding that microbial activity and propagation rates in the biofilms must exist in a balance with the biomineralization rates, which adversely affect the availability of nutrients. The goals of the study were to: (i) directly measure diffusive transport and consumption of dissolved O<sub>2</sub> within geothermal acidic hydrous ferric oxide microbial mats in situ; (ii) to quantitatively compare mass transfer and metabolic rates associated with biomineralization of Fe(III)-oxide and microbial mat formation; and (iii) use a multi-scale in situ measurement approach to



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interconnect mRNA expression, metagenomics, and physical transport phenomena to establish the significance of dissolved O<sub>2</sub> mass transfer limitations on the overall nutrient requirement and ecology of the geothermal microbial mat system.

### **Extremophilic fungi for sustainable energy**

*Presenter:* Rich Macur, Assistant Professor, Chemical & Biological Engineering

*Co-presenter:* Mark Kozubal, Postdoctoral Researcher, Land Resources & Environmental Sciences

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

In the production of cellulosic ethanol, lignocellulosic feedstocks such as wheat straw, corn stover, and bagasse are pretreated at high temperature and low pH to decrystallize cellulose and release it from the protective cover of lignin and hemicellulose. These pretreatment conditions are analogous to acidic geothermal environments in Yellowstone National Park, which harbor a broad diversity of both prokaryotic and eukaryotic life. It is well known that fungi produce a myriad of lignocellulose degrading enzymes (e.g., cellulases, xylanases, laccases, peroxidases) and these enzymes are commonly used in the biofuel industry. However, the use of acid-thermo-tolerant enzymes from extremophilic fungi is relatively unexplored. Use of these enzymes for the production of biofuels has the potential to increase the efficiency and rates of conversion, and thereby decrease costs. We will present information on one extremophilic fungi that was isolated from Yellowstone and provide preliminary information on its capabilities for use in the biofuels industry. The novel strain, referred to as Strain MK7, is capable of directly converting (consolidated bioprocessing) a variety of lignocellulosic substrates under acidic culture conditions to lipids, hydrogen, and ethanol.

## **SESSION 3: Industrial Biofilms**

### **Innovative use of biofilms in water and wastewater treatment**

*Presenter:* Zbigniew Lewandowski, Professor, Civil Engineering

*Co-author:* Joshua P. Boltz, CH2M Hill

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

Biofilms were used for the treatment of municipal water and wastewater before the invention of the activated sludge process, and long before they were called biofilms; e.g., in slow sand filters and trickling filters. The application of fundamental principles of microbial process engineering to design biofilm reactors represents a paradigm shift from the historical approach, which was based on empirical criteria and design formulations.

Process designers have used the newfound understanding of biofilm processes to improve biofilm reactors and supporting control system designs; fundamental principles describing biofilms exist as a result of focused research, and biofilm technologies in water and new technologies have been designed based on the understanding of the biological processes, structure, and function of various components of biofilms. As a result, several new biofilm-based technologies have been designed and implemented in wastewater treatment. Some old technologies have been hybridized with the biofilm-based technologies, which addressed many problems troubling the traditional technologies relying on the use of suspended biomass only, and also provided as with an array of new names and acronyms, such as the Integrated Fixed Film Activated Sludge (IFAS), or a similar technology, HYBAS—similar to the IFAS hybrid process that combines the AnoxKaldnes biofilm technology with activated sludge, the Submerged Fixed Film Technology (ACCU FAS), the Moving Bed Biofilm Reactor (MBBR) and the Fluidized Bed Biofilm Reactor (FBBR).

Despite the successful implementation of biofilm-based technologies in the water and wastewater treatment industries, many aspects of biofilm processes remain poorly understood, namely the fate or particulate organic matter, dynamics and rate of biofilm detachment, and factors influencing concentration gradients external to the biofilm surface, to name a few. While it is expected that the results of the currently

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conducted large scale studies on the existing biofilm-based treatment plants will be used to improve the design and operation of biofilm reactors, there is a need to guide the future microscale biofilm research to the same end. Unfortunately, little information exists to bridge the gap between our current understanding of biofilm fundamentals and reactor-scale empirical information. Therefore, there is a clear dichotomy in literature and practice: micro- (biofilm) and macro- (reactor) scales. This presentation shows examples of the new technologies and reveals the directions of future research that are needed to improve the level of understanding of biofilm processes with the overall goal of reaching the comprehensive level of designing biofilm technologies analogous to the design of the traditional activated sludge treatment plants. Limited time will allow for a rather sketchy exposition of the accomplishments and the remaining problems. More specific information on that topic can be found in our recent works, such as: Lewandowski Z and Boltz JP, (2011) "Biofilms in Water and Wastewater Treatment." In: Peter Wilderer (ed.) *Treatise on Water Science*, Vol. 4, pp. 529–570 Oxford: Academic Press; this presentation summarizes the main points of these discussions.

**Metabolic cooperation in methanogenic biofilms: Cellular biomass or cellular energy**

*Presenter:* Kristen Brileya, PhD student, Microbiology

*Co-authors:* Matthew Fields, Associate Professor, Microbiology, MSU

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

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 7 days, based upon protein levels and methane mass flux. Biofilm establishment was dependent upon initial colonization by *D. vulgaris* that was followed by recruitment of *M. maripaludis* into the biofilm matrix. The initial *Desulfovibrio:Methanococcus* biofilm ratio was approximately 375:1 but steady-state biofilm reached a ratio of 4:1. 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. Key structural signatures were observed that confirmed the cooperative nature of the community, using a newly developed model. Colorimetric assays indicated cell-associated carbohydrate was composed of .035  $\mu\text{g}$  hexose/ $\mu\text{g}$  protein, .017  $\mu\text{g}$  pentose/ $\mu\text{g}$  protein and .011  $\mu\text{g}$  uronic acid/ $\mu\text{g}$  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. Syntrophic biofilm 3-D structure appears to be initialized by *D. vulgaris* and provides an advantageous environment for *M. maripaludis* to establish micro-colonies throughout the *D. vulgaris* scaffold. The coculture biofilm growth mode resulted in a 10-fold higher methane production per *M. maripaludis* biomass than the planktonic-only growth mode, indicating that the structure of cooperative interactions between a bacterium and archaeon positively impacts function.

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### **Special Presentations**

#### **Highlights from recent European biofilm meetings**

*Presenter:* Phil Stewart, CBE Director

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

Highlights from two European biofilm meetings of 2011 will be presented. The first meeting was a European Cooperation in Science and Technology workshop entitled *Biofilm: Friend or Foe?* that took place in Berlin in June. It was attended by 45 participants from 18 countries. The second was the EuroBiofilms 2011 meeting held in Copenhagen in July with over 300 attendees from 31 countries. One recurrent theme was interactions of microbial biofilms with all types of other organisms. Karen Tait explained how barnacle zoospores home in on acyl homoserine lactone signals released by marine biofilms. Ivan Kennedy spoke about extensive field work showing that beneficial bacteria seeded into the rhizosphere can increase the health and productivity of crops. Joanna Azeredo described the use of bacteriophage to control biofilms. A number of presenters also sought botanical inspiration to control unwanted biofilms with plant-derived chemistries such as essential oils or quorum sensing inhibitors. For example, Michael Givskov discussed the quorum sensing inhibitory activity of a chemical found in garlic. Jonathan Leder described antagonistic interactions between different genera of bacteria. Another strong theme dealt with the use of antibiotics against biofilms. Simon Lynch outlined a strategy for discovering and optimizing antibiotics specifically for their efficacy against biofilms and provided examples of such drugs. Werner Zimmerli recapped years of animal model investigation into antibiotic therapies to cure biofilm infections. Tom Coenye presented molecular genetic data on how biofilms tolerate antibiotics. Iona Ciofu described the application of pharmacokinetic and pharmacodynamic models and analyses to biofilm treatment. Other interesting topics or themes included dispersal of biofilms by carbon starvation (Tim Tolker-Nielsen), extracellular electron transfer (Lars Peter Nielsen), and mutation and evolution in biofilms (multiple presentations).

#### **Analytical challenges of microbial biofilms on medical devices**

*Presenter:* K. Scott Phillips, Regulatory Research Scientist

*Affiliation:* Division of Chemistry and Materials Science, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, United States Food and Drug Administration, Silver Spring, MD

There are many technologies, in both research and production stages, that are designed to prevent or reduce biofilm formation on medical devices. While these technologies promise to improve safety for the consumer, the claims being made are challenging from the perspective of regulatory science. Not only are there many obstacles to the measurement of biofilms in a quantitative and reliable manner, but it is also difficult to relate in vitro biofilm assays to in vivo and clinical outcomes.

The goal of our regulatory research is to identify biofilm analysis strategies with high accuracy and reliability, improved correlation with in vivo results and clinical outcomes, and cost effectiveness. Because each type of medical device is affected uniquely by biofilms, there may not be a "one-size-fits-all" solution to biofilm testing. This talk will briefly touch on some of the basic aspects of biofilms that are important from a regulatory science perspective.

**abstracts****SESSION 4: Green Biofilm Control Strategies****Biofilm shielding: The role of quorum sensing, rhamnolipids, and eDNA***Presenter:* Michael Givskov*Affiliations:* Professor, International Health, Immunology, & Microbiology, University of Copenhagen; Professor, Singapore Centre on Environmental Life Sciences Engineering

The biofilm lifestyle plays a key role in many chronic bacterial infections. *Pseudomonas aeruginosa* is known as a notorious biofilm former. During establishment, bacteria emerge in a “harmless” state (no expression of virulence factors) as they increase in numbers and build biofilms. In the biofilm mode, *P. aeruginosa* uses “quorum sensing (or QS)” communication to inform its fellow bacteria about progress in the infectious process. When the QS system signals, the biofilm launches a cocktail of destructive virulence factors which in turn attract phagocytic white blood cells (PMNs), a major cellular component of the innate immune system. To offer protection, a rhamnolipid based “shield” is also launched, which on contact kills attaching PMNs. The magnitude of this “shield” is carefully adjusted in response to as yet unknown signals originating from the incoming PMNs. The rhamnolipids cause PMN lysis, which subsequently liberates large amounts of DNA that is capable of directly interacting with antibiotics and consequently mitigates their antimicrobial action. Both protective mechanisms are controlled by QS. Lysis of the PMNs also releases oxygen radicals, metalloproteases, and other degrading enzymes. In the host organism this creates a vicious cycle, the result of which is that neither immune system nor administration of antibiotics can mediate biofilm eradication, a situation that goes hand in hand with development of inflammation and collateral host tissue damage. Cross communication between bacteria and the immune system seems to play an important role in this complex scenario. Small molecule signal blockers (QS inhibitors) can be introduced, which do not kill bacteria but block QS and bacteria-immune system cross communication. The shield is not formed and the PMNs can actively break down the bacterial biofilm. QS inhibition chemistry is available in a variety of ecological niches, and I will present some new data on inhibitors from herbs.

**Bioinspired topographies for bacterial inhibition strategy***Presenter:* Rhea May, PhD*Co-authors:* Rhea M. May, PhD<sup>1</sup>; Matt G. Hoffman, BS<sup>1</sup>; Shrvanthi T. Reddy, PhD<sup>1</sup>; Kenneth K. Chung, MSE<sup>1</sup>; and Anthony B. Brennan, PhD<sup>2</sup>*Affiliations:* <sup>1</sup>Sharklet Technologies, Inc. Aurora, Colorado, USA<sup>2</sup>Department of Materials Science and Engineering and J. Crayton Pruitt Family, Department of Biomedical Engineering, University of Florida, Gainesville, Florida, USA

It has long been known that surface roughness affects wettability, which has motivated the study of topography to control bioadhesion for randomly roughened surfaces and some ordered patterns. Dr. Anthony Brennan at the University of Florida observed that the surface roughness associated with the unique structure of shark skin denticles matched his roughness estimates for an effective anti-fouling surface using the engineered roughness index (ERI) model he developed. The ERI is a function of three variables: Wenzel roughness factor ( $r$ ), number of unique features ( $n$ ), and area fraction of feature tops ( $\Phi_s$ ). This model has been used to design surface topographies that are unfavorable for microorganism adhesion, and thus the Sharklet micro-pattern was created as a non-toxic, non-biocidal surface designed to inhibit biofilm formation of undesirable microorganisms. The initial goal of this biomimetic strategy was to reduce the occurrence of algae and barnacle growth on underwater surfaces to potentially replace existing toxic solutions. As predicted, the Sharklet micro-pattern was demonstrated to be effective against marine algal zoospores, barnacle cyprids and marine bacteria. Since then, the Sharklet pattern has been shown to perform against numerous clinically relevant pathogens on several materials and in a range of conditions. The pattern has been shown to inhibit attachment of the eight organisms that it has been tested against by at least 50% when compared to un-patterned controls. It reduces the migration of *Escherichia coli* and *Serratia marcescens* by up to 92% and decreases the area coverage of *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* biofilms by up to 70% after 14 days in a static growth environment. We are

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currently developing methods to utilize confocal scanning laser microscopy to quantify the volumetric differences in biofilm formation on Sharklet micro-pattern surfaces compared to un-patterned controls. Based on previous data from scanning electron micrographs, we hypothesize that micro-colonies on the Sharklet surface are smaller, more dispersed and have less depth than biofilms formed on un-patterned control surfaces. Preliminary evidence has validated this hypothesis as four-day static *S. aureus* biofilms on an un-patterned surface appears to be several microns deep as opposed to those on the Sharklet surface, which appear to be dispersed and composed of one cell layer. The slowing of biofilm formation and disaggregation of microbes may have a major impact on medical device related infections if the Sharklet micro-pattern is manufactured onto the surfaces of these devices.

### Microbe-microbe interactions and application to MBR flux enhancement

*Presenter:* Jonathan Leder, PhD, Technical Director

*Co-authors:* Sarah C. McHatton and Seth D'Imperio

*Affiliations:* Novozymes Biologicals, Inc, Salem, Virginia, USA

Membrane bioreactor systems are increasingly used in treatment of industrial and municipal wastewater. A key factor limiting faster adoption of this technology is build-up of biofilms on membrane surfaces, which limits flux and necessitates frequent cleaning via air scouring and periodic chemical disinfection. We have explored the addition of selected bacteria to membrane systems, which decrease fouling and improve flow rates. Results of testing in laboratory model systems and pilot scale membrane bioreactors are described, along with studies to explore the distribution of the bioaugmentation strains within the bioreactors.

## SESSION 5: Medical Biofilms

### Considering the host immune response for vaccine development against *Staphylococcus aureus* biofilms

*Presenter:* Mark E. Shirtliff, Associate Professor, Dept. of Microbial Pathogenesis, Dental School

*Affiliation:* University of Maryland, Baltimore, Maryland, USA

*Staphylococcus aureus* is a major cause of infection occurring after orthopedic surgical interventions. One of the most important mechanisms that *S. aureus* uses to cause orthopedic infection is through a biofilm mode of growth that, when mature, results in both host immunity and antibiotic therapy being unable to clear the infection. The best way to resolve a biofilm infection is to prevent it from occurring without intervention. During an early *S. aureus* biofilm infection, an intense inflammatory response is produced by the host. *S. aureus* is readily able to resist clearance from the host through a large number of virulence factors that specifically attack the host and promote immuno-avoidance. The expression of *S. aureus* virulence factors, timed by the quorum sensing system, promotes the host to release Th1 cytokines including IL-12, IFN- $\gamma$ , and TNF- $\alpha$ , resulting in a shift of the adaptive immune system to an ineffective overactive inflammatory immune response. By preventing this initial severe inflammation, the host is then able to mount an effective Th2 antibody-mediated response that is readily effective at clearing the infection in the early phase of biofilm formation.

Therefore, administering biofilm up-regulated antigens as a vaccine preoperatively to patients undergoing implantation of an indwelling medical device may demonstrate protective efficacy, since the host immune response is primed for an early, effective antibody response prior to the destructive effects of inflammation. The Shirtliff lab has targeted biofilm formation by creating a vaccine against four protein antigens that are (1) expressed constitutively during *S. aureus* biofilm growth in vitro, (2) cell-surface associated, and (3) immunogenic throughout all infection stages in a rabbit model of osteomyelitis. When used together as a prophylactic quadrivalent vaccine and combined with post-infection vancomycin treatment to eliminate planktonic bacteria, 87.5% of animals cleared the infection completely, while no significant clearance was attained with vaccination or antibiotic treatment alone. Furthermore, there were

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significant reductions in radiological and clinical signs of infection in the vaccinated versus non-vaccinated groups.

While demonstrating future potential, orthopaedic infections continue to occur and will expand with the increased use of indwelling devices and an aging population. Therefore, diagnosis of these infections continues to be an important priority for today's clinicians. Current means of diagnosis of biofilm-mediated orthopedic infection are lacking. While biopsy and culture remain the gold standard of diagnosis due to ineffective imaging alternatives, contamination of the biopsy or sampling from a draining sinus tract (instead of directly from the bone), can lead to aberrant results. In addition, culturing all too often results in false negative results because the culture sample frequently is obtained from an area close to, but not at, the localized biofilm nidus of infection.

In order to develop better diagnostic tools, one should consider the particular phenotype of biofilms. Biofilm cells exhibit a radically altered phenotype with regard to growth, gene expression, and protein production as compared to identical microorganisms growing planktonically. This altered phenotype presents a unique opportunity for diagnosis of such infections by discovery of specific antigenic markers of the biofilm phenotype and development of diagnostics targeting these markers. We have used the antigenic distinctiveness of *S. aureus* biofilm cells to allow for the detection of biofilm-specific and immunogenic proteins. These proteins were used in a system that detects host anti-biofilm antibodies from host serum samples to act as clinical markers for ex vivo detection of biofilm infections by lateral flow immunoassay (LFI). In addition, biofilm-specific labeled antibodies enabled the in vivo detection and localization of biofilm infections. Our long-term objective is to discover antigenic proteins specific to other pathogenic microbial biofilms and demonstrate their efficacy as clinical markers of biofilm infection by LFI as well as labeled antibodies for the in vivo localization of biofilm infections. Diagnostic tools that can determine whether or not the patient is suffering from a *S. aureus* biofilm infection as well as one that localizes at the infection location would provide increased sensitivity and specificity while also decreasing the need for biopsy and culture. This would lead to an earlier inception of the proper antibiotic treatment, less need for surgical intervention, identifying infections in cases of supposed aseptic loosening, and result in an overall lessening of patient morbidity, mortality, and cost of care.

**Application of the nematode *Caenorhabditis elegans* as a model for multi-kingdom biofilm interactions of *Staphylococcus epidermidis* and *Candida albicans***

*Presenter:* Birthe Venø Kjellerup, Assistant Professor, Microbiology

*Co-authors:* Asia Houston, Sarah J. Edwards, and Birthe Venø Kjellerup

*Affiliation:* Goucher College, 1021 Dulaney Valley Road, Baltimore, Maryland 21204, USA

Multi-kingdom biofilms are often the cause of chronic infections, many that are non-responsive to antimicrobial therapy. The bacterium *Staphylococcus epidermidis* and the fungus *Candida albicans* are present in humans as commensal organisms. In addition, they are often found coexisting in biofilms as opportunistic pathogens in immuno-compromised patients. Though the need to examine multi-kingdom interactions within biofilms is great, this field of study is still in its infancy. In this study an in-vivo biofilm model using *C. albicans* and *S. epidermidis* was developed to propagate biofilms within the nematode *Caenorhabditis elegans* for examination of mechanisms driving such an interaction. Since slime production is an important characteristic of staphylococcal pathogenicity it was here evaluated if the interaction between slime-producing *S. epidermidis* coexisting with *C. albicans* in a biofilm was more pathogenic to *C. elegans* when compared to multi-kingdom biofilms formed by either a wild-type *S. epidermidis* or a slime-negative *S. epidermidis* mutant, respectively and *C. albicans*.

*C. elegans glp-4;sek-1* was grown on NGM agar and *E. coli* OP50, removed, and placed in groups of fifty onto five plates of overnight *S. epidermidis*, *C. albicans*, and dual culture plates grown at 37°C, respectively. The nematodes were fed for 1-2 days at 15°C after which they were assessed using PNA-FISH and LIVE/DEAD BacLight. The organisms were examined using fluorescence microscopy. The morphology and growth patterns were recorded and imaged.

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*C. elegans* consumed both *C. albicans* and wild type *S. epidermidis* cultures and formed biofilms inside *C. elegans*. The nematode survival rate showed that the survival rate was significantly reduced (35-50%) within two days of observation for both *S. epidermidis* and *C. albicans* compared to *E. coli*. Biofilms formed by both *S. epidermidis* and *C. albicans* remained alive even when the nematode was dead shown by the LIVE/DEAD staining, while the consumed *E. coli* were dead. Observations of established *C. albicans* biofilm in *C. elegans* showed that both hyphal and yeast forms existed. Similar experiments are currently being performed with slime-producing and slime-negative strains of *S. epidermidis* in co-existence with *C. albicans*. In the study it was shown that application of the nematode *C. elegans* as a competitive host model system for the study of multi-kingdom infectious biofilms has great potential.

### **Animal models useful for efficacy screening of antimicrobial and anti-biofilm drugs and coatings**

*Presenter:* Paul Attar, PhD, President

*Affiliation:* BRIDGE PTS, Inc.

BRIDGE PTS is a Contract Research Organization (CRO) specializing in animal models of device-related (biofilm-related) infections and wound healing. Over the years, BRIDGE PTS has tested hundreds of implants, coatings, formulations and pharmaceutical actives using animal models. While no single animal model can answer all questions, our presentation will focus on two of our most popular and most versatile models for early-stage antimicrobial/anti-biofilm efficacy assessments.

The first model we will discuss is our rat subcutaneous implant and infection protocol. This model is useful for screening medical device coatings as well as local and systemic therapeutics, and is performed by inserting a medical device into the subcutaneous space coincident with infection by one or more microbial species. The model has been successfully utilized for evaluations lasting up to six months and is a low-cost, rapid turn-around screening tool. In addition to describing how the model is run, we will present experimental data from antimicrobial, anti-adhesive, and injectable antibiotic studies.

The second model that we will highlight is our porcine (pig) model of infection. Researchers often focus on the pig as a wound healing model, but our presentation will instead highlight its use for the evaluation of topical and surgical intervention therapies. Using this model, a series of wounds is created along the backs of the pigs and then infected with one or more microbial species. Therapeutic intervention can begin at any time, but generally commences within 0–48 hours of the application of the contaminating bacteria. It can then be maintained for up to 21 days. We will demonstrate the utility of this model by presenting experimental data from topical antimicrobials, antiseptic/wound cleaners, and surgical debriders in studies that we have run.

Our presentation will conclude with a small list of other popular animal models available through BRIDGE PTS's contract services.

**abstracts****Poster Abstracts****Center for Biofilm Engineering posters****CBE Poster #504***Date:* 08/2009*Title:* **Characterization of methane-producing communities in deep coal seam***Authors:* **Elliott Barnhart**<sup>1</sup>, Wheaton J, Cunningham A, and Fields M*Affiliation:* Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA*Sponsored by:* US Department of Energy

We have conducted initial phylogenetic diversity studies using inoculated coal from methane producing wells in the Powder River Basin (PRB) of southeastern Montana and northeastern Wyoming. Methane generating enrichments were grown with coal as the only energy source and compared to enrichments with acetate. Preliminary data revealed an extremely diverse bacterial community established in coal cultures compared to enrichments without coal. DNA sequences indicative of methanogens (methane-producing archaea) were detected in both enrichments. These findings offer a compelling motive for further investigations of the biogeochemical processes controlling coal bed methane (CBM) production. The research is aimed at enhancing the fundamental understanding of the ecology and physiology of methane producing communities with the intent of identifying strategies for enhancement of in situ CBM production.

**CBE Poster #531***Date:* 03/2010*Title:* **EFRI-HyBi: Fungal processes for direct bioconversion of cellulose to hydrocarbons***Authors:* **Natasha Mallette**<sup>1,2</sup>, Peyton B<sup>1,2</sup>, Carlson R<sup>1,2</sup>, Strobel G<sup>3</sup>, Hunt K<sup>1,2</sup>, Strobel S<sup>4</sup>, Smooke M<sup>4</sup>*Affiliation:* <sup>1</sup> Center for Biofilm Engineering, <sup>2</sup> Dept. of Chemical and Biological Engineering, and <sup>3</sup> Dept. of Plant Sciences, Montana State University, Bozeman, MT, USA<sup>4</sup> Yale University, New Haven, CT, USA*Sponsored by:* NSF Emerging Frontiers in Research & Innovation (EFRI)

While considerable national effort has been focused on ethanol production, very little research—beyond characterization of cellulolytic fungal enzymes—has examined the potential role of fungi in renewable fuel production. *Ascocoryne sarcoides* (NRRL 50072) is an endophytic fungus recently isolated from Northern Patagonia by Gary Strobel (MSU). *A. sarcoides* produces and excretes “mycodiesel,” an extensive series of straight chained and branched medium chain-length hydrocarbons including heptane, octane, undecane, dodecane, and hexadecane (Strobel et al., 2008). This organism has the potential to produce petroleum directly using a cellulose fermentation process that is essentially carbon neutral. The goal of this research is to determine kinetic parameters of optimal fungal growth and hydrocarbon production through fermentation experiments. Experimental results from shake flask and 5 L reactor runs have verified hydrocarbon compound production under many different growth conditions. Biomass yields have improved from 0.05 g/L to 4.8 g/L. The pH tolerance of *A. sarcoides* is in the acidic range, and optimal temperature is between 16–23°C. These preliminary results confirm the ability of *A. sarcoides* to produce valuable fuel compounds. Future research will focus on product chemistry and yields, and completing the mass balance for the system.



**abstracts****CBE Poster #536***Date:* 05/2011*Title:* **Antimicrobial penetration and efficacy in an in vitro oral biofilm model***Authors:* Audrey Corbin<sup>1</sup>, Pitts B, Parker A, and **Philip S. Stewart**<sup>2</sup>*Affiliation:* <sup>2</sup> Center for Biofilm Engineering and Dept. of Chemical and Biological Engineering, Montana State University Bozeman MT, USA<sup>1</sup> Current Address: ALCIMED, 75016 Paris, France*Sponsored by:* This work was sponsored in part by Colgate-Palmolive Company.

The penetration and overall efficacy of six mouthrinse actives were evaluated using an in vitro flow cell oral biofilm model. The technique involved pre-loading biofilm cells with a green fluorescent dye that leaked out as the cells were permeabilized by a treatment. The loss of green color, and of biomass, was observed by time-lapse microscopy during 60 min of treatment under continuous flow conditions. The six actives analyzed were: ethanol, sodium lauryl sulfate (SLS), triclosan (TRN), chlorhexidine digluconate (CHX), cetyl pyridinium chloride (CPC), and nisin. Each of these agents effected loss of green fluorescence throughout biofilm cell clusters, with faster action at the edge of a cell cluster and slower action in the cluster center. The time to reach half of the initial fluorescent intensity at the center of a cell cluster, which can be viewed as a combined penetration and biological action time, ranged from 0.6 min to 19 min for the various agents. These times are much longer than the predicted penetration time based on diffusion alone, suggesting that anti-biofilm action was controlled more by the biological action time than by the penetration time of the active. None of the agents tested caused any removal of the biofilm. The extent of fluorescence loss after 1 h of exposure to an active ranged from 87% to 99.5%, with CHX being the most effective. Extent of fluorescence loss in vitro, but not penetration and action time, correlated well with relative efficacy data from published clinical trials.

**CBE Poster #550***Date:* 04/2011*Title:* **Transcriptome analysis of *Pseudomonas aeruginosa* biofilm subpopulations***Authors:* **Michael Franklin**<sup>1</sup>, Williamson KS<sup>1</sup>, Stewart PS<sup>1</sup>, Perez-Osorio AC<sup>2</sup>, McInerney K<sup>1</sup>*Affiliation:* <sup>1</sup> Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA<sup>2</sup> Washington State Department of Health, Shoreline, WA, USA*Sponsored by:* This work was supported by Public Health Service grant AI-065906 from the NIAID.

Bacteria in biofilms are heterogeneous with respect to cell physiology. As nutrients diffuse into biofilm and are utilized by the bacteria, chemical concentration gradients of nutrients, waste products, and signaling compounds are established. These gradients may intersect, creating many unique microenvironments within biofilms. In this study, we used laser capture microdissection (LCM) and Affymetrix<sup>®</sup> microarrays to characterize bacterial adaption to local environmental conditions within biofilms. RNA was purified from cells isolated from the top and bottom 30  $\mu\text{m}$  of *P. aeruginosa* biofilms. As controls, eight genes with differing expression levels were also assayed by LCM and qRT-PCR. The microarray results showed that most genes had higher mRNA abundances at the top compared to the base of the biofilms. Among the genes showing highest mRNA levels at the biofilm top were genes involved in general cell metabolism, including ATP biosynthesis, cell division, and lipid production, suggesting that cells at the top of the biofilm are involved in cell growth. mRNA for genes regulated by Anr and oxygen limitation stress were highly abundant in cells at the top of the biofilm, suggesting that these cells may be in a transition state from oxygen-sufficient to hypoxic conditions. Cells deeper in the biofilms showed little mRNA for Anr-regulated genes, and have likely experienced long-term anoxia. Other transcripts that were highly abundant at the top of the biofilms, but below detection at the bottom of the biofilms, were for genes involved in stationary phase growth and quorum sensing. Ribosomal RNAs were highly abundant throughout the biofilms, but mRNA for ribosomal proteins was only observed at the top of the biofilms, suggesting that de novo ribosome synthesis occurs in cells near the air-biofilm interface, but ribosomes are stably maintained throughout the biofilm. Consistent with these results was the identification of mRNAs for ribosome hibernation factors, which were highly abundant at both the top and bottom of the biofilms. The results suggest that in thick *P. aeruginosa* biofilms, cells are physiologically distinct spatially, with cells near the

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air-biofilm interface in a transition state from exponential to stationary phase, while cells deep in the biofilm may be dormant, possibly due to long-term oxygen starvation.

**CBE Poster #553**

*Date:* 07/2011

*Title:* **Evaluation of human hand bacterial communities with different techniques**

*Authors:* **Carrie A. Zapka**<sup>1</sup>, Ramsay B<sup>2</sup>, Rackaityte E<sup>1</sup>, Lauber C<sup>3</sup>, Weimer BC<sup>4</sup>, Desai P<sup>4</sup>, Fierer N<sup>3</sup>, Macinga DR<sup>1</sup>, Fields MW<sup>2</sup>

*Affiliation:* <sup>1</sup> GOJO Industries, Inc, Akron, OH, USA

<sup>2</sup> Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

<sup>3</sup> University of Colorado at Boulder, Department of Ecology and Evolutionary Biology, Boulder, CO, USA

<sup>4</sup> University of California at Davis, School of Veterinary Medicine, Davis, CA, USA

*Sponsored by:* GOJO Industries, Inc.

Skin of the human hand, one of many microbial habitats of the human body, is of particular interest because it plays an important role in the transmission of pathogens due to constant interaction with external surroundings and other parts of the body. Previous studies have shown that the skin microbiota can be diverse, dependent upon body location, skin type, and subject. The objective of our study was to compare culture-dependent to culture-independent methods (clonal sequences, pyrosequences, and PhyloChip) for the assessment of hand skin microbiota for 3 test subjects. In addition, we also assessed the bacterial communities post-treatment. A whole-hand sampling method was used on both hands of 6 participants who had not used antimicrobials for 2 days and who had not washed for at least two hours prior to sampling. For the post-sanitizing treatment, hands were re-sampled under the same conditions two days later, immediately after treatment with sanitizing solution. When different cultivation media were used, 7 to 17 different genera were isolated from three individuals and the cultivated populations were present at levels between <500 and 3,000,000 CFU/hand. Viable colonies of *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* sp. were isolated from all three individuals. Cultivated isolates differed between individuals and represented a subset of the sampled diversity observed with sequence-based techniques. As expected, clonal sequence libraries underestimated the community diversity (between 11 to 29 genera), but differentiated microbial communities for the tested subjects. Pyrosequencing libraries were compared at different depths of coverage (approximately 1,000 versus 10,000) and gave varying results with estimated OTU levels ranging from 70 to 1,000 per individual. Predominant sequences were detected at similar relative distribution levels (e.g., *Propionibacterium*, *Staphylococcus*, and *Streptococcus*), while less predominant populations (e.g., *Moraxella*, *Haemophilus*, *Kocuria*, and *Eubacterium*) were only observed with deeper coverage. The PhyloChip detected similar sequences, but estimated the number of predicted OTUs between 165 and 585 OTUs. As expected, pyrosequencing and PhyloChip analyses provided improved community characterization and indicated that individuals displayed distinct communities as well as variation between left and right hands. All bacterial communities were altered post-treatment wash to varying degrees, and the response was subject-dependent.

**CBE Poster #554**

*Date:* 07/2011

*Title:* **Optimization of cell yield and triacylglycerol accumulation for a Yellowstone diatom**

*Authors:* **Karen Moll**, Gardner R, Eustance E, Macur R, Gerlach R, Peyton B, Cooksey K

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

With increasing global demand for petroleum, microalgae may soon become a viable biodiesel source due to their higher oil yield per hectare compared to other biofuels. Optimizing the accumulation of lipids as triacylglycerol (TAG) for biodiesel production is critical to decreasing production costs. Some algal strains are capable of producing large quantities of TAGs under stressed conditions, such as nutrient limitation or other factors (e.g., pH). This study investigated the role of varying silica concentrations on cell yield, growth kinetics, and TAG accumulation for a pinnate diatom isolated from Yellowstone National Park. Additional

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results are presented on the ability of  $\text{NaHCO}_3$  to increase the rate of production and accumulation of TAG. Silica concentration was varied to increase diatom cell density. Growth was monitored using direct cell counts; pH, chlorophyll, nitrate, and silica utilization were quantified. TAG measurements were monitored by Nile Red fluorescence. Increasing the silica concentration in the growth medium resulted in higher diatom cell yield and cellular dry weight, but decreased diatom growth rate. This indicates an optimum silica concentration for growth. At silica depletion, lipid accumulation was promoted. It was found that cultures with added  $\text{NaHCO}_3$  enhanced the rate of lipid production and total TAG accumulation. Following  $\text{NaHCO}_3$  addition, diatoms reached the same TAG concentration in a shorter time. When silica is fully utilized, cells redirect energy and  $\text{CO}_2$  into storage molecules (TAGs). The addition of  $\text{NaHCO}_3$  increased the rate of TAG accumulation. The combination of increased cell yield and specific TAG accumulation rate significantly increased total lipid production. Results are industrially relevant because they demonstrate decreased time and cost for biodiesel production, improving algal biofuel viability as an alternative energy source.

**CBE Poster #555***Date:* 07/2011*Title:* **Microbial ecology of biofilms on two types of water distribution pipe materials***Authors:* **Gem Encarnacion**, Camper AK*Affiliation:* Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

This study analyzed differences between bacterial populations in biofilms that formed on polyvinyl chloride (PVC) and copper coupons in reactors that simulate premise plumbing. Here we used modified CDC reactors that had been actively nitrifying for six years. Biofilms were collected for carbohydrate and protein analysis as well as for DNA extraction. Polymerase Chain Reaction (PCR) targeting the 16S ribosomal RNA gene, followed by denaturing gradient gel electrophoresis (DGGE) was performed. Carbohydrate to protein ratio of the biofilms from the copper and PVC were found to differ, suggesting a difference in the assemblage of organisms. This result is also reflected in the molecular analysis. DGGE profiles of the inoculum (Bozeman tap water), biofilm from the copper, and PVC coupons were compared and were found to significantly differ from each other. This demonstrated that pipe material affects bacterial diversity, since the reactors with the copper and PVC coupons were given the same inocula, and microbial assemblages distinct from the source developed in each. To further assess the diversity, bands were excised from the denaturing gel from both copper and PVC samples and were re-amplified, cloned, and sequenced. Sequencing revealed the presence of nitrite oxidizing bacteria (NOB), but not ammonia oxidizing bacteria (AOB) in these actively nitrifying systems. As AOB are the commonly implicated organisms in ammonia oxidation in drinking water distribution systems, results may mean that the techniques used (i.e., DNA extraction, PCR primers), failed to detect their presence or that another group of organisms is responsible for this step in the nitrification process in this specific system.

**CBE Poster #560***Date:* 11/2011*Title:* **Potential of microbes to increase  $\text{CO}_2$  storage security***Authors:* **Robin Gerlach**<sup>1,2</sup>, Mitchell AC<sup>2,3</sup>, Ebigo A<sup>4</sup>, **Adrienne Phillips**<sup>1,2</sup>, Spangler L<sup>5</sup>, Cunningham AB<sup>2</sup>*Affiliation:* <sup>1</sup> Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA<sup>2</sup> Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA<sup>3</sup> Institute of Geography and Earth Sciences, Aberystwyth University, UK<sup>4</sup> Dept. of Hydromechanics and Modeling of Hydrosystems, University of Stuttgart, DE<sup>5</sup> 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  $\text{CO}_2$  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  $\text{CO}_2$ , particularly near well bores and potential leakage pathways. This poster presents concepts and results from bench- to meso-scale experiments

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focusing on the utility of attached microorganisms and biofilms to enhance storage security of injected CO<sub>2</sub>, via mineral trapping, solubility trapping, formation trapping, and leakage reduction. Batch and flow experiments at atmospheric and geologic CO<sub>2</sub> storage-relevant pressures have demonstrated the ability of microbial biofilms to decrease the permeability of natural and artificial porous media, survive the exposure to sc CO<sub>2</sub>, and facilitate the conversion of CO<sub>2</sub> into long-term stable carbonate phases as well as increase the solubility of CO<sub>2</sub> 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<sub>2</sub> storage security. As a result, reactive transport models describing the influence of biological processes on CO<sub>2</sub> storage security have been developed and are continuously being modified to include relevant processes.

**CBE Poster #561**

*Date:* 01/2012

*Title:* **Diffusion and microbial consumption of oxygen in acidic geothermal iron-oxide mats**

*Authors:* **Bernstein HC**, Beam JP, Carlson RP, Inskeep WP

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

*Sponsored by:* NSF IGERT

The role of dissolved oxygen as a primary electron acceptor for microbially mediated iron oxidation was investigated within the primary flow path of an acidic geothermal spring in Norris Geyser Basin, Yellowstone National Park. Previous data has suggested that Fe(II)-oxidizing microbial populations (e.g., *Metallosphaera sp* and potentially other novel members of the domain *Archaea*) represent the primary-producers within these microbial communities. Consequently, the availability of oxygen is hypothesized to limit microbial Fe(II)-oxidation and primary-productivity in this system. In situ measurements of oxygen profiles were obtained perpendicular to the direction of convective flow across the aqueous phase-Fe(III)-oxide interface using oxygen microsensors. Dissolved oxygen concentrations drop below detection by ~600 μm into the Fe(III)-oxide mat, indicating reactive oxygen consumption and defined spatial gradients. Evaluation of the oxygen flux across the liquid-mat boundary showed that convection was negligible compared to diffusive transport in the mat. Reaction-diffusion models were evaluated assuming both zero and first-order reaction kinetics. The in situ measurements and models suggest that the rate of oxygen consumption exceeds the rate of diffusion. Thus, microbially mediated Fe(II)-oxidation in this system is likely limited by oxygen diffusion, resulting in an active surface layer of Fe(III)-oxide biomineralization.

**CBE Poster #562**

*Date:* 02/2012

*Title:* **Quality-score refinement of SSU rRNA gene pyrosequencing differs across gene region for in situ samples**

*Authors:* **Kara Bowen De León**, Ramsay BD, and Fields MW

*Affiliation:* Department of Microbiology, and Center for Biofilm Engineering,  
Montana State University, Bozeman, MT, USA

Thermal Biology Institute, 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

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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<sub>15%</sub>, Q30<sub>10%</sub>, and Q32<sub>15%</sub>, respectively—and the most stringent Q tested (Q32<sub>10%</sub>) 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  $\alpha$ -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<sup>1,2</sup>, **Christine Foreman**<sup>1,2</sup>, McConnell J<sup>3</sup> and Priscu J<sup>1</sup>

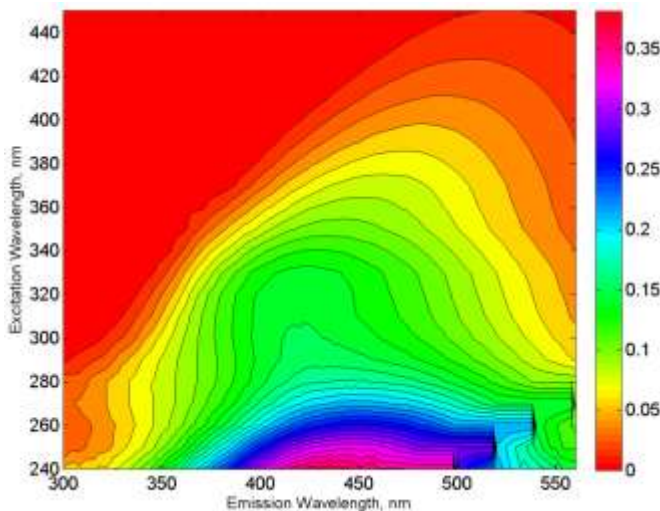
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<sup>3</sup> Desert Research Institute, Reno, NV, USA

*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 (see Figure 1 for fluorescent regions of interest). 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.

**Figure 1: Representative Excitation Emission Matrix of Pony Lake DOM that shows the major fluorescing components of DOM. A and C are humic-like components, M is a marine humic-like signature, and B and T both denote the protein-like fluorescing components tyrosine and tryptophan.**

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.

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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.

**CBE Poster #564**

*Date:* 01/2012

*Title:* **Structure impacts function for a syntrophic biofilm of *Methanococcus maripaludis* and *Desulfovibrio vulgaris***

*Authors:* **Kristen A. Brileya**<sup>1</sup>, Sabalowsky A<sup>1,2</sup>, Ramsay B<sup>1</sup>, Zane G<sup>3</sup>, Wall JD<sup>3</sup>, Fields MW<sup>1,2</sup>

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*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  $\mu\text{g}$  hexose/ $\mu\text{g}$  protein, .017  $\mu\text{g}$  pentose/ $\mu\text{g}$  protein and .011  $\mu\text{g}$  uronic acid/ $\mu\text{g}$  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  $\Delta\text{flaG}$  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  $\Delta\text{flaG}$  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,  $\Delta\text{flaG}$  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 haptotaxis for incorporation into the biofilm.

## abstracts

### **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

**Aims:** 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 40–80 µg ml<sup>-1</sup> 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.

### **CBE Poster #566**

Date: 01/2012

Title: **Imaging biofilm and microbially induced CaCO<sub>3</sub> precipitation in porous media reactors**

Authors: **James Connolly**<sup>1,2</sup>, Iltis G<sup>4</sup>, Wildenschild D<sup>4</sup>, Cunningham A<sup>1,3</sup> and Gerlach R<sup>1,2</sup>

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Sponsored by: National Science Foundation

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

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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 100 $\mu$ m 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.

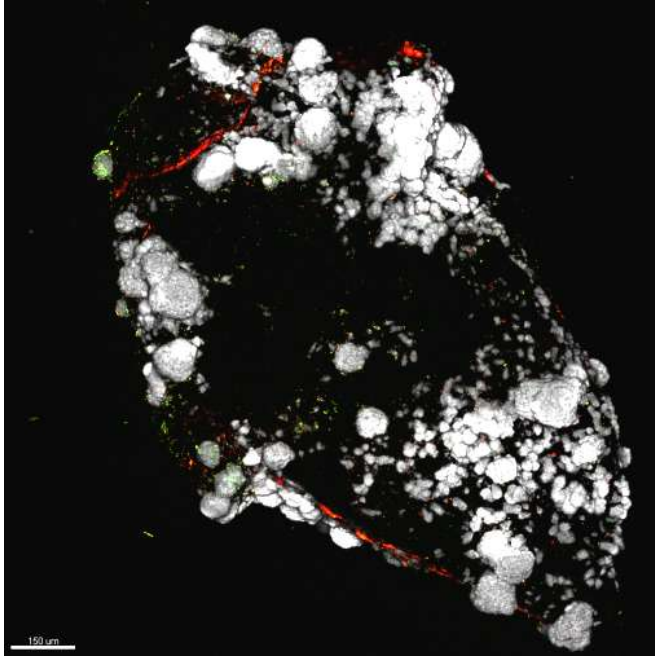


Figure 1. A CLSM reconstruction of a sand grain colonized by *Sporosarcina pasteurii* under urealytic conditions where calcium carbonate (shown in white) has been precipitated. The sample was stained with Invitrogen LIVE/DEAD so areas with healthy cells are shown in green. Regions with cells that have 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 = 150 $\mu$ m.

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. Biofilm 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.

### **CBE Poster #567**

Date: 01/2012

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

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

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

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

This poster will present the results of evaluating 3M Petrifilm™ 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™ Aerobic Count plates and drop plated on R2A plates. The Petrifilm™ and R2A plates were incubated at 36°C and colonies enumerated after 24 and 48 hours. The experiment was replicated three times by two technicians. The time required for both plating methods was recorded to help assess the efficiency of both methods. The results from this study may



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demonstrate that Petrifilm™ could replace drop-plating as a more efficient and cost effective method for bacterial enumeration.

**CBE Poster #568**

*Date:* 01/2012

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

*Authors:* **Jacob Valenzuela**<sup>1,5,6</sup>, Mazurie A<sup>2,3</sup>, Carlson RP<sup>4,6</sup>, Gerlach R<sup>4,6</sup>, Cooksey KE<sup>2</sup>, Bothner B<sup>1</sup>, Peyton BM<sup>4,6</sup>, and Fields MW<sup>2,6\*</sup>

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

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

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

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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 right 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 through-put, 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.

**CBE Poster #547**

*Date:* 01/2012

*Title:* **Bacterial biofilms are oxygen sinks in murine and in vitro models of wound infection**

*Authors:* **Garth James**<sup>1</sup>, Nguyen HD<sup>2</sup>, Beyenal H<sup>2</sup>, Zhao AG<sup>3</sup>, Agostinho AM<sup>1</sup>, deLancey Pulcini E<sup>1</sup>, Usui M<sup>3</sup>, Underwood B<sup>3</sup>, Fleckman P<sup>3</sup>, Olerud J<sup>3</sup>, and Stewart PS<sup>1</sup>

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Local oxygen (O<sub>2</sub>) concentration is a critical parameter in wound healing. Tissue injury, in some cases combined with pre-existing ischemia, creates hypoxic niches in wounds. The presence of biofilms in chronic wounds has been demonstrated, but their role in delayed wound healing is unclear. We hypothesized that bacterial biofilms exploit hypoxic niches in wounds and function as O<sub>2</sub> sinks, perpetuating anoxia and preventing O<sub>2</sub>-dependent wound healing processes. We used microelectrodes to measure O<sub>2</sub> concentration profiles of biofilms grown in vitro using bacteria isolated from human chronic wounds, as well as wound scabs and wound beds, in a murine model for delayed healing of biofilm-infected wounds. Biofilms formed in vitro by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, as well as mixtures of these species were all capable of depleting O<sub>2</sub> to less than 10% of air saturation within distances of a fraction of a millimeter. Mice challenged with *P. aeruginosa* biofilm had the

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largest populations of bacteria associated with the wound scabs. O<sub>2</sub> profiling was performed in situ using scabs on both live and euthanized mice, as well as in excised scabs. O<sub>2</sub> profiling demonstrated steep oxygen gradients similar to those measured in the in vitro models in the all of the scabs. O<sub>2</sub> profiles from scabs were more complex than from in vitro biofilms, which may correlate with the heterogeneous ultrastructure of the scab. These O<sub>2</sub> gradients were eliminated by heat-killing. In contrast, a wound bed probed after removal of the scab did not have a significant O<sub>2</sub> gradient. These results demonstrate that bacterial biofilms function as oxygen sinks in both the in vitro and murine models. Perpetuation of anoxic conditions by biofilms may be an important barrier to wound healing.

**CBE Poster #571**

*Date:* 01/2012

*Title:* **Impact of biofouling on transport dynamics measured by magnetic resonance displacement relaxation correlation**

*Authors:* **Alexis B. Sanderlin**<sup>1,2</sup>, Vogt SJ<sup>1,2</sup>, Codd SL<sup>1,3</sup>, Seymour JD<sup>1,2</sup>

*Affiliation:* <sup>1</sup> Center for Biofilm Engineering, <sup>2</sup> Dept. of Chemical and Biological Engineering, and <sup>3</sup> 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.

**CBE Poster #572**

*Date:* 01/2012

*Title:* **Magnetic resonance relaxation of alginate solutions and gels**

*Authors:* **Sarah J. Vogt**<sup>1</sup>, Fabich HT<sup>1</sup>, Sherick ML<sup>1</sup>, Brown JR, Seymour JD<sup>1,2</sup>, and Codd SL<sup>1,3</sup>

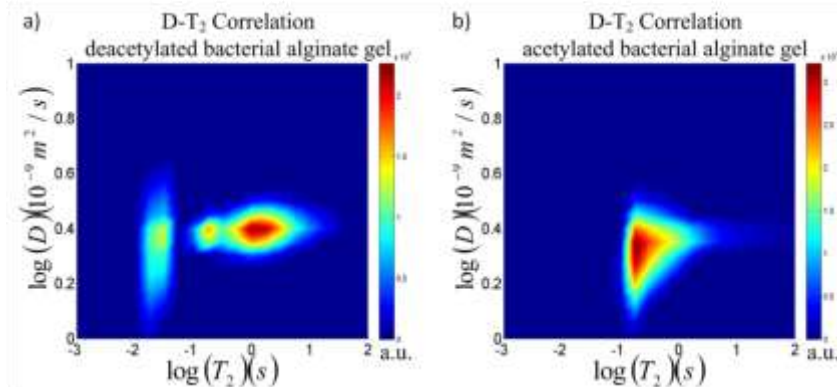
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*Sponsored by:* MLS and HTF are funded by INBRE Grant Number P20RR016455 from the National Center for Research Resources (NCRR), NIH. DOE EPSCOR DE-FG02-08ER46527

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

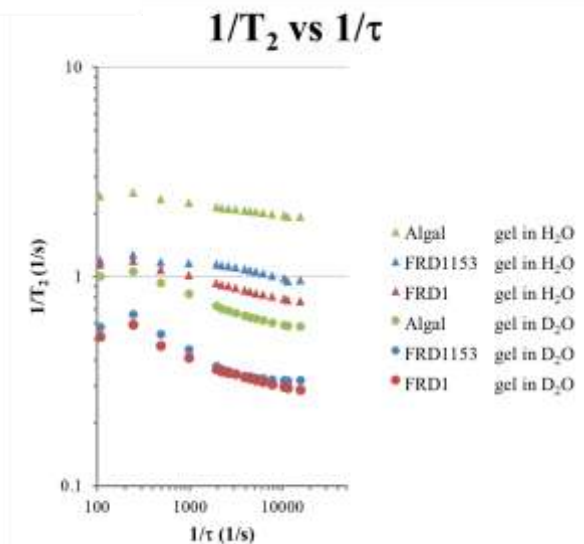
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algal- and microbial-produced alginates are investigated. Of particular interest is the difference in gel structure and formation between acetylated alginate formed by a *P. aeruginosa* isolate FRD1 from CF patients and de-acetylated alginate from 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  $\text{CaCl}_2$  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  $\delta$ -lactone and  $\text{CaCO}_3$  from 1%wt. alginate solutions for three different genetic variants of *P. aeruginosa*. Gels were formed in both distilled water ( $\text{H}_2\text{O}$ ) and 99.9%  $\text{D}_2\text{O}$  to investigate the effect of proton exchange between the polymer matrix and the bulk water.**



### **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 P2ORR016455 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*

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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.

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5. Maneval JE, et al, "Magnetic resonance analysis of capillary formation reaction front dynamics in alginate gels," in press, *Magnetic Resonance in Chemistry*, 2011.

**CBE Poster #574**

*Date:* 01/2012

*Title:* **Microbial community dynamics in groundwater and surrogate sediments during HRC<sup>®</sup> biostimulation of Cr(VI)-reduction**

*Authors:* **Kara Bowen De León**<sup>1,2</sup>, Ramsay BD<sup>2</sup>, Newcomer DR<sup>3</sup>, Faybishenko B<sup>4</sup>, Hazen TC<sup>5,6</sup>, and Fields MW<sup>1,2</sup>

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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<sup>®</sup>) 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<sup>®</sup>. 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 SSU rDNA gene fragments were sequenced via multiplex pyrosequencing. Sequences were refined by length, primer errors, and Ns, and sequences with a high percentage of low Phred quality score values were removed. Python scripts were developed to filter the pyrotag data with respect to quality scores, and the filtering technique was validated with environmental samples. Soil samples 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. Predominant populations for the sediments were *Pseudomonas*, *Acidovorax*, *Clostridium*, *Aquaspirillum*, *Methylibium*, and *Anaeromyxobacter*; while predominant populations for groundwater were *Pseudomonas*, *Pleomorphomonas*, *Ramlibacter*, *Arthrobacter*, and *Herbaspirillum*. Genera observed only in the sediment included *Marinomonas* while genera observed only

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in the groundwater included *Desulfonauticus*, *Desulfomicrobium*, and *Syntrophobacter*. 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. However, a prevalence of core genera and rare genera were observed across 34 samples, while urban and rural genera were less abundant. There was a shift from *Acidovorax* to *Aquaspirillum* from upstream (non-stimulated) to downstream soil both pre- and post-injection. Surrogate soil samples indicated similar changes in the soil community in the injection (Well 45) and downstream (Well 41) wells across time, while water samples seem to indicate more of a pre- and post-injection grouping instead of gradual changes across time. Furthermore, while post-injection soil samples indicate a continuing dominance of *Aquaspirillum*, corresponding water samples indicate *Pseudomonas* as a dominant genus. 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.

**Other Academic Posters**

*Date:* 02 / 2012

*Title:* **Characterization of two *Staphylococcus aureus* nucleases and their relationship to biofilm formation**

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Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is emerging as a significant cause of biofilm-related infections. CA-MRSA USA300 strain LAC encodes two thermonucleases, the major secreted nuclease (Nuc) and a second predicted nuclease (Nuc2). Considering reports that extracellular DNA (eDNA) is an important biofilm matrix component, we hypothesized that one or both of these nucleases may impact LAC biofilms. We first investigated the role of Nuc. Regulation studies showed that *nuc* expression is induced in a *sigB* mutant and repressed under biofilm-forming conditions. A LAC *nuc* mutant displayed enhanced biofilm formation and accumulated more high molecular weight eDNA than the WT and regulatory mutant strains, and mutation of *nuc* consistently results in enhanced biofilm formation across other *S. aureus* strain lineages. Inactivation of *nuc* in the biofilm-deficient LAC *sigB* mutant background partially restored biofilm formation, suggesting *nuc* expression contributes to the inability of a *sigB* mutant to form a biofilm. These findings confirm the important role of eDNA in the *S. aureus* biofilm matrix and indicate that Nuc regulates biofilm formation. Currently, little is known about the second nuclease, Nuc2. Topology programs predict the N-terminus of Nuc2 to be membrane-anchored, with the staphylococcal nuclease domain located in the C-terminus of the protein. Preliminary experiments have confirmed that Nuc2 is membrane-associated with the C-terminus facing the extracellular environment, and purified Nuc2 protein has activity against double-stranded DNA. We are working to further characterize Nuc2 to learn more about its activity and determine whether Nuc2, like Nuc, has a role in regulation of *S. aureus* biofilm development.

## abstracts

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*Title:* **N,N-dodecyl,methyl-PEI coatings that inhibit biofilms and support bone healing during infection**

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Adhesion of microorganisms to biomaterials with subsequent formation of biofilms on such foreign bodies as orthopedic trauma hardware is a critical factor in implant-associated infections; once a biofilm has been established, its microorganisms become recalcitrant to the host's immune surveillance and markedly resistant to drugs. We have previously reported that painting with the hydrophobic polycation N,N-dodecyl,methyl-PEI (PEI = polyethylenimine) renders solid surfaces bactericidal in vitro. Herein we observe that N,N-dodecyl,methyl-PEI-derivatized titanium and stainless steel surfaces resist biofilm formation by *S. aureus* compared to the untreated ones. Using imaging, microbiology, histopathology, and scanning electron microscopy (SEM) experiments in a clinically relevant large-animal (sheep) trauma model, we subsequently demonstrate in vivo that orthopedic fracture hardware painted with N,N-dodecyl,methyl-PEI not only prevents implant colonization with biofilm but also promotes bone healing. Functionalizing orthopedic hardware with hydrophobic polycations thus holds promise in supporting bone healing in the presence of infection in veterinary and human orthopedic patients.