Montana State University

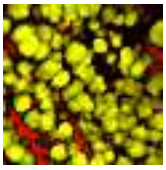
Center for Biofilm Engineering

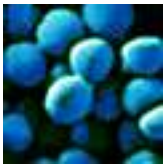
Bozeman

montana biofilm SCIENCE & TECHNOLOGY meeting

February 8–9, 2011

proceedings





Montana Biofilm Science & Technology Meeting: February 8–9, 2011

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Speaker Abstracts

SESSION 1: Novel Antimicrobials

Next-gen antimicrobial strategies enabled by synthetic biology

Presenter: Michael Koeris, President
Affiliation: Novophage Therapeutics, Inc.

Increased speed and reduced cost for deciphering and manipulating the informational content encoded in biological systems enable the application of pure engineering design principles. Rational engineering and design of biologic antimicrobials can focus on a desirable subset of characteristics for novel approaches: i) highly specific microbial targeting, ii) modular and quickly customizable setup, and iii) flexible formulation and delivery without compromising efficacy. Bacteriophages are ideal substrate to start work in this field because they are highly ordered, uniform in composition, robust (low variability in outcomes), yet genomically small enough to engineer readily.

Surface active systems afford insights for biofilm control agents

Presenter: Joe Sauer, Senior R&D Advisor
Co-authors: Cook GW, Jr and Doerrler WT
Affiliation: The Albemarle Corporation

Quaternary ammonium salts have been used historically as hard surface disinfectants. While several classes of these materials have been utilized, a systematic investigation of the structural features of these classes provides insights as to what might be necessary for a good disinfectant system. Albemarle's core technologies provide novel approaches to the synthesis of quats that might have value as disinfectants and biofilm control agents. One extremely important bacterial genus to consider is *Mycobacterium* spp. (*M. tuberculosis, M. leprae, M. avium, M. bovis*). Many members of this genus have been resistant to the effects of quaternary biocides. A large part of this resistance is due to the cell wall structure of mycobacteria—although a significant additional part of this resistance may well be due to their ability to form single species biofilm structures or exist as members of multispecies communities in which other species may have the primary role of biofilm construction and promotion. This presentation will cover Albemarle's novel approach to development of "new" quaternary ammonium biocides—a synthetic overview of what is possible. Additionally, a view of why this approach has not been tackled by other companies within the disinfectant community will be covered. Results from initial trials of a library of over 200 novel quats will be discussed as well as implications for biofilm control, with particular emphasis on *Mycobacteria*. This approach gives us new avenues toward construction of innovative disinfectants. . . but, does it also give us the ability to generate structures that are capable of more than that?

Research and development of antimicrobial coatings using combinatorial/high-throughput methods

Presenter: Bret Chisholm, Senior Research Scientist
Affiliation: Center for Nanoscale Science and Engineering; Director, Combinatorial Materials Research Laboratory, North Dakota State University, Fargo, ND

Combinatorial chemistry has been well established in the pharmaceutical industry for the development of new drugs. More recently, combinatorial and high-throughput methods have been applied to the development of non-drug materials such as catalysts, sensing materials, and materials for electronic devices. At North Dakota State University, we have developed a combinatorial/high-throughput (C/HT) workflow for the research and development of organic polymers and surface coatings. The C/HT workflow possesses unique tools for each component of the experimental process including experimental design, polymer synthesis, polymer characterization, coating preparation, coating application, coating characterization, data analysis, and data management. A primary focus of the C/HT workflow is the research and development of antimicrobial coatings for marine and biomedical applications. HT characterization of antimicrobial properties is achieved using a robotic platform designed around 24 element coating arrays. The primary HT assays used for screening antimicrobial efficacy are based on measurements of biofilm retention. Using the C/HT workflow, extensive structure-antimicrobial activity relationships have been developed for a variety of coating systems. The most heavily investigated system is based on crosslinked polysiloxane coatings containing chemically bound (i.e., tethered) quaternary ammonium salt moieties. The results of the studies showed that antimicrobial activity was highly dependent on multiple compositional variables, including higher order interactions between variables. A strong correlation between antimicrobial activity and coating surface morphology was observed, which indicated that antimicrobial activity was largely influenced by the effect of the compositional variables on segregation of quaternary ammonium salt groups to the coating-air interface during film formation. Due to the extensive structure-antimicrobial activity relationships derived from the study, optimized coating compositions were readily identified and used to produce coated urinary catheters that exhibited broad spectrum antimicrobial activity.

Antibacterial properties of cold electrically generated plasma and of plasma treated materials

Presenter: Gary Friedman, Professor
Affiliation: School of Biomedical Engineering, Drexel University, Philadelphia, PA

Gas phase plasma is known to produce highly oxidative species such as ozone, superoxide, and hydroxyl radicals in addition to ions, electrons, and electronically excited atoms and molecules. Combination of ions and reactive oxygen species entering aqueous phase can directly oxidize organic molecules or generate organic peroxides that can begin a chain of reactions ending in oxidation. As a result, plasma appears to be highly effective as an antibacterial treatment.

Different types of cold plasma treatment will be reviewed in this talk. These types fall into two major classes: non-thermal plasma treatment and treatment by cooled effluent from thermal plasma in the presence of different gases. In air, non-thermal plasma tends to produce more reactive oxygen species such as ozone, while thermal plasma creates greater concentrations of reactive nitrogen species such as nitric oxide. In addition, non-thermal plasma can be used in direct contact with heat sensitive surfaces.

Effects of different plasma treatments on bacteria will be reviewed in this talk, demonstrating that non-thermal plasma treatment, particularly in direct contact, appears to have the strongest effect when bacteria are treated on the surface of agar. Oxygen appears to be necessary, and moisture significantly enhances antibacterial effects. Effects of charges in plasma were tested separately using corona discharges. Effects of

plasma treatment on bacteria suspended in aqueous solutions will also be reviewed. It will be demonstrated that treated liquids can retain strong antibacterial activity correlated with their acidity. Possible applications of plasma antibacterial technology will be discussed.

The surprising biological activity of volatile organic compounds from *Muscodor* spp.

Presenter: Gary Strobel, Professor Emeritus
Affiliation: Department of Plant Sciences, Montana State University, Bozeman, MT

A novel fungal genus is described that produces extremely bioactive volatile organic compounds (VOCs). The initial fungal isolate was discovered as an endophyte in *Cinnamomum zeylanicum* in a botanical garden in Honduras. This endophytic fungus was named *Muscodor albus* because of its odor and its white color. This fungus produces a mixture of VOCs that are lethal to a wide variety of plant and human pathogenic fungi and bacteria. It also is effective against nematodes and certain insects. The mixture of VOCs has been analyzed using GC/MS and consists primarily of various alcohols, acids, esters, ketones, and lipids. Final verification of the identity of the VOCs was carried out by using artificial mixtures of the putatively identified compounds and showed that the artificial mixture possessed the identical retention times and mass spectral qualities as those of the fungal derived substances. Artificial mixtures of the available VOCs mimicked some but not all of the biological effects of the fungal VOCs when tested against a wide range of fungal and bacterial pathogens. Other species and isolates of this genus have been found in various tropical forests in Australia, Bolivia, Ecuador, and Thailand. The most recent discovery is *Muscodor crispans* whose VOCs are active against many plant and human pathogens. Potential applications for "mycofumigation" by members of the *Muscodor* genus are currently being investigated and include uses for treating plant diseases, buildings, soils, agricultural produce, and much more. This report will describe how the fungus was discovered, identified, and found potentially useful to agriculture, medicine, and industry.

SESSION 2: Biofilm Methods

Chlorine and chlorine dioxide: Comparing the disinfection of detached biofilm particles

Presenter: Sabrina Behnke, PhD Candidate, Microbiology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Unilever U.K. Central Resources Limited

The goal of this study is the evaluation of the disinfection susceptibility of detached cells and cell clusters of *Burkholderia cepacia* and *Pseudomonas aeruginosa* in comparison to planktonic cultures grown as either single species or in co-culture. This comparison allows for the detection of differences in disinfection susceptibilities between the two species and also shows how the two species influence each other when grown together. In addition, differences in susceptibilities between the two reactor samples (chemostat cells and biofilm reactor effluent/detached biofilm cells) were investigated.

Log reductions with chlorine and chlorine dioxide have been assessed for all species scenarios (single and dual species cultures) at incrementally increasing concentrations. In addition, CT curves have been established to determine the kinetics of the treatments.

Update on the validation of a biofilm disinfectant efficacy test

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

The CBE's Standard Biofilm Methods Laboratory (SBML) is collaborating with the EPA to develop a standard method for testing the efficacy of liquid disinfectants against biofilm bacteria. The EPA Microbiology Laboratory Branch has developed a draft protocol known as the "Single Tube Method." In this method, biofilm grown on a test coupon is exposed to disinfectant and neutralized within a 50 mL conical vial. The vial is then vortexed and sonicated to remove the biofilm from the coupon and disaggregate the clumps. The bacterial suspension is serially diluted, spread plated, and enumerated. Although this efficacy method could be used for biofilm grown in any of the standardized reactor systems, the efficacy test is being developed using a *Pseudomonas aeruginosa* (ATCC 15442) biofilm grown on borosilicate glass coupons in the CDC biofilm reactor according to a slightly modified version of ASTM Method E2562-07.

Recently, the SBML and EPA Microbiology Laboratory Branch completed a two-laboratory study to determine the repeatability, reproducibility, resemblance, and responsiveness of the Single Tube Method. Testing showed that the untreated log densities exhibited resemblance; they were highly repeatable across multiple experiments at each laboratory separately, and highly reproducible between the two laboratories. At both the presumed low and high concentrations for all three disinfectants tested, the log reductions were acceptably repeatable across multiple experiments. All three disinfectants at all concentrations tested exhibited log reductions that were acceptably reproducible between the two labs. The Single Tube Method was statistically significantly responsive to the increased efficacy for two of the disinfectants tested.

The experimental design employed in this study allowed for the investigation of possible effects due to either processing order of the treatments or coupon position within a rod in the CDC reactor. No such effects were detected on either the untreated or treated log densities. On average, the untreated log densities were statistically equivalent across the different processing orders and coupon positions.

The Single Tube Method exhibits the qualities of repeatability, reproducibility, resemblance, and responsiveness. Based upon these results, the method will be written as a standard test method and submitted to a standard-setting organization where it can be reviewed by other experts and validated through a full collaborative study.

SESSION 3: Energy Related Biofilms

MSU energy research

Presenter: Lee Spangler, Director
Affiliation: The Energy Research Institute, Montana State University, Bozeman, MT

MSU has over 40 faculty members working on research programs in a large number of energy-related areas. It is an international leader in carbon sequestration and lead institution for the Big Sky Carbon Sequestration Partnership (one of seven DOE Region Partnerships) and the Zero Emissions Research and Technology Collaborative (ZERT), and has four other sequestration related federally funded projects. Additionally, MSU has strong funded programs in biofuels (both algae and crop based), wind (including one of the first six DOE funded Wind Application Centers), and fuel cells. This talk will provide an overview of these activities.

Characterization of algal triacylglycerol accumulation for biodiesel production

Presenters: Brent Peyton, Professor, Chemical and Biological Engineering
Co-authors: Carlson R, Cooksey KE, Eustance E, Fields M, Gardner R, Macur R, Mus F, Moll K, Gerlach R, and Valenzuela J
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Economic production of fuels and other products from algae is limited by the ability to maintain sustained growth in pond-type reactors. In these open systems, long-term productivity of desired organisms may be limited by (1) contamination by competing microbial species or predators, (2) mass transfer of carbon dioxide, and (3) sufficient availability of quality water. Lower quality water can be used for growth, and ponds can be built on non-arable land, such that issues of food-for-fuel are eliminated. Results from MSU's Algal Biofuels Group will be presented on the characterization of lipid-producing microalgae isolated from a variety of environments. Further, a major goal of the DOE Aquatic Species Program in the 1980s was the identification of the so-called "lipid trigger," a set of circumstances or a signaling molecule that mediates lipid synthesis or accumulation. No new definitive answers were found (growth medium N-limitation was already known), but there were indications that interference of the algal cell cycle by monofluoroacetic acid or elevated medium pH were possible candidates, albeit for a single species of alga, i.e., *Chlorella* Chlor-1. Here the results of studies on cellular lipid accumulation will be presented. Triacylglycerol (TAG) accumulation, nitrate remaining in the growth medium, and pH were monitored in biologically buffered and unbuffered cultures. Data include culture optimization, algal growth kinetics, growth yields, and lipid production rates and yields. This bio/chemical engineering approach is critical to the optimization of lipid production and the future design and scale-up of large algae-to-fuel and chemical systems.

This work is funded by the Air Force Office of Scientific Research (AFOSR grant FA9550-09-1-0243), US Department of Energy (Office of Biomass Production grant DE-FG36-08GO18161), and partial support for RG was provided by NSF IGERT Program in Geobiological Systems (DGE 0654336) at Montana State University.

Ascocoryne sarcoides: Exploration of potential fuel production

Presenter: Natasha Mallette, PhD candidate, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Whereas a large national effort has focused on ethanol production, very little research has examined the potential role of fungi in renewable fuel production beyond characterization of cellulolytic fungal enzymes. *Ascocoryne sarcoides* (NRRL 50072) is an endophytic fungus recently isolated from Northern Patagonia by Gary Strobel (MSU, Bozeman). *A. sarcoides* produces and excretes "mycodiesel," an extensive series of straight-chained and branched medium chain-length hydrocarbons, including heptane, octane, cyclodecane, 1-heptanol, and 2-methyl-3-pentanone (Griffin et al., 2010). 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.

Utility of biofilms and biologically induced mineralization in geologic carbon sequestration

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

Geologic carbon sequestration involves the injection of CO_2 into underground formations including oil beds, deep un-minable coal seams, basaltic rocks, and deep saline aquifers, with temperature and pressure conditions such that CO_2 will often be in the supercritical state.

Four trapping mechanisms are proposed to play significant roles in the deep geologic sequestration of CO_2: formation trapping, capillary trapping, solubility trapping, and mineral trapping.

Our research has shown that, independent of the host rock, microbial biofilms are capable of enhancing formation trapping, solubility trapping, and mineral trapping.

i) We have demonstrated that engineered microbial biofilms are capable of reducing the permeability of rock cores at pressures and temperatures, which would be found in the presence of supercritical CO_2.

ii) The biofilms have been demonstrated to be resistant to supercritical CO_2.

iii) Biofilms precipitate CO_2 in the form of calcium carbonate ($CaCO_3$), which resists dissolution by brine and $scCO_2$.

iv) Microbial activity can increase CO_2 solubilization, thus improving solubility trapping.

Recent activities have begun to focus on practical aspects related to the implementation of biofilm-enhanced geologic carbon sequestration technologies in field situations.

SESSION 4: Biofilm Imaging/Microscopy

Optical microscopy of biofilms: The pursuit of more relevant imaging conditions

Presenter: Betsey Pitts, Research Associate and Microscope Facilities Manager
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Light and fluorescence microscopy (often grouped together and called "optical microscopy," which includes transmitted light, epifluorescent, and confocal microscopy) are powerful tools for the examination of biofilms. Whether qualitative or quantitative, microscopy can provide unique data and observation unavailable by any other means. This is especially true at the Center for Biofilm Engineering, where microscopy has been adapted to accommodate the specific imaging requirements of fully hydrated, thick, uneven, *live* samples, which are often accompanied by human tissue, plant matter, minerals, and commonplace objects such as toothbrushes and fabric. As the imaging conditions are made more relevant to the *in-situ* biofilm conditions, the value of the data obtained from a microscope image increases. This presentation will highlight some of the imaging situations CBE researchers have encountered in the past year and the exceptional images they have collected using optical microscopy. In addition, the CBE received funding this past year for nearly $1 million in new confocal microscopy equipment, which is expected to arrive in early 2011. The new confocal equipment will have a tremendous impact on the quality and relevance of optical microscopy of biofilms at the CBE and will offer CBE researchers and colleagues many imaging capabilities that are currently unavailable. The new microscope equipment and imaging possibilities will also be discussed.

Nanoscale manipulation by immunoimmobilization of *living* bacteria

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

This talk is about tethering (by leashing, using nano ropes) and nanoscale manipulation of *living* bacteria on flat material surfaces. Tethering (hereafter referred to as immunoimmobilization) of living bacteria on flat material surfaces in their physiological environment offers potential applications of practical and fundamental interest. Our work in the last five years on the immunoimmobilization of selected mutants of *Salmonella* and *E. coli* suggests that the most efficient and reliable immunoimmobilization involves a limited number of specific surface antigens such as the pili, flagella or O-antigens of bacteria and the corresponding antibodies. Such an efficient and specific immobilization method for living bacteria opens up opportunities for conducting fundamental studies on individual cells or small groups of localized bacterial cells. For example, our work has proven that multiple puncturing of the cell wall of a bacterium by means of an AFM tip does not kill the organism, which opens up the possibility of introducing macromolecules and nanoparticles into the cytoplasm of an individual living bacterium. The high efficacy and specificity of immunoimmobilization can also be utilized for the rapid detection and determination of pathogenic species. This can be done by capturing potential pathogenic entities using a microarray that is composed of antibodies against various phenotypes. The talk will focus on the physics, chemistry, and biology of immunoimmobilization technology and on its potential use in fundamental and practical applications.

In situ measurement of biofilm activity by FISH: Implications for the therapy of infective endocarditis

Presenter: Judith Schmiedel, Institute for Microbiology and Hygiene
Co-authors: Petrich A, Mallmann C, Musci M, Hetzer R, Göbel UB, and Moter A
Affiliation: Charité University Hospital, Berlin, Germany

Infective endocarditis is a rare but life-threatening disease, in which microorganisms form so-called vegetations on the endocardium of the heart. The treatment of choice for endocarditis is high-dosage antibiotic therapy. In case of failure, the heart valves need to be replaced. However, concentrations of the antibiotics are chosen based on minimal inhibitory concentrations (MICs) estimated from planktonic cells. Here we investigate the activity of bacterial communities within human heart valves under adequate antibiotic therapy by fluorescence in situ hybridization (FISH). The signal intensity of FISH correlates to a high ribosomal content of the bacteria, indicating metabolic activity at the time of surgery.

In 22 endocarditis cases we succeeded in visualizing rich, well structured, and highly organized bacterial communities. We also found FISH-positive bacteria in culture-negative samples and samples from patients under antibiotic therapy.

To detect the activity of single bacterial cells more precisely, we developed FISH probes for the 16S-23S internal transcribed spacer that is only present in actively transcribing cells. Using this spacer FISH, we detected positive cells in heart valves of patients under adequate therapy.

In summary, these findings confirm the higher resistance of bacteria toward antibiotic treatment in biofilms in the clinical setting. They stress the point that our current diagnostic techniques regarding cultivation and antibiotic resistance testing of planktonic cells in vitro are not satisfactory. In situ techniques might allow visualizing the effect of antimicrobial therapy on in vivo grown biofilms in situ.

SESSION 5: Healthcare Related Biofilms

In vitro and in vivo testing of antimicrobial central venous catheters

Presenter: Garth James[1], CBE Medical Biofilm Laboratory Manager
Co-authors: Ryder MA[2], Gunther RA[3], Breznock EM[4], deLancey Pulcini E[1], and Bickle L[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT
[2]Ryder Science, San Marcos, CA
[3]University of California Davis, Davis, CA
[4]BioSurg, Inc., Winters, CA
Sponsored by: Teleflex Medical, Inc.

Catheter-related blood stream infections (CRBSI) are costly in terms of health care spending and, more important, patient morbidity and mortality. One approach to preventing these infections is the use of antimicrobial catheters. We evaluated a chlorhexidine-coated peripherally inserted central venous catheter (PICC) in comparison to a non-coated PICC for preventing intraluminal colonization by *Staphylococcus aureus* using in vitro tests and an ovine model of CRBSI. The in vitro tests were used to determine the effect of inoculum density on initial bacterial attachment and to evaluate microscopy techniques. In these in vitro tests, the antimicrobial catheters (AC) had less bacterial colonization (CFU/cm^2) than the control catheters (CC). Subsequent in vivo experiments involved eight sheep, which were randomized to CC or AC groups. Each sheep received one catheter inserted into the jugular vein. Post-operatively catheters were locked with a 10^6 *Staphylococcus aureus* inoculum for three hours and the inoculum was then removed. Catheters were infused daily with Lactated Ringer's solution over 8 hours for 7 days and removed on Day 8. One control sheep was removed from the study due to a persistently high fever (>105°F, 2days). Clinical assessments indicated higher temperatures, higher white blood cell counts, and bacterial counts from blood cultures for the AC group relative to the CC group. Blood cultures were collected through the catheters and were only positive for the AC group in the first sample after catheter inoculation. At the end of the study, AC had significantly less bacterial colonization than CC at the tip and middle of the catheter. Colonization of the catheter hub and extension tubing was also lower for the AC relative to the CC. In most cases bacteria on the AC were below detection limits, while CC had populations as high as 6.19 log(10) CFU/cm^2. Scanning electron microscopy confirmed that there was less bacterial colonization on AC compared to CC samples. Overall, these results indicate that the AC prevented intraluminal colonization and infection by *Staphylococcus aureus* in an ovine model of CRBSI.

Bacterial inter-species interactions in multispecies biofilm communities

Presenter: Alex Rickard, Assistant Professor of Biological Sciences
Affiliation: Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI

Biofilms are typically complex communities that can contain hundreds of bacterial species. The component species are not solitary units; rather, they are able to interact with one another. Importantly, inter-species interactions can promote biofilm development and enable species to maintain a niche in the community. Two types of inter-species cell-cell interactions are associated with biofilm development: physical interactions and chemical interactions. Chemical interactions, through metabolic or cell-cell signaling, have received much attention, but studies of physical interactions are still in their relative infancy. It is the aim of this talk to describe the role of one type of physical interaction, often referred to as coaggregation. Coaggregation is distinct from other forms of aggregative interactions as it mediates inter-species adhesion between specific species of bacteria. As such, coaggregation can promote biofilm integration and intimately juxtapose specific biofilm species. The impact of coaggregation interactions in freshwater biofilms, human oral dental plaque, and in human chronic wound biofilms will be discussed in this presentation, and

attention will be focused on differences and similarities between mechanisms mediating coaggregation. For example, we have found that coaggregation can be growth phase dependent, is able to mediate competitive interactions in biofilms, can only be detected by testing with specific buffers, and—depending upon the environment being studied—be blocked by the addition of certain sugars. Thus, strategies to detect, characterize, and inhibit coaggregation will be described. We propose that coaggregation is an ecologically relevant mechanism to promote multispecies biofilm development and that this has medically relevant and environmentally germane ramifications.

Urinary conditioning film components—A new target for preventing bacterial biofilm formation on urinary devices

Presenter: Dirk Lange, The Stone Centre at Vancouver General Hospital,
Affiliation: University of British Columbia, Vancouver, Canada

The urinary tract presents a unique environment for pathogens to interact with the surfaces of indwelling ureteral devices, facilitating bacterial attachment and subsequent biofilm formation. Infection and encrustation of devices such as catheters and stents related to biofilm formation are common problems in urology, limiting their long-term use. The formation of a conditioning film on the surface of urinary devices by the deposition of urinary components has been proposed to facilitate bacterial biofilm formation directly by providing adhesion points for bacteria, but also indirectly by rendering biomaterial coatings and drug eluting mechanisms designed to prevent bacterial colonization ineffective. Our work with a triclosan-eluting stent has shown to significantly decrease bacterial adhesion to stents by various uropathogens *in vitro*, and to decrease the number of UTIs and the device-associated bacterial load in a rabbit model of cystitis caused by *Proteus mirabilis*. In clinical trials using long-term stented patients, however, the triclosan-eluting stents did not show a clinical benefit in terms of urine and stent cultures, which may be attributed to the deposition of urinary components and encrustation preventing effective triclosan elution over time. Therefore designing biomaterials that prevent the deposition of urinary components to the stent surface warrants further investigation. Current studies in our laboratory are aimed at identifying urinary conditioning film components that deposit on the surfaces of stents, specifically determining whether they differ between stent biomaterials and patients. This is a new angle at preventing bacterial biofilm formation on ureteral devices, as it targets a mechanism that not only promotes bacterial biofilm formation, but also renders any promising biomaterial design inactive. Preventing its deposition will not only limit specific bacterial adhesion points but will render drug eluting technology more effective at killing bacteria before they have a chance to interact with the device surface.

This talk is aimed at giving an overview of our work on stent biomaterial design—specifically promising coatings and drug eluting technology—and to provide new opportunities aimed at preventing bacterial colonization of urinary devices by discussing our most recent work studying conditioning film deposition as a nidus for bacterial adhesion and biofilm formation.

SaeR/S-mediated regulation of *hla* promotes USA300 pathogenesis

Presenter: Tyler Nygaard, Postdoctoral Researcher
Co-Author: Jovanka Voyich-Kane, Assistant Professor
Affiliation: Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT

The SaeR/S two-component sensory system of *Staphyloccocus aureus* regulates the expression of numerous virulence genes important for pathogenesis. Our laboratory has confirmed the transcriptional regulation of alpha-toxin (Hla) by the SaeR/S two-component system and examined role of this virulence factor during USA300 pathogenesis. Strong saeR/S-mediated transcription of hla corresponded to significantly larger

and more severe soft-tissue infections in mice. Survival of an USA300 isogenic deletion mutant of hla (USA300Δhla) in human blood was comparable to that of the parental wild-type strain and saeR/S-mediated cell membrane damage on polymorphonuclear leukocytes did not require hla. However, cytokine transcript levels were diminished during infection of human blood with USA300 relative to USA300Δhla. Flow cytometry analysis revealed USA300 expressing hla generated T cell plasma membrane damage in a concentration-dependent manner. Unexpectedly, rapid hla-mediated T cell proliferation was observed following exposure to USA300 supernatants. These findings demonstrate saeR/S-mediated transcription of hla compromises human T cell plasma membrane integrity and promotes rapid human T cell expansion.

Poster Abstracts

Center for Biofilm Engineering posters

CBE Poster #504

Date: 08/2009
Title: **Analysis of methane producing communities within underground coal beds**
Authors: ***Elliot Barnhart***[1], Wheaton J, Cunningham A, and Fields M
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
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 #521

Date: 07/2010
Title: ***In situ* microbial reduction of selenium as source control in phosphate mine waste**
Authors: ***Lisa Bithell Kirk***[1], Peyton B[1], Childers S[2], and Gerlach R[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Dept. of Geological Sciences, University of Idaho, Moscow ID
Sponsored by: Inland Northwest Research Alliance, EPA Science to Achieve Results, Montana Water Center, and Idaho Mining Association

This study of subsurface microbial ecology investigates selenate reduction by indigenous micro-organisms, using naturally available carbon in backfilled phosphate mine waste at sites in southeast Idaho, with an ultimate goal of defining how backfilled mine pits can be ecologically engineered to reduce toxic and mobile selenate to insoluble and non-toxic elemental selenium. Several *Dechloromonas*-like, indigenous facultative ß-proteobacteria rapidly reduce selenate within a consortium of cold-tolerant hydrocarbon-degrading microbes. Temperature, lithology, and oxygen availability influence extent and rate of selenate reduction. More selenate-reducing organisms live in anaerobic shale than chert or mudstone, and almost no selenate reduction occurs when oxygen is present. Microbial reduction is distinguished from abiotic processes by evidence of biotic stable isotope fractionation and comparison with killed controls. Operational waste management strategies that promote Se(VI)-reduction by indigenous organisms using native carbon offer a sustainable, design-based approach to natural attenuation of selenium in mined rock.

CBE Poster #522

Date: 08/2009
Title: **Detection of uranium oxidation and solubility using NMR**
Authors: ***Sarah J. Vogt***, Seymour JD, Stewart BD, Peyton BM, and Codd SL
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US DOE, NSF, and The Murdock Trust

The conversion of soluble uranyl ions (UO_2^{2+}) by bacterial reduction to insoluble uraninite (UO_2) is being studied as a way of immobilizing spent uranium waste [1-3]. Under anaerobic conditions, several known iron-reducing types of bacteria have been shown to also use the uranyl ion as an electron acceptor. Preliminary tests using a suspension of uraninite (UO_2) particles produced by *Shewanella putrefaciens* CN-32 bacteria show a dependence of the T_1 and T_2 on the oxidation state and solubility of the uranium. Gradient echo and spin echo images were compared to quantify the T_2* effect caused by the magnetic field fluctuations of the uraninite particles and soluble uranyl ions. Since the precipitate studied is suspended in liquid water, the effects of concentration and particle aggregation are also being explored. A suspension of uranium particles was injected into a polysaccharide gel, which simulates the precipitation of uraninite in the extracellular biofilm matrix. A reduction in the T_2 of the gel surrounding the particles was seen [4]. Therefore it may be possible to detect the presence of uraninite precipitate within a biofilm during the bacterial reduction reaction.

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CBE Poster #523

Date: 04/2010
Title: **Magnetic resonance analysis of physically crosslinked biopolymer gels**
Authors: ***Hilary T. Fabich***[1], Maneval JE[2], Bernin D[3], Seymour JD[1], and Codd SL[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
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Sponsored by: NIH, INBRE

Using the non-invasive properties of nuclear magnetic resonance (NMR), the mobility of water in a biopolymer gel can be examined by measuring diffusion and magnetic relaxation. Understanding the molecular role of water in physical gelation and water distribution on gel material properties has great potential to increase understanding of biological function [1]. Alginate and the impact of a divalent cation on gelation has been extensively studied using NMR [2,3]. During formation of the gel under certain conditions, small capillaries are formed inside the gelled structure [4]. These may provide molecular transport pathways through the entangled biopolymer network and can control how water and ions are transported through the gel. Another point of interest in this system stems from the role of acetylated and deacetylated alginates in biofilm formation [1]; NMR data on the relaxation and diffusion of water in model alginates provide baseline data for exploring the role of water in biofilm formation.

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CBE Poster #524

Date: 07/2010
Title: **Colocalization of syntrophs in a methanogenic biofilm**
Authors: ***Kristen A. Brileya***[1], Hatzenpichler R[2], Arkin AP[3], Hazen TC, and Fields MW[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2] Universitat Wien, Vienna, Austria;
[3]Lawrence Berkeley National Lab, Berkley, CA
Sponsored by: NIH, INBRE

Transfer of reduced carbon and electrons between microbial community members is of interest in methanogenic systems that represent natural mediators of atmospheric carbon flux. The current work uses a dual-culture approach to examine the structure of syntrophic biofilm formed by the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough and the methanogenic archaeon *Methanococcus maripaludi*s. We hypothesized that biofilm structure would reflect the energetic benefits of living in close association; the aim of the study is to visualize intact 3-dimensional biofilm structure to make testable predictions of structure-function relationships. Biofilm was grown in a continuously stirred biofilm reactor where cells could attach to a silica surface or remain suspended. Intact biofilm was fixed for fluorescence in situ hybridization (FISH) and embedded in agarose to maintain 3-dimensional structure. FISH revealed a framework of *D. vulgaris* with both single cells and large micro-colonies of *M. maripaludis* interspersed within the biofilm. FISH also confirmed steady-state biofilm irregularity, with ridge, valley, and spire macro-architecture. SybrGreen counterstaining confirmed the presence of extracellular material. Colorimetric assays indicated cell-associated carbohydrate was composed of .035 μg hexose/μg protein, .017 μg pentose/μg protein and .011 μg uronic acid/μg protein, similar to *D. vulgaris* mono-culture biofilm and approximately 5 times less than *M. maripaludis* biofilm. 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 driven by *D. vulgaris* while providing an advantageous situation for *M. maripaludis* to establish presumably active micro-colonies throughout the *D. vulgaris* scaffold.

CBE Poster #526

Date: 07/2010
Title: **Comparing the disinfection of planktonic cells, biofilms, and detached biofilm particles in single species and dual species cultures**
Authors: ***Sabrina Behnke*** and Camper AK
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Unilever U.K. Central Resources Limited

Purpose: The goal of this study is the evaluation of the disinfection susceptibility of detached cells and cell clusters of *Burkholderia cepacia* and *Pseudomonas aeruginosa* in comparison to planktonic cultures and attached biofilms grown as either single species or in co-culture. This comparison allows for the detection

of differences in disinfection susceptibilities between the two species and also shows how the two species influence each other when grown together.

Methods: For the purpose of testing and comparing biocide susceptibilities of planktonic cells, cells in biofilms and detached cell aggregates, we designed experiments as follows: *B. cepacia* and *P. aeruginosa* (either as single species or in co-culture) were grown in standardized laboratory reactors for enhanced repeatability. Planktonic cultures were grown in a continuously stirred chemostat, while biofilm was obtained from a tube reactor. Detached aggregates were sampled from the outflow of the tube reactor.

Chlorine disinfection: As anticipated, chlorine disinfection data suggests that planktonic and detached cells and particles are more susceptible than biofilm cells, which require about 10x more hypochlorite for complete inactivation of the culture. *B. cepacia* chemostat cells are more susceptible to chlorine than the *B. cepacia* detached cells and clusters, but *P. aeruginosa* chemostat cells are less susceptible to chlorine than *P. aeruginosa* detached cells and particles. Interestingly, when *B. cepacia* and *P. aeruginosa* are grown together in co-culture, they have very similar disinfection tolerances with the chemostat cells being more susceptible than the detached cells and clusters. When *B. cepacia* is grown in a dual species biofilm with *P. aeruginosa*, the biofilm is more tolerant to disinfection than the single-species biofilms which results in a significant advantage for both species. There is no advantage in co-culture with regard to the chemostat and the detached cells and particles.

Ozone disinfection: Ozone disinfection to date has been done with only single species *B. cepacia*. The difference between susceptibilities of chemostat cells and detached cells and clusters are similar to chlorine disinfected *B. cepacia* samples although much lower concentrations of ozone are required since O_3 is a very strong oxidizing agent. The biofilm however could not be completely inactivated with the concentrations we were able to achieve with the ozone generator.

CBE Poster #527

Date: 07/2010

Title: **Microbial growth and diversity in a humic-free environment on the Cotton Glacier, Antarctica**

Authors: ***Heidi Smith***[1], ***Christine Foreman***[1], Sattler B[2], Chin Y-P[3], and McKnight D[4]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]University of Innsbruck, Austria;
[3]The Ohio State University;
[4]University of Colorado–Boulder

Sponsored by: NSF OPP-0838970

A supraglacial stream forms annually on the Cotton Glacier, Antarctica. Analysis of dissolved organic matter (DOM) from this stream in 2004–05 and again in 2009–10 showed that the concentration was low (44–48 μM C), and lacked humic signatures, unlike typical DOM of microbially based ecosystems. Our results indicate that DOM in this system is seasonally formed from soluble microbial products and that a reservoir of recalcitrant humified DOM does not pre-exist. In most aquatic ecosystems, humic DOM acts as a natural sunscreen and the absence of humics may thus represent an additional stressor influencing the microbial community. Nonetheless, the stream contained an active microbial assemblage with bacterial cell abundances from 2.94×10^4–4.97×10^5 cells ml^{-1}, and bacterial production ranging from 58.8–293.2 ng C l^{-1} d^{-1}. Chlorophyll-a concentrations ranged from 0.3 to 0.53 μg l^{-1}, indicating that algal phototrophs were the probable source of the DOM. Microbial isolates produced a rainbow of pigment colors, suggesting adaptation to UV stress, and were similar to those from other cryogenic systems (Cytophagales and β-Proteobacteria lineages). Clone library analysis of the microbial assemblages from the stream water, ice, sediments and aeolian communities were significantly different, but still related to organisms from other

cold temperature environments. Taken together, these results suggest that the occurrence of related phylotypes from diverse glacial environs is due to similar survival strategies and that UV stress due to the absence of humics is important in supraglacial streams. Supraglacial streams provide an example of contemporary microbial processes on the glacier surface and a natural laboratory for studying the microbial adaptation to the absence of humics, as well as chemical processes controlling the eventual genesis of humic DOM.

CBE Poster #532

Date: 01/2011
Title: **Imaging biofilm and microbially induced $CaCO_3$ precipitation in 2D porous media reactors**
Authors: ***James Connolly***, Phillips A, Bugni S, and Gerlach R
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: National Science Foundation and the United States Department of Energy

Biological processes in the subsurface environment are important to understand in relation to many engineering fields 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 to study these processes, but supplementing microscopic imaging provides additional information and validation to these data sets.

Confocal scanning laser microscopy (CSLM), field emission scanning electron microscopy (FEM), and visible light stereoscopy were used to study processes in two dimensional reactors with regular etched pore structures. Two kinds of reactors were used. The first (Fig. 1A and Fig. 2A, B) has uniform 1.0mm square pore structures and is designed for direct observation under a stereoscope or destructive sampling

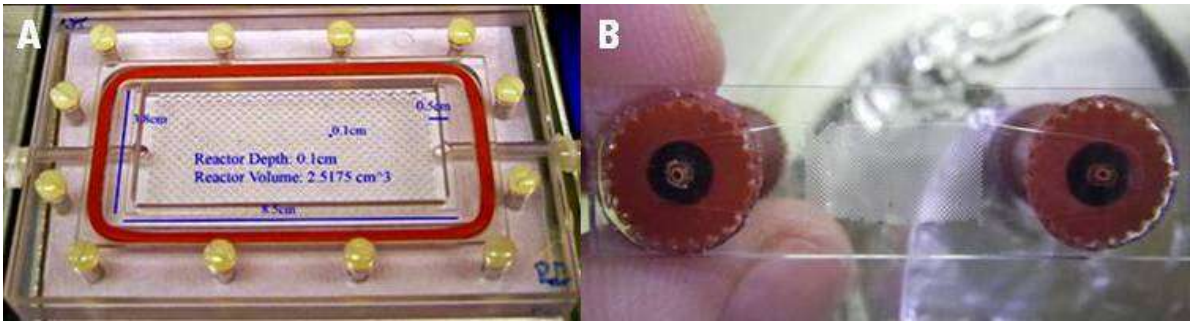

Figure 1 - (A) 2D porous media reactor with 1mm pore structures. (B) 2D porous media reactor with 100µm pore structures designed for CSLM imaging.

and imaging with other techniques. The second reactor (Fig. 1B and Fig. 2C, D) has 100µm pore structures and is specifically designed for CSLM imaging. Samples imaged under CSLM are generally prepared by staining the biofilm with various fluorescent stains. However, since staining may cause deleterious changes to metabolic processes, organisms with fluorescent protein are also imaged with CSLM so as to study basic biofilm functions. Finally, CSLM and FEM imaging are used in conjunction to obtain the most complete sets of images and data from a sample.

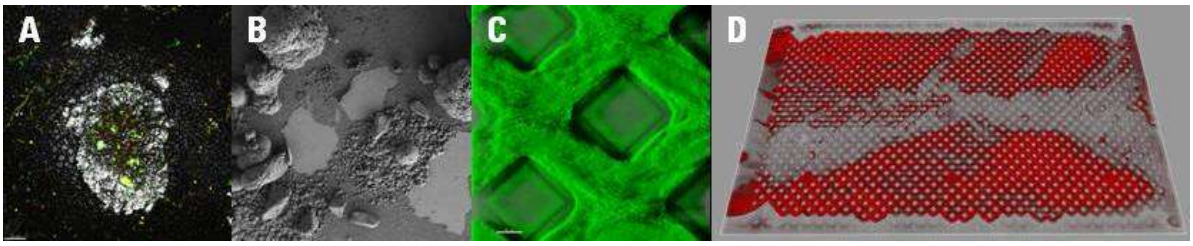

Figure 2 – (A) CSLM image of a microbially induced $CaCO_3$ crystal (gray) with cells attached (green and red). (B) FEM micrograph of microbially induced $CaCO_3$ precipitation. (C) Living bacterial biofilm grown in a porous media reactor with 100µm pore structures. The bacterial strain is expressing green fluorescent protein, so no staining was required. (D) 3D CSLM reconstruction of a stained bacterial biofilm grown in the reactor pictured in Fig 1B.

CBE Poster #533

Date: 01/2011
Title: **Using synthetic biology to engineer microbial consortia based on syntrophic metabolite exchange**
Authors: ***Hans C. Bernstein***, Paulson SD, Carlson RP
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: National Institutes of Health

A combination of synthetic biology and metabolic engineering was used to construct artificial microbial consortia comprised of engineered *Escherichia coli* strains. The design was based on biomimicry of key ecological roles found in stable, naturally occurring microbial consortia. The constructed consortia were demonstrated to partition resources based on design and were then studied under batch, chemostat, and biofilm growth conditions. The consortium culturing strategy enabled an increased biomass yield, as compared to traditional mono-culturing, for all three experimental systems. The artificial community metabolic interactions dampened chemostat oscillations associated with the production of inhibitory compounds like acetate, highlighting ecological and bioprocess implications of consortia interactions. The engineered community, when cultured as a biofilm, self-assembled into micron-scale spatial regions highlighting a new tool for engineering multi-reaction bioprocess systems.

CBE Poster #534

Date: 01/2011
Title: **Impact of biofilm structure on biofilm-fluid interactions**
Authors: ***Bryan Vadheim***, Heys JJ
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Vice President for Research, Creativity and Technology Transfer

More than eighty percent of human infections involve biofilms—aggregates of microorganisms that form a structure in a moist environment by adhering to both a surface and each other. As a result, the impact of biofilms on human health is enormous. Biofilm colonies present a serious challenge to medicine due to the dense extracellular polymeric substance that surrounds a living colony. Computer modeling of biofilms represents a powerful resource that has yet to be fully tapped. Though the concept is not new, the computing power required to achieve a sufficient level of accuracy in dealing with a complex physical interaction between a fluid and a biofilm structure is only relatively recent.

The immersed boundary method was first used in 1977 to model blood flow in a compliant heart ventricle[1]. Recently, it has been utilized for the modeling of biofilm-fluid interactions based on the idea of

representing a collection of particles connected by springs, which, in some cases, can break if stretched beyond a specified distance, resulting in detachment from the biofilm[2]. Immersed boundary models of biofilm were recently experimentally validated[3].

The computational algorithm employed here is based on the C++ programming language, and it examines how changing select biofilm properties affects the overall structure and detachment of the microbe colony. In the model, colonies of organisms are attached to one another by springs. Varying the spring constant (i.e., biofilm stiffness), biofilm geometry, and extracellular matrix density all affect the response of the biofilm subjected to a moving fluid. These variables were chosen because they can be difficult to vary in experiments, but they are easy to vary in a numerical model. The model is used to predict the impact of the variables on the magnitude of biofilm displacement (i.e., maximum strain) and detachment of the biofilm from the surface. The overall objective of this research is to improve understanding of fluid-biofilm interactions.

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CBE Poster #535

Date: 02 / 2011
Title: **Time-lapse imaging at the Center for Biofilm Engineering**
Author: ***Betsey Pitts***
Affiliation: Center for Biofilm Engineering, Montana State University Bozeman MT

Much of the optical microscopy at the Center for Biofilm Engineering is performed on live, fully hydrated biofilm samples. In the ideal live-cell imaging situation, the imaging process has as little impact on the biofilm as possible, so that time-lapse imaging can be a means of watching biofilm bacteria interact with each other and the environment. 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 time-lapse microscopy. The CBE's earliest transmitted light movies showed streamer development and surface migration, and allowed for investigation of biofilm rheology and detachment processes. With the development of fluorescent proteins and fluorescent probes for bacterial activity in the last ten years, the breadth of application of time-lapse microscopy has increased dramatically. Some of the processes that time-lapse is used to capture include: biofilm accumulation and development beginning with initial attachment of cells; detachment of clusters in response to treatment; pathogen capture by existing biofilms; diffusion of antimicrobials into a biofilm; the impact of an antimicrobial on a single cell; motility of biofilm cells; the viscoelasticity of biofilm clusters and how they can be chemically altered; and the impact of biofilm as compared to planktonic bacteria on human tissue culture. This poster summarizes some recent highlights of the application time-lapse microscopy to biofilm science at the CBE.

CBE Poster #537

Date: 02/2011
Title: **SSU rDNA gene sequence region and quality-checking are essential for species richness and diversity estimates via pyrosequencing**
Authors: ***Kara B De León*** and Fields MW
Affiliation: Center for Biofilm Engineering, Thermal Biology Institute, and Department of Microbiology, Montana State University, Bozeman, MT

Due to errors during sequencing, pyrosequencing can overestimate the diversity of a system. The traditional sequence refinement method of removing sequences that contain ambiguous nucleotides, primer errors, and sequences that are less than one standard deviation from the mean length is not sufficient to account for this overestimation. Recent in silico and single organism studies have revealed the importance of SSU rDNA region selection and sequence quality score cutoffs in the estimation of diversity, respectively. This is the first study to validate these findings with an *in situ* environmental sample via the comparison of species richness and diversity estimates to a corresponding clone library. A clone library (1,113 sequences) and pyrosequencing library (18,628 sequences) were generated for two regions of the SSU rDNA, one that slightly overestimates (V4) and one that underestimates (V6) the diversity of a sample. Sequence refinement included the traditional refinement method as mentioned above and all sequences were trimmed to the mean length and checked for chimeric sequences. Additionally, the pyrosequences were subjected to varying quality score cutoffs ranging from 20 to 32, corresponding to an error probability rate of 0.063% to 1%. At each quality score cutoff either 10% or 15% of the nucleotides were allowed to be below the cutoff, the minimum and maximum allowable as suggested by Pyrotagger, an online program for sequence refinement (hereafter designated as a subscript of the quality score)(Kunin and Hugenholtz, 2010). The additional refinement resulted in 30.1–95.1% of total sequences removed. Sequences were clustered at 97% and rarefaction data and Chao1 diversity estimates were generated to compare the species richness and diversity at each quality score cutoff to the clone library data. For both the V4 and V6 SSU rDNA regions, a quality score cutoff of less than 25 resulted in an overestimation of species richness and diversity. The most stringent quality cutoff of $32_{10\%}$ for the V4 region was comparable to the clone data for species richness and diversity. A quality cutoff of $27_{10\%}$ for the V6 region corresponded best to the species richness and diversity estimates for the clone library data. The species richness and diversity estimates were underestimated for the V6 region when quality score cutoffs of $30_{10\%}$ and $32_{10\%}$ were used. These results indicate that pyrosequencing data must be thoroughly filtered and that a quality score cutoff is not universal across the SSU rDNA gene, likely due to differing proportions of conserved and variable regions. Using an environmental sample, our results further stress the importance of quality-checking pyrosequencing data in a region-dependent manner for the estimation of species richness and diversity.

CBE Poster #538

Date: 02/2011
Title: **The extracellular metabolome of *Staphylococcus aureus* biofilms assessed using NMR- and MS-based metabolite profiling**
Authors: ***Pat Secor[1/3], Laura Jennings[2],*** *Bothner B[3], Hilmer J[3], Copié V[2], James G[1], and Stewart P[1]*
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]Metabolomics Facility and [3]Mass Spectrometry Facility, Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT
Sponsored by: NIH NCRR CoBRE and the National Institute for General Medical Sciences

The formation of biofilm in diseased skin (i.e., atopic dermatitis) and in cutaneous infections such as non-healing ulcers and surgical wounds is a major barrier to healing. *Staphylococcus aureus* is an important

human pathogen and among the most common bacterial species associated with cutaneous infection. Knowledge of the *S. aureus* biofilm extracellular metabolome may provide insight into mechanisms of pathogenesis and the persistence of infection in chronic wounds. Nuclear magnetic resonance (NMR) and high performance liquid chromatography/mass spectrometry (HPLC/MS) were used to measure the extracellular metabolome of *S. aureus* biofilms compared to planktonic cells *in vitro*. Metabolomics is an emerging field that enables the quantification of small molecule metabolites (<1000 Da) in biological systems. Metabolite profiling analysis revealed metabolites unique to biofilms. Results indicated that glucose and amino acids were selectively consumed by *S. aureus* biofilms, while mixed-acid fermentation products (lactate, acetate, and formate) were produced. The results agree with previously published findings from proteomics and transcriptomics that suggest that anaerobic or microaerobic metabolism is important to the *S. aureus* biofilm phenotype. The presence of the virulence regulators, aureusimines A and B, were detected at higher levels in biofilms compared to planktonic cells. Aureusimines are recently described regulators of virulence that were found to be required for infection in mice. In this study, aureusimine A and B were found to be produced at higher levels under low oxygen, suggesting a link between the anoxic core within *S. aureus* biofilms and aureusimine production.

CBE Poster #539

Date: 02/2011

Title: **Differential effects of planktonic and biofilm MRSA on human fibroblasts**

Authors: ***Kelly R. Kirker***[1], James GA[1], Fleckman P[2], Olerud JE[2], and Stewart PS[1]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Division of Dermatology, University of Washington, Seattle, WA

Sponsored by: National Institute of General Medical Sciences (NIGMS), Grant 1P20GM078445

Despite the prevalence of biofilms in wounds, the role of biofilms in chronic wound pathogenesis remains ambiguous. It was recently demonstrated that planktonic *Staphylococcus aureus* and *S. aureus* biofilms have different effects on human keratinocytes (HK). Soluble products from *S. aureus* biofilms induced dramatic HK morphological changes, reduced HK viability, and increased HK apoptosis compared to soluble products from planktonic *S. aureus*; however, chronic wounds are not epithelialized tissues. The biofilm is most likely to colonize dermal tissue, and it may be more appropriate to investigate the effects of bacterial biofilms on the dermal fibroblast. Thus, in this investigation, the effects of a predominant wound pathogen, methicillin-resistant *S. aureus* (MRSA), on normal human, dermal fibroblasts (HF) were examined.

The soluble products from both planktonic and biofilm MRSA had deleterious, and similar, effects with respect to HF migration, viability, and apoptosis. Differences between the planktonic and biofilm groups were not evident until the presence of HF derived growth factors, cytokines, and proteases in the media were examined. Soluble products from planktonic MRSA, relative to biofilm MRSA, induced increased levels of interleukin-6 (IL-6), IL-8, vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TFG-β1), heparin-bound epidermal growth factor (HB-EGF), matrix metalloproteinase-1 (MMP-1), MMP-3, and MMP-7. Only tumor necrosis factor-α (TNF- α) was elevated in HF exposed soluble products from MRSA biofilm compared to planktonic MRSA.

The effect of bacteria on human cells has predominantly been investigated using planktonic bacteria; however, bacteria colonizing chronic wounds generally exist as biofilm, rather than planktonic communities. Recently, we reported the differential effects of planktonic and biofilm MRSA on HK. The results presented herein demonstrate there are also differential effects on HF. Overall, the results illustrate that to fully characterize how bacteria participate in chronic wound pathogenesis, bacterial biofilms should be investigated.

CBE Poster #540

Date: 02/2011
Title: **Lectin staining of a human wound specimen**
Authors: ***Margaret Elm Campbell***, James GA, Marçal Agostinho A, and deLancey Pulcini E
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: NIH (NIGMS) 1P20GM078445

When examining wounds or other tissue, we would like to be able to distinguish the bacterial extracellular polymeric substance (EPS) from the host tissue, including host extracellular matrix (EM). This is difficult because both matrices have a polysaccharide backbone and it is likely that the bacterial EPS will incorporate or be closely associated with host EM components (or visa-versa). Lectins are carbohydrate-binding proteins that are highly specific for certain sugars and are commonly used for blood typing and are also components of commercially available microbiological stains (e.g., ViaGram™). Lectins have also been used to characterize the EPS of environmental biofilms. We evaluated a variety of fluorescent lectins for staining a chronic wound specimen. This specimen had been previously characterized as harboring a multispecies biofilm, based on epifluorescence microscopy after staining with ViaGram™ and results from16S sequencing. We evaluated 16 different fluorescent lectins in combination with the DNA stains Sytox™ green or propidum iodide. None of the lectins provided clear discrimination of EPS from EM. However, some of the commonly used lectins (e.g., concanavalin A and Wheat Germ Agglutinin) and other lectins (e.g., Potato and Jacalin) provided an effective counter-stain to the DNA stains and enabled visualization of fine structure in the wound specimen.

CBE Poster #541

Date: 02/2011
Title: **Siderophore production by haloalkaliphilic bacteria under varied physiologic conditions**
Authors: ***Luis O. Serrano Figueroa***,[1,2] and Richards A[2,3]
Affiliation: [1]Department of Microbiology, [2]Center for Biofilm Engineering, [3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT
Sponsored by: NIH (NIGMS) 1P20GM078445

Siderophores are small organic compounds, produced by many microorganisms under iron stress, which act as iron chelators. In this project, we investigated siderophore production by two isolates (SL01 and SL28) from Soap Lake, an alkaline lake in Washington State. Soap Lake media (SLM) was prepared at different NaCl concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 % w/v) and cultures were incubated at either 37° C or room temperature (RT). Siderophore quantification was achieved by the Chrome Azurol Sulfonate assay (CAS). Growth curves were obtained by measuring optical densities at 600 nm. Extraction of siderophores from the media supernatant was accomplished using a Varian Solid Phase C2 cartridge. Purification of siderophores was done by HPLC, and collected fractions were lyophilized to obtain crystals for future structure characterization, for SL01, with mass spectrophotometry. The HPLC system was composed of a C-4 column, Dionex AD20 Absorbance Detector, and acetonitrile (10-70 %) and nano-pure water as mobile phases. The optimum siderophore concentration of SL01 was 16.3 µM and occurred at 10.0 % w/v NaCl and 37° C. However, SL28 optimum siderophore concentration of 12.7 µM occurred at 2.5 % w/v NaCl and RT. As concluding remarks, siderophores were quantified for both strains and purified by HPLC. Structural identification via mass spectrometry is underway.

CBE Poster #542

Date: 02/2011
Title: **Evaluation of bulk soap dispenser washing procedures**
Authors: ***Lindsey Lorenz[1]***, Goeres D[1], and Zapka C[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]GOJO Industries, Inc. Akron, OH
Sponsored by: GOJO Industries, Inc.

A field study of soap dispensers demonstrated that up to 25% of open refillable bulk hand soap dispensers are contaminated with approximately 6 LOG_{10}(CFU/mL) heterotrophic bacteria based upon samples collected from the bulk soap[1]. In 2009 the CBE completed a research project to determine if biofilm growth within the dispensers contributed to bulk soap contamination. Plastic counter-mounted, plastic wall-mounted, and stainless steel wall-mounted dispensers collected from various locations in Ohio were analyzed for suspended and biofilm bacteria using heterotrophic and coliform viable plate counts and total cell counts. Bacterial identifications from the plate counts were performed using biochemical profiling of isolated colonies. Results indicated that the bulk soap was contaminated with 4–7 LOG_{10}(CFU/mL) bacteria and 4–7 LOG_{10}(CFU/cm^2) biofilm bacteria from the inside of the dispensers (n=6), independent of dispenser type or construction material. Overall, the biochemical profiling identified 14 unique bacterial species, and 11 different genera, from all the dispensers tested. Bacterial populations were also identified using 16s SSU rRNA gene sequencing for the plastic and stainless steel wall-mounted dispensers to confirm organism identifications. No significant differences in bacterial genera were observed. All microorganisms identified are considered opportunistic pathogens.

The goal of the current project was to determine how the presence of biofilm impacts the ability to clean and sanitize the dispensers. Two dispenser washing experiments were performed.

Three washing procedures were evaluated for plastic wall-mounted dispensers:

1) a simple rinsing technique,
2) a rinse and scrubbing technique, and
3) a rinse, scrub, 5,000 mg/L bleach treatment, rinse combination.

Three additional washing procedures were evaluated for stainless steel wall-mounted dispensers:

4) a rinse, scrub, 5,000 mg/L bleach treatment with 10 minute soak, rinse combination,
5) a rinse, scrub, 8 mL/L Quat treatment with 10 minute soak, rinse combination, and
6) a rinse, scrub, full strength mildew remover treatment with 10 minute soak, rinse combination.

The washing study results showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks of cleaning a dispenser then treating it with any of the methods tested.

These studies showed that dispensers contaminated with bacteria in the bulk soap also had high levels of biofilm bacteria that would be available to re-contaminate a dispenser, even if the old soap is emptied and the dispenser washed and treated with bleach/quat/mildew remover before new soap is added.

[1]Gerba CP, and Maxwell SL, "Bacterial contamination of liquid hand soaps used in public restrooms," Poster Presentation at NEHA 71st Annual Educational Conference & Exhibition, Atlantic City, NJ, 2007.

CBE Poster #393

Date: 07/2007
Title: **Cost-benefit analysis of microbial resource allocation: Implications for intracellular pathogens**
Authors: ***Ross P. Carlson,*** Taffs RL, Folsom J
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: National Institutes of Health

Background and Objective: Persisting intracellular pathogens must adapt their metabolic functioning to cope with harsh environments, including low pH and high oxidative stress, while utilizing limited resource pools. Robust metabolic networks possess a wide range of options, which complicates prediction of pathogen stress response. This study uses systems biology to predict and interpret competitive pathogen resource allocation strategies that are likely necessary for intracellular colonization.

Methods: A cost-benefit analysis of an in silico *Escherichia coli* network model was performed using ecologically relevant resource allocation strategies. The strategies were identified by decomposing the metabolism into mathematically defined biochemical pathways (elementary flux modes) and assessing the resource investment cost-benefit properties for each pathway.

Results: The cost-benefit analysis revealed competitive molecular-level relationships between pathway enzyme investment, pathway efficiency, and enzyme functionality. The study identifies novel competitive network design principles which can be used to counter microbial strategies by accounting for the inherent trade-offs of investing finite resources like iron into different enzymes.

Discussion and Conclusions: The interpretation of bioinformatics data in terms of cellular function is a major challenge facing systems biology. The current study establishes a competitive relationship between resource allocation and metabolic fitness. This relationship is likely essential for pathogen adaptation to low nutrient environments like the phagosome. Understanding competitive strategies provides a rational basis for countering intracellular pathogens.

CBE Poster #544

Date: 02/2011
Title: **Medium pH and nitrate concentration effects on triacylglycerol in two Chlorophyta**
Authors: ***Robert Gardner,*** Peters P, Peyton B, and Cooksey KE
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Air Force Office of Scientific Research, US Department of Energy (Office of Biomass Programs). Partial support for RG was provided by NSF IGERT Program in Geobiological Systems at Montana State University.

Identifying a so-called "lipid trigger"—an environmental circumstance or production of a signaling molecule that cued lipid synthesis/accumulation—was one of the main goals of the former DOE Aquatic Species Program in the 1980s. While no new definitive trigger was found (growth medium N-limitation was already known), there were indications that interference of the algal cell cycle by monofluoroacetic acid or elevated medium pH were possible candidates, albeit for a single species of alga, i.e., *Chlorella* Chlor-1. Here we present the results of studies on two more Chlorophytes: *Coelastrella sapiensis* PC-3 (similar to *Chlorella* Chlor-1,) and *Scenedesmus obliquus* WC-1 (dissimilar to Chlor-1), where we seek to answer whether the pH stress effect is specific to Chlor-1 or general for Chlorophytes. Time-courses of triacylglycerol (TAG) accumulation were measured together with N– as nitrate remaining in the growth medium, and medium pH. Medium buffered at specific pH values and unbuffered medium gave differential results. This poster

documents the timing of TAG accumulation during growth (monitored by Nile Red fluorescence and confirmed by gas chromatography) and separates the causal effects of this phenomenon, i.e., N-depletion in the growth medium versus pH changes therein. The organisms studied began to accumulate TAG before they became N-limited. This suggests a control point for TAG accumulation that is independent of N-depletion and thus provides a new potential focus for genetic manipulation designed to increase TAG accumulation.

CBE Poster #505

Date: Revised 02/2011

Title: **Utility of biofilms and biologically induced mineralization in geologic carbon sequestration**

Authors: ***Robin Gerlach[1]***, Mitchell AC[1], Spangler LH[2], and Cunningham AB[1]

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Geologic carbon sequestration involves the injection of CO_2 into underground formations including oil beds, deep un-minable coal seams, basaltic rocks, and deep saline aquifers, with temperature and pressure conditions such that CO_2 will often be in the supercritical state.

Four trapping mechanisms are proposed to play significant roles in the deep geologic sequestration of CO_2: formation trapping, capillary trapping, solubility trapping, and mineral trapping.

Our research has shown that, independent of the host rock, microbial biofilms are capable of enhancing formation trapping, solubility trapping, and mineral trapping.

i) We have demonstrated that engineered microbial biofilms are capable of reducing the permeability of rock cores at pressures and temperatures, which would be found in the presence of supercritical CO_2.

ii) The biofilms have been demonstrated to be resistant to supercritical CO_2.

iii) Biofilms precipitate CO_2 in the form of calcium carbonate ($CaCO_3$), which resists dissolution by brine and $scCO_2$.

iv) Microbial activity can increase CO_2 solubilization, thus improving solubility trapping.

Recent activities have begun to focus on practical aspects related to the implementation of biofilm-enhanced geologic carbon sequestration technologies in field situations.

Industry Poster

Date: 02/2011

Title: **Anti-biofilm compounds provide a new approach to treating cystic fibrosis**

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Agile Sciences' technology focuses on a family of organic compounds that have anti-biofilm properties. Biofilms impact a variety of industries and our molecules have a broad spectrum of activity against these biofilms. More specifically, our molecules exhibit anti-biofilm properties against *Pseudomonas aeruginosa*, which is the predominant cause of chronic airway infections in cystic fibrosis (CF). Bacterial biofilms are up to 1000 times more resistant to antibiotic treatment, and while treatment with antibiotics in CF patients may result in improvement, complete eradication of the infection is rare. Recent findings show that Agilyte not only has the ability to inhibit and disperse PA14, but is also active against a variety of clinical isolates, including both multi-drug and aminoglycoside resistant isolates.