CENTER FOR BIOFILM ENGINEERING

Montana Biofilm SCIENCE & TECHNOLOGY Meeting **Proceedings**

July 7–9, 2009

MONTANA STATE UNIVERSITY

Montana Biofilm Science & Technology Meeting: July 7–9, 2009

Table of Contents: Speaker Abstracts

Table of Contents: Poster Abstracts

Speaker Abstracts

SESSION 1: Phenotypic Heterogeneity

Biofilms, genetic diversity, and the insurance hypothesis

Presenter: Pradeep Singh, Associate Professor of Medicine and Microbiology
Co-authors: B.R. Boles[2] and P.K. Singh[1]
Affiliation: [1]Division of Pulmonary and Critical Care Medicine, University of Washington School of Medicine, Seattle, WA;
[2]University of Iowa College of Medicine, Iowa City, IA

A number of investigators have found that biofilm growth results in the genetic diversification of bacterial populations. In experiments using the opportunistic pathogen *Pseudomonas aeruginosa*, we found that many traits diversify after relatively short periods of biofilm growth. For example, biofilm-grown bacteria displayed much greater variance in swimming distance (caused both by increased and decreased swimming) than colonies that had not been grown in biofilms. We also found that biofilm growth induced diversity in bacterial pigment synthesis and surfactant production, and produced auxotrophs at a high rate. Furthermore, some of the biofilm-induced variants manifest specialized biofilm phenotypes. Some variants show accelerated biofilm formation, and others premature detachment. The presence of these functionally diverse bacteria could increase the ability of biofilms to resist environmental stress. A key question is whether the observed diversity is solely caused by the strong and varied selective pressures inherent to the biofilm growth mode, or whether biofilm growth also increases the propensity for mutation. Current data relating to this question will be discussed.

Distinct physiological cell-subpopulations and antimicrobial tolerance in biofilms

Presenter: Sünje J. Pamp, Postdoctoral Research Fellow, Microbiology and Immunity
Affiliation: Stanford School of Medicine, Stanford University Medical Center, Palo Alto, CA

Explaining the pervasive features of microbial biofilms, such as their persistence towards antimicrobial compounds, has been challenging for scientist. Of significance for a fundamental understanding is knowledge about the genetic determinants important for differentiation of biofilms, and about the inherent characteristics of the participating biofilm cells.

Our investigations on *Pseudomonas aeruginosa* biofilm anatomy and physiology reveal that these biofilms are composed of at least two distinct phenotypic cell-subpopulations. Through the use of confocal laser scanning microscopy (CLSM), flow-cell technology, single and mixed colour-coded biofilms (Gfp, Cfp, Yfp), and fluorescent reporters, we provide evidence that differential cellular metabolic activity, as well as type IV-pili, secreted biosurfactants, flagella, and chemotaxis are all involved in the differentiation of the two distinct phenotypic cell-subpopulations. Interestingly, one of the two cell-subpopulations exhibited increased sensitivity to membrane-targeting compounds, such as the antimicrobial peptide colistin, whereas the other cell-subpopulation was able to develop tolerance. Tolerance to colistin was found to depend on cellular metabolic activity, the *pmr*-LPS-modification system and *mexAB-oprM*-mediated antimicrobial efflux. Intriguingly, the colistin-tolerant subpopulation exhibited sensitivity to conventional antimicrobial agents, such as ciprofloxacin and tetracycline. By targeting the two physiologically distinct cell-subpopulations by a systematic combined antimicrobial treatment, nearly all biofilm cells could be eradicated.

Diverse phenotypes in biofilms: Implications for antimicrobial control

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

Multiple lines of evidence from the biofilm literature are consistent with the hypothesis that biofilms, even those of a single species, harbor subpopulations of differing physiological status and also differing antimicrobial susceptibility. This is apparent in biphasic survival versus time data in which an initial rapid drop in cell viability slows drastically with continued exposure to the agent. Microscopy of antimicrobial-treated biofilms stained for viability sometimes reveals obvious spatial segregation of killed and tolerant populations. Given the phenotypic diversification that occurs in biofilms, a useful strategy may be to use combinations of antimicrobial agents to target each of the distinct populations within the biofilm. This concept is illustrated with unpublished data and examples from the literature. For example, experiments with biofilms grown under different nutrient conditions reveal large differences in antibiotic susceptibility depending on the nutrient and oxygen status of the cell. Simple mathematical models for analyzing the behavior of non-uniform populations, particularly with regard to antimicrobial survival were derived and applied to develop insight. One of these models simulates a population of bacteria that contains a mix of active and dormant cells. Simulations suggest that the resuscitation process is a critical step controlling antimicrobial efficacy. Slow resuscitation from dormancy is a powerful protective mechanism. Physiological heterogeneity is emerging as an important characteristic of microbial biofilms; it explains the robust protection from antimicrobials and motivates development of combination therapies.

SESSION 2: Environmental Biofilms

Image and tracer analysis of bio-affected porous media

Presenter: Logan Schultz, MS Candidate, Chemical and Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

The development and application of subsurface porous media biotechnologies (e.g. Contaminant Remediation/Immobilization, Enhanced Oil Recovery, Carbon Sequestration) demands a fundamental understanding of fluid and substrate transport. The ability to non-invasively visualize the spatial and temporal evolution of microbes, minerals, and biofilms in laboratory porous reactors provides invaluable insight. In this presentation, research utilizing several laboratory reactor designs will be discussed, each allowing for unique methods of macroscopic and microscopic characterization and quantification. Specific techniques to be highlighted include stereo microscopy, fluorescent microscopy, confocal scanning laser microscopy, and dye tracer analysis. The discussion will highlight ways that these methods, supported with image and chemical analysis, can provide the means for transport characterization and predictive modeling.

Phase-field model of biofilm-flow interaction

Presenter: Tianyu Zhang, Assistant Professor, Mathematical Sciences
Co-authors: A. Cunningham, A. Mitchell, L. Schultz, and S. Parks
Affiliation: Center for Biofilm Engineering and Department of Mathematical Sciences, Montana State University, Bozeman, MT

We derive a set of phase-field models for biofilms using the one-fluid two-component formulation in which the combination of extracellular polymeric substances (EPS) and the bacteria are effectively modeled as one fluid component, while the collective ensemble of nutrient and the solvent are modeled as the other. The biofilm is assumed an incompressible continuum. The dynamics of the biofilm are governed by a modified Cahn-Hilliard equation. Numerical simulations are carried out, and biofilm growth, expansion,

streaming, rippling, and detachment in shear cells are captured. Viscoelastic properties of the biofilm are investigated as well.

Organisms responsible for nitrification in drinking water

Presenter: Gem D. Encarnacion, PhD Candidate, Microbiology
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Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman

Nitrification episodes in the drinking water distribution system (DWDS) are becoming increasingly problematic as water utilities switch the secondary disinfectant from chlorine to chloramine to avoid the production of disinfection by-products (DBP). The degradation of chloramine leaves residual ammonia which is the substrate for microbial nitrification (Baribeau, 2006; Regan et al., 2003). A nitrification episode in the DWDS is typically defined as a decrease in the chloramine residual concomitant with an increase in nitrite concentration and heterotrophic plate counts (HPC) (Wilczak, 2006). Unfortunately, nitrification in the DWDS is nearly impossible to stop once it has begun, and there are currently no effective methods available, short of shock chlorination, to reverse a nitrification event.

Nitrification is assumed to be primarily caused by autotrophic bacteria harbored in biofilms in the DWDS (Regan et al., 2002; Regan et al., 2003; USEPA, 2001; Wolfe et al., 1990). The conversion of ammonia to nitrite provides an energy source for the proliferation of autotrophic ammonia oxidizing bacteria. Subsequently, nitrite can be oxidized to provide an energy source for nitrite oxidizing bacteria.

Research has focused exclusively on the contribution of autotrophic ammonia oxidizing bacteria and nitrite oxidizing bacteria to nitrification episodes seen in DWDS (Lipponen et al., 2002; Regan et al., 2002; Regan et al., 2003; USEPA, 2001; Wilczak, 2006; Wolfe et al., 1990). Recent studies have shown that in environments such as soils and oceans, other groups of microorganisms contribute to nitrification more than what was previously known. Thus, the contribution of other nitrifying organisms to nitrification in drinking water systems should be evaluated as well. We have isolated heterotrophic nitrifying bacteria from both an actual drinking water distribution system biofilm as well as from a reactor that simulates premise plumbing. We have also found evidence for the presence of nitrifying archaea from our reactors. These data may be crucial in redefining how we view nitrification in drinking water.

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SESSION 3: Dental Biofilms

Microbiology of oral biofilms—Present and future

Presenter: Robert J. Palmer, National Institute of Dental and Craniofacial Research
Affiliation: National Institutes for Health, Oral Infection and Immunity Branch, Bethesda, MD

Oral biofilms have been investigated for decades and were some of the first biological systems in which spatiotemporally resolved data on biofilm topography were integrated with taxonomic data. These studies documented the development of a multispecies biofilm within a human host, but detailed information on the arrangement of species within the biofilm was not available until recently. Modern in situ taxonomic approaches (immunofluorescence and FISH coupled with laser confocal microscopy) have shown that the early biofilm is composed of biomass islands, each of which typically comprises more than one phenotype or species. The early colonizers are adept at recognition of the protein coating on the tooth surface, and highly specific adhesin-receptor pairs mediate this binding. Furthermore, species composition within initial colonies appears to be heavily influenced by the cell-cell recognition event known as coaggregation, likewise mediated by adhesin-receptor pairs. Specific recognition of one organism by another brings together organisms that are physiologically complementary. The process of community evolution is in turn likely to be a driving force for evolution of coaggregation traits. From a molecular taxonomic standpoint, oral biofilms are more diverse than those found on skin (another easily accessible human biofilm habitat), but significantly less diverse than the gut flora. The vast majority of the phylotypes are closely related to those already known from culture work, but some "novel" organisms do occur. Isolation and characterization of unique organisms is important to our understanding of communities, but characterization of other, more typical oral bacteria is of equal, if not greater, importance. The complex biofilm present subgingivally, or in late stages of supragingival plaque, develops primarily through growth and unification of uniting of the separate colonies rather than adhesion of new organisms. Small-molecule-based communication processes may operate on a broader scale in transitions of early commensal biofilms into later pathogenic communities. From the standpoint of human health, an understanding of oral microbial ecology is paramount.

Diffusion of macromolecules in model oral biofilms

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

The diffusive penetration of fluorescently tagged macromolecular solutes into model oral biofilms was visualized by time-lapse microscopy. Mixed-species biofilms of *Streptococcus oralis*, *Streptococcus gordonii*, and *Actinomyces naeslundii* were grown in glass flow cells. Solute access into the center for biofilm cell clusters was visualized by confocal scanning laser microscopy. All of the solutes tested—including dextrans, proteases, GFP, and IgG—accessed the interior of cells clusters one- to two hundred microns in diameter within 3 minutes or less. The effective diffusion coefficient in biofilm ranged from 22% of its value in water (IgG, MW 150,000) to 90% of its value in water (dextran, MW 3,000). These results suggest that while macromolecule diffusion is moderately slower inside a cell cluster, macromolecules are not excluded from the biofilm.

Environmental modulation of in vitro antimicrobial efficacy of oral care products

Presenters: Ositadinma Ona, and Raymond Ignar
Co-authors: O. Ona[1], R. Ignar[1], A.M. Middleton[2]
Affiliation: [1]GlaxoSmithKline, Parsippany, NJ, USA; [2]GlaxoSmithKline, Weybridge, UK

Dental caries and associated tooth decay (demineralization) are correlated with the disruption of the stable microbial communities associated with health, due to altered environmental conditions. In this study we monitored in vitro biofilm growth and the efficacy of various oral care products on *Streptococcus mutans* biofilms in relation to environmental conditions such as the availability of a carbon source and aeration. Results are discussed with a view to improving antimicrobial efficacy test methods used for oral care products.

Dental biofilm control

Presenter: Harsh Trivedi, Senior Technical Associate, Early Research Oral Care
Affiliation: Colgate-Palmolive Company, Piscataway, NJ

Dental biofilms, commonly known as plaque, are a cause of several oral conditions—caries, gingivitis, periodontitis and candidacies. The control of these biofilms has been traditionally through antimicrobial agents formulated in oral care products: i.e., Triclosan in Colgate Total® toothpaste and Chlorohexidine in Peridex™ mouthrinse.

Over the course of several years we built a wealth of knowledge on antimicrobials; during our searches we learned that at times we needed to develop new methods and conduct an iterative process when we went from single-active to formulations to maintain efficacy. Throughout this process we gathered a list of "actives" that failed to show efficacy using our traditional assays, yet these "actives" were suggested in literature to provide the benefits we sought. A program was set up to look in depth at this packet of actives and determine whether they truly do provide anti-plaque benefit, and if so, by what mode of action. The talk will be divided into four parts a) an introduction to oral biofilms, b) approaches to biofilm control, c) an example of alternate technology, and d) hierarchy of testing from the laboratory to clinical settings, as well as a suggested mode of action. A specific example will be shown to share the process that Colgate-Palmolive follows in identifying novel technologies, how it goes about validating a technology and then providing suggestions for its mode of action.

An in vitro model for the study of bad breath

Presenter: Alessandra Agostinho, Research Scientist, CBE Medical Biofilm Laboratory
Co-authors: E.Pulcini[1], G. James[1], R. McNab[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]GlaxoSmithKline, Weybridge, UK.

Halitosis is a problem that affects a large proportion of the population. It is estimated that about 50% of the adults in the USA suffer from persistent oral malodor. Halitosis is a general term used to describe unpleasant breath, regardless of its sources, oral or non-oral. Oral malodor or bad breath are the terms specifically used to describe the odor from the oral cavity.

Oral malodor is associated with microbial putrefaction of proteins, peptides and mucins found in saliva, blood, gingival crevicular fluid, lysed neutrophils, desquamated epithelial cells and any residual food retained on the oral surfaces. Malodor generation is particularly associated with periodontal pockets and dorsal surface of the tongue posterior to the circumvallate papillae.

Effective new oral care products have significant commercial potential for the control of bad breath therefore, an *in vitro* model to study oral malodor generation and reduction would be beneficial for the evaluation of different treatment strategies.

The Medical Biofilm Lab at the CBE performed research, funded by GlaxoSmithKline (GSK), to develop an *in vitro* model system for testing potential new treatments and approaches to combat bad breath. The model system consists of a drip flow reactor (DFR) which was inoculated with pooled saliva and tongue scrapings from human volunteers. Modified Bradshaw Marsh Medium was utilized to form biofilms on hydroxyapatite-coated glass slides within the reactor. Total volatile sulfur compounds production was monitored using a commercially available Halimeter® (Interscan, Chatsworth, CA).

Treatment effectiveness of several antimicrobials was evaluated against odor production, culturable microbial populations and biofilm architecture by confocal scanning laser microscopy.

Formation of communities within oral biofilms

Presenter: Robert J. Palmer, National Institute of Dental and Craniofacial Research
Affiliation: National Institutes for Health, Oral Infection and Immunity Branch, Bethesda, MD

Receptor-adhesin interactions mediate early biofilm formation in the oral cavity. Individual bacteria recognize particular molecules on the tooth surface to promote adhesion. After initial adherence, organisms begin to interact with one another. Coaggregation is defined by in vitro parameters, but has recently been shown to play an important role in bringing together oral bacteria that can function as a community. Community fitness cannot be directly assessed solely with molecular data; physiological characterization of the bacteria under in situ conditions is required. Understanding the physiological traits of individual bacteria is important, but these traits may be of limited significance in predicting community interactions. Model communities composed of different oral bacteria can be used to follow success and failure within the oral biofilm.

SESSION 4: Biofilm Mechanics

Development of a microcantilever method for measuring the cohesive strength of biofilms

Presenter: Raymond M. Hozalski[1], Associate Professor, Civil Engineering
Co-authors: P.S. Stewart[2], D.A. Miller[2], Srijan Aggarwal[1] and R. Hozalski[1]
Affiliation: [1]University of Minnesota, Minneapolis, MN;
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT

Biofilms are responsible for troublesome fouling in water distribution systems and industrial equipment and for persistent infections in medicine and dentistry. Unfortunately, the use of antimicrobial agents to remove biofilms is frustrated by the reduced susceptibility of microorganisms in biofilms. Mechanical removal is effective, but requires physical access to the biofilm and can be labor intensive or involve equipment downtime. The proposed approach represents a paradigm shift in control of unwanted biofilms from "kill" and "scrape" to weakening the biofilm structure and promoting detachment. The goal of this fundamental research is to develop effective strategies for controlling and removing microbial biofilms based on an improved understanding of the mechanisms of cohesion of the biofilm extracellular matrix. In order to achieve our goals, a method was needed to measure the mechanical properties of bacterial biofilms. Thus, a significant accomplishment of this research was the development of a micromechanical method to quantify the cohesive strength of microbial aggregates (i.e., flocs and detached biofilm fragments). A journal article on the method has been published (Poppele and Hozalski, 2003) and with this method we provided some of the first rigorous estimates of biofilm cohesive strength. A novel testing platform was developed for application of the method to intact biofilms, and both methods were applied to investigate the effects of fluid shear on cohesive strength. Recent work has demonstrated an effect of strain rate on biofilm strength that is characteristic of viscoelastic materials. Ongoing work concerns viscoelastic modeling and parameter estimation, investigation of the mechanisms of cohesive strength by testing the

effects of strength modifiers (e.g., divalent cations, enzymes such as proteases), and investigation of the effects of environmental conditions (e.g., variations in nutrient or electron acceptor availability, fluid shear) on strength. Experiments involve biofilms grown from single species cultures (*Staphylococcus epidermidis*, *Pseudomonas fluorescens*) and undefined mixed cultures.

Measuring changes in biofilm mechanical properties due to chemical and enzymatic treatments

Presenter: Eric Brindle, Masters Candidate, Mechanical Engineering
Co-authors: P.S. Stewart and D.A. Miller
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Bacterial cells in a biofilm are surrounded by protective extracellular polymeric substances (EPS) that provide the mechanical stability for these biofilms. Little is known about the material properties of attached biofilms, thus making it difficult to predict how a biofilm will behave in response to an applied force. The ability to alter the mechanical properties of a biofilm with various chemical and enzymatic treatments was investigated. Treatments tested included urea, Dispersin B ®, chlorhexidine, and iron chloride. Biofilm material properties were measured by applying a force to the biofilm before and after treatment. Two methods of applying forces to a biofilm were employed. The first method used time lapse confocal microscopy to measure the deflection of a biofilm in response to a constant, elevated fluid shear stress within a capillary flow cell reactor. The second method utilized atomic force microscopy to create force-displacement curves from indentation of the biofilm structure. Through very different loading paths, these two methodologies both show the viscoelastic nature of the biofilm. All four treatments tested resulted in alterations of biofilm viscous and elastic properties, some strengthening and some weakening the biofilm. By considering the biofilm as a mechanical structure that can support loads and recover deformations, we can generate viscoelastic models and perform experiments to determine the influence of treatments on biofilm mechanical properties.

Modeling fluid structure interactions with application to biofilms

Presenter: Jeff Heys, Assistant Professor, Chemical and Biological Engineering
Co-authors: I. Klapper, T. Harrer, and G. Vo
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

The purpose for developing a model of a physical system is, ultimately, to be able to either make predictions about that system without having to perform numerous and expensive experiments or to make a prediction in a situation where an experiment is not possible. In the past, a number of different models of diffusion and transport within biofilms have been developed with the goal of predicting, for example, the concentration of an antimicrobial agent within the biofilm or the local growth of the biofilm. These types of models have focused on physical phenomena on the time scale of hours, days, or even weeks. A few mathematical models have also been developed to predict the physical interaction between a moving fluid and a flexible biofilm. The goal is to predict biofilm deformation, permeability, and detachment. These types of physical phenomena play an important role in membrane fouling, the physical removal of a biofilm, and the spread of microorganisms from the biofilm to other regions of the fluid system.

We have developed a mathematical model of the physical interaction between a fluid and a biofilm that is roughly based on the immersed boundary method originally developed by Peskin to model blood flow in the heart. The method begins by solving the Navier-Stokes equations, which describe viscous fluid flow, on the entire domain of interest. The fluid region is then overlaid with a set of point that roughly approximate a microorganism and then these points are connected with springs to approximate the extra-cellular matrix of the biofilm. A force balance is then utilized to match the forces between the moving fluid, which is typically water, and the immersed points and springs, which represent the biofilm. In this way, the biofilm is 'immersed' in the fluid and exerts a force on the fluid. If the points are sufficiently close to one another, the biofilm becomes practically impermeable to the fluid; but, if the points are distant from each other, the biofilm has some permeability. Thus, the permeability of the biofilm can be controlled, and the permeability

can change as the biofilm is displaced. This type of model was originally developed by Dillon and Fauci [1] and later extended by Alpkvist and Klapper [2].

Our focus has been on the experimental validation and tuning of a pair of biofilm models to enable them to be used as a predictive tool in biofilm research. Specifically, the deformation of the biofilm predicted by the mathematical model is compared to the experimental measurements of Brindle. We are able to determine the optimal spring stiffness in the model, optimal failure strain for detachment in the model, and the importance of viscoelasticity. The model predictions are consistent with the experimental measurement when the optimal model parameters are used, and we expect this model to provide important predictions about the physical interaction between a fluid and biofilm in the future.

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Magnetic resonance determines degree of biofouling in porous media

Presenter: Jennifer Hornemann, Recent PhD Graduate, Chemical and Biological Engineering
Co-authors: S. Codd and J. Seymour
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Due to the complicated nature of studying living bacterial communities, Magnetic Resonance Microscopy (MRM) is a necessary tool providing unique data that is complementary to other techniques such as confocal microscopy and microelectrodes. MRM has the ability to probe an opaque system non-invasively and collect velocity measurements, imaging data, diffusion, and relaxation values and thus is an asset in the quest to learn how biofilms establish, grow, and die.

This presentation will summarize current biofilm MRM research which investigates transport phenomena over a hierarchy of scales, from the microscopic diffusion level to the macroscopic bulk flow. Results for three projects will be discussed:

(1) Diffusion measurements determined the impact of environmental and chemical challenges on the biomacromolecular dynamics in medically relevant *Staphylococcus epidermidis* biofilm material. This demonstrated MRM's ability to characterize molecular dynamics in biofilms, providing a basis for sensors that can indicate the state of the biofilm after thermal or chemical treatment and provide information to further understand the molecular level mechanisms of such treatments.

(2) Analysis of bulk advection in capillary bioreactors, also using *S. epidermidis* biofilms, indicates the prevalence of secondary flows. The data clearly supports the conclusion that reactor size impacts studies of spatially distributed biological activity, and the idea that scaling of transport models in biofilm impacted devices is possible but requires more study.

(3) Due to the role of biofilms as bio-barriers in environmental remediation and the potential role they may play in CO_2 sequestration to combat the impacts of global warming, studies were conducted to understand how biofilms grown in porous media impact pore structure and connectivity. The resilience of *Bacillus mojavensis* biofilms to super critical CO_2 is documented, and thus, this bacteria was chosen for the porous media studies. Results indicate that by varying exchange times, T_2-T_2 exchange experiments can determine the extent of biofilm growth in an opaque porous media as demonstrated in multiple model glass bead pack configurations.

SESSION 5: Industrial Biofilms

Monitoring biofilms in industrial applications and processes

Presenter: Michael V. Enzien, Lead. Research and Development Specialist
Affiliation: Dow Microbial Control, Buffalo Grove, IL

Over the last 10 years there have been many advances in the characterization of biofilms. Techniques such as confocal microscopy and even more recently, two-photon lasers, have enhanced the ability for researchers to study biofilm structure and community diversity at the microscopic and mesoscopic scales. Combined with modern molecular microbial ecology techniques and the advances in microscopy, many insights into biofilm microbial community structure and function have been elucidated. While these advances have helped improve the fundamental knowledge of biofilm and even study the impacts on novel biofilm control technologies, they are not practical or economic tools for monitoring biofouling under industrial processes. This presentation will give examples of some the various biofilm monitoring techniques that are used in industries such as Paper Making, Cooling Water and Oil and Gas.

Biofilm control can be divided into three basic categories: prevention, removal, and kill. Problems caused by biofilms in industrial process waters are not limited to the effects of microbial activity, but the mere presence of a biofilm structure, dead or alive, can cause severe process perturbations. Physical presence of biofilms can have dramatic impacts on flow across heat exchangers, RO membranes, and through hydrocarbon-bearing reservoirs. Industrial biofilm monitoring can be as simple as monitoring these process changes in flow, heat transfer, permeability and pressure drop. Other times it is also necessary to know the impact of biofouling control agents, such as biocides, on microbial activity. Online monitoring technologies have been developed which can measure microbial activity in situ within large recirculating systems. This technology is based on diffusible bioreporter molecules, which change their fluorescence spectra after being metabolized by microorganisms. Other technologies can measure oxygen consumption at surfaces as a measure of biofilm microbial activity. Devices that measure biofilm thickness using optical transmission through clear substrates are also used successfully in many applications. Online biofilm monitoring with a modified Quartz Crystal Microbalance (QCM) that can measure viscoelastic deposits has been developed; however it may be too sensitive for most industrial biofilm monitoring. In the case of biofilms associated with Microbiologically Influenced Corrosion (MIC), the key performance indicator is localized or pitting corrosion rates. While many online localized or pitting electrochemical corrosion monitors have been developed, currently there are no standard accepted monitors. The current standard for MIC monitoring involves metal coupons removed from side-stream devices that can be analyzed for both corrosion and microbial characterization. While many techniques are used to monitor biofilms in industrial process waters, only a few have enough reliability that they can be used for feedback of biofilm control agents. There are still many opportunities for new online biofilm monitoring technologies.

Fouling and cleaning science: Direct detection of biofilms and CIP-related problems in liquid process systems

Presenter: Mark Fornalik, Director, Analytical Chemistry and Biofouling Science
Affiliation: Ethox International, STS Life Sciences Division, Rush, NY

Biofilms are well known to be pervasive in many environments and can be found on the internal surfaces of pipes, tanks, and process equipment in a wide range of industries: pharmaceutical, food/beverage, ultrapure water, chemicals manufacturing, etc. Biofilms, though, remain remarkably difficult to detect by traditional microbiological testing and can be even more difficult to control. Negative microbial testing results do not imply that the manufacturing process is clean. Early and direct detection of biofilms and chemical fouling can be accomplished by employing surface-sensitive analytical measurements and removable witness plates (fouling cells). This presentation details use of fouling cell technology for early detection of biofilms and Clean-In-Place (CIP) problems, with case studies from several industries.

Imaging industrial samples at the CBE: How do we do it?

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

An image of an industrial biofilm is often a critical component in communicating the impact of fouling and inadequacy of antifouling treatments and cleaning methods to operators, plant managers and others responsible for controlling fouling in a process. However, imaging industrial biofilms can be challenging; most microscopes are set up for examination of flat, thin, coverslipped samples prepared in oil. A few simple additions and modifications to an existing epifluorescent microscope or confocal can essentially re-design it for use with industrial biofilms. This part of the presentation will offer some suggestions and ideas for such modifications, and show images obtained from some non-standard sample configurations.

Comparing the disinfection of planktonic cells, biofilms, and detached biofilm particles

Presenter: Sabrina Behnke, PhD Candidate, Microbiology
Co-author: A.K. Camper
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Although the detachment of aggregated cells from biofilms is of fundamental importance to the dissemination of contamination and infection in both public health and clinical settings, the disinfection efficacies of commonly used biocides on detached particles have not been adequately investigated. Therefore, the question arises: Can cells in detached aggregates be killed with disinfectant concentrations sufficient to kill planktonic cells? The goal of this study is the evaluation of the chlorine susceptibility of detached cells and cell clusters of *Burkholderia cepacia* in comparison to planktonic cultures and attached biofilms grown as a single species. Another aspect is to grow the organism in co-culture with *Pseudomonas aeruginosa* to determine how a second bacterial species influences the survival of *B. cepacia.* Experiments showed that *B. cepacia* is the dominant species in the dual species co-cultures with percentages ranging from 95 –97.5% of total cells.

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* (alternatively in co-culture with *P. aeruginosa*) is grown in standardized laboratory reactors for enhanced repeatability. Planktonic cultures are grown in a continuously stirred chemostat, while biofilm is obtained from a tube reactor. Detached aggregates can be sampled from the outflow of the tube reactor.

SESSION 6: Molecular Methods in Biofilm Ecology

Detection of opportunistic pathogens in drinking water and biofilms in rural Montana

Presenter: Crystal Richards, PhD Candidate, Microbiology
Co-authors: M. Eggers1 S. Broadaway[1], B. Pyle[1], T. Ford[2] and A.K. Camper[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]University of New England
Sponsors: National Center for Environmental Research (NCER) EPA STAR, Montana INBRE

Public health has been shown to be directly related to water quality, and although drinking water quality has improved in much of the United States there are potentially many areas with aging water systems. Underserved and rural areas with water systems that are not heavily regulated, monitored, and updated could have drinking water that poses a health risk. The purpose of this research is to identify and characterize the distribution of selected human opportunistic pathogens in drinking water systems in rural Montana. The pathogenic bacteria of interest are *Helicobacter pylori*, *Legionella pneumophila*, and

Mycobacterium avium. This research utilizes community-based participatory research during all aspects of data collection and management. Water and biofilm samples were collected from public buildings and private residences in Big Horn and Gallatin Counties. The pathogens of interest were detected by Polymerase Chain Reaction (PCR) and amplifications were performed using 16s rDNA primers specific for genus and/ or species. All three organisms were identified in Big Horn County, while *Mycobacterium avium* was the only pathogen identified in Gallatin County. *Helicobacter pylori* and *Legionella pneumophila* were detected in 14% and 41% of Big Horn samples, respectively. *Mycobacterium avium* was detected in 33% of Gallatin County samples while 50% of Big Horn County samples tested positive. Rural water sources appear to have different distributions of pathogenic bacteria when compared to more developed water sources. This research has shown that opportunistic pathogens such as Helicobacter, Legionella and Mycobacterium can be found in drinking water and associated biofilms in rural and developed areas of Montana.

Contaminated bulk soap dispensers: Comparison of two methods of analysis

Presenter: Lindsey Lorenz, CBE Research Assistant
Co-authors: B. Ramsay, CBE Research Assistant
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Bulk, refillable soap dispensers can become highly contaminated over time and are challenging to clean and sterilize. This can result in transfer of opportunistic bacteria to the hands after washing, and has been shown to subsequently transfer bacteria from the hands to other surfaces.

Experiments were conducted to determine the presence of biofilm on the surfaces of contaminated dispensers and to identify the contaminating microorganisms. Counter-mounted, plastic wall-mounted, and stainless steel wall-mounted dispensers, were sent to the CBE and analyzed using two distinct methods of analysis: 1) a traditional microbiological approach that used heterotrophic plate counts, coliform counts, and total cell counts; and/or 2) a community analysis molecular approach. According to the viable plate counts, the counter-mounted dispensers contained roughly 3.5–5 LOG_{10}(CFU/mL) in the bulk soap, and approximately 3.5–5 LOG_{10}(CFU/cm^2) bacteria were surface associated. The plastic wall-mounted dispensers contained roughly 5.4–6.7 LOG_{10}(CFU/mL) in the bulk soap, and approximately 3.3–6.8 LOG_{10}(CFU/cm^2) bacteria were surface associated. The stainless steel wall mounted dispensers contained roughly 4.9–5.2 LOG_{10}(CFU/mL) in the bulk soap, and there were approximately 5.1–6.4 LOG_{10}(CFU/cm^2) surface associated microorganisms. The total cell counts were generally 2–4 logs greater than the viable counts.

It was determined that there was a reasonable agreement in the results from each approach tested, although the community analysis approach identified more contaminating organisms than did the traditional microbiological approach. Most of the organisms identified were gram negative, and overall, there was not a largely diverse number of differing isolates found in the dispensers, even though the dispensers were from different locations.

The study showed that, regardless of dispenser design or construction material, dispensers with high levels of bacteria in the bulk soap also had high levels of surface associated bacteria that would be available to re-contaminate a dispenser if the old soap is emptied and new soap added.

Some statistical considerations in molecular methods

Presenter: Al Parker, CBE Statistician
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Molecular methods such as micro-array analyses generate large amounts of data from a relatively small number of samples. This talk discusses some statistical tools, such as cluster and principle components

analysis, which can be used to extract meaningful information from such data sets. In addition to microbial species identification, a common issue is to discover relationships between the microbes found and the relevant environmental factors. A statistical approach to this problem is presented as well.

Molecular methods for analyzing microbial function in constructed wetlands

Presenter: Jennifer Faulwetter, PhD Candidate, Microbiology
Co-authors: M.Burr, O.Stein, and A.K. Camper
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Constructed wetlands (CWs) are ecologically based water treatment systems that provide cost-effective amelioration of waterborne pollutants originating from a wide variety of sources. Relatively low capital costs, very low operating costs and associated benefits such as pleasing aesthetics and wildlife habitat make this technology highly attractive for agricultural producers, small municipalities, and other rural inhabitants. Previous research has confirmed that operational factors (e.g., plant species selection and hydraulic operation) influence effluent water quality and mediate the effect of season on CW operation. Our focus has been on obtaining a better description of the microbial influence on the carbon, nitrogen and sulfur cycles. Traditionally, this influence has largely been inferred by coupling the measurement of changes in water chemistry with known removal mechanisms in other kinds of treatment systems. As a result, the types, densities, and distributions of microbial communities present in CW environments are poorly understood. Basic information gaps exist with regard to microbial community structure and function and their relationship to plant species, season, and wastewater type.

An extensive sampling protocol was developed to capture a comprehensive and accurate view of the microbial community present throughout the CW. Molecular techniques were used to determine the microbial community structure and activity in the rhizosphere biofilms of the CW using a variety of 16S group-specific PCR primer combinations. Denaturing gradient gel electrophoresis (DGGE) fingerprints initially obtained from rhizosphere biofilm samples using universal bacterial primers revealed a poor resolution of differences between microbial communities largely because real differences were likely masked by the great complexity of the profiles. Breaking down this complex community into smaller, more specific groups made further analysis and visualization by DGGE more informative. However, this approach still revealed only a qualitative view of the resident biofilm populations.

Therefore, current investigation of these populations using the most recent techniques in molecular biology has three components: 1) distinguishing between live and dead microbial communities (using propidium monoazide (PMA) 2) estimating population size with quantitative PCR, and 3) using primer sets specific for functional microbial groups of interest. These groups include: nitrifying bacteria (targeting the *amoA* gene) and sulfate reducing bacteria (targeting the *dsrB* gene). These populations are being observed and compared across plant species and season. This most recent microbial assay work is ongoing. It is our hope that we will be able to correlate an improvement in water quality to the presence of particular microbial populations by detecting specific genes.

In summary, our research is focused on utilizing molecular technology to identify major microbial functional groups of interest (e.g., nitrifying bacteria, denitrifying bacteria, sulfate reducing bacteria), the location of these groups within CW biofilms (e.g., gravel, basal roots, root tips, etc.), and the changes in activity (growth) of these groups as influenced by plant species selection and season.

Rapid taxonomic classification and analysis of complex microbial communities using a microarray

Presenter: Seth D'Imperio, Postdoctoral Researcher, Chemical and Biological Engineering
Co-authors: E.K. Field, J.G. Moberly, and B.M. Peyton
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

The comprehensive characterization of mixed microbial communities presents problems of scale, cost, and time. A 16S-based microarray (PhyloChip) was designed with the goal of rapidly cataloging the diversity of several complex microbial communities. This phylogenetic information can, in turn, be used for comparative analysis of community structures from distinct environmental sites, growth conditions, or treatments.

Data from two environmental studies will be presented: i) Sediment samples from Lake Coeur d'Alene in Idaho that were impacted by upstream metal mining on the Coeur d'Alene River and comparative samples from a non-metal-contaminated region of the same lake. ii) Regions both up- and downstream of a mercury mine on the River Idrijca, Slovenia. The technique effectively elucidated family-level differences in the phylogenetic composition between associated sites and can be used in conjunction with related relevant data for a clearer understanding of microbial activities in complex communities.

Microbial community analysis of a low-level waste site using the PhyloChip, a novel microarray

Presenter: Erin K. Field, PhD Candidate, Microbiology
Co-authors: E.K. Field[1], S. D'Imperio1, M. VanEngelen[1], B.M. Peyton[1], R. Gerlach[1], B.D. Lee[2], A. Miller[2], W. A. Apel[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]Idaho National Laboratory, Idaho Falls, ID
Sponsors: Department of Energy, Environmental Remediation Sciences Program

Low-level radioactive waste sites frequently contain cellulosic waste in the form of paper towels, cardboard boxes, or wood contaminated with heavy metals and radionuclides such as chromium and uranium. To understand how the soil microbial community is influenced by the presence of cellulosic waste products, multiple soil samples were obtained from a non-radioactive model low-level waste test pit at the Idaho National Laboratory. Samples were analyzed using 16S rDNA clone libraries and 16S rRNA gene microarray (PhyloChip) analyses. Both the clone library and PhyloChip results revealed changes in the bacterial community structure with depth. In all samples the PhyloChip detected significantly more unique Operational Taxonomic Units (OTUs), and therefore more relative diversity, than the clone libraries. Calculated diversity indices suggest that diversity is lowest in the Fill and Fill Waste layers and greater in the Wood Waste and Waste Clay layers. Principal coordinates analysis and lineage specific analysis determined that Bacteroidetes and Actinobacteria phyla account for most of the significant differences observed between the layers. Overall, these results suggest that the presence of the cellulosic material significantly influences the bacterial community structure in a stratified soil system. This study demonstrates the value of using PhyloChip and clone library analyses to complement each other to gain more information about the microbial community. Current and future studies include flow-through column studies in which the influence of metal mobility on the microbial community as metal contaminated cellulosic waste is broken down will be assessed through the use of PhyloChip and GeoChip (a functional gene microarray) analyses.

Elucidating possible relationships within bacterial community dynamics and abiotic parameters

Presenter: Kara De Leon, PhD Candidate, Microbiology
Co-authors: M. Fields, C. Hwang
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Microbial community composition and structure has typically been done via clone libraries of well conserved genes, but the recent pyro-sequencing technology can allow for a far greater number of sequences. Furthermore, the wealth of information that can be obtained from clone libraries and 454 pyro-sequencing is not limited to microbial community surveys and identification but can also allow for elucidation of relationships between biotic and abiotic parameters. Multivariate analysis techniques are available to identify possible relationships between the population distributions and the geochemical and geophysical parameters of a system of interest. From these analyses, hypotheses can be formed about possible causal relationships. Data will be presented about the use of multivariate analysis to track bacterial community dynamics (Hwang et al., 2009) as well as recent results for using pyro-sequencing data.

Poster Abstracts

CBE Poster #452

Date: 07/2008
Title: **Confocal laser microscopy on biofilms: Successes and limitations**
Authors: ***Betsey Pitts*** and P.S. Stewart
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

This poster presents a shorter version of an article by the authors from *Microscopy Today*, July 2008.

Imaging of bacterial biofilms with microscopes has been an essential and transformative method in biofilm research. Fluorescence microscopy can elucidate specific biofilm components and cellular activities that cannot be separated otherwise. In particular, confocal fluorescence microscopy extends that examination through the thickness of a fully hydrated, *in situ* biofilm, affording the potential for 3D, non-invasive, time-lapse imaging. This article discusses some striking examples of the insight provided by confocal fluorescence microscopy into biofilm structure, composition, and heterogeneity, and it will also enumerate some limitations of this imaging process.

CBE Poster #466

Date: 07/2008
Title: **Biofilms on ice: "Unveiling" a new matrix stain?**
Authors: ***Christine M. Foreman***[1,2], M. Dieser[1,2] and B. Pitts[1]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2] Land Resources and Environmental Sciences, Montana State University, MT
Sponsor: National Science Foundation

Organisms that exist in icy environments possess mechanisms to protect themselves from extremes of thermal and radiative conditions that would cause severe damage to non-adapted organisms. While evaluating the potential of bacterial pigments to serve as cryo- or solar radiation protectants in our Antarctic bacterial culture collection, we came across an interesting phenomenon involving a control organism, *Escherichia coli* K12. Broth cultures of *E. coli* were subjected to a series (0, 1, 2, 6, 10, 15, 20, 30, 40, 50, 70, and 100) of 12-hour freeze-thaw cycles, rotating between -20°C and 6°C. After two freeze-thaw cycles, viability of *E. coli* decreased significantly (CFUs dropped three orders of magnitude), and by 40 cycles there was 100% mortality (as determined by culturability). Over the course of the freeze-thaw cycles the organisms produced an enormous amount of what appears to be extracellular polymeric substances (EPS), presumably as a protective mechanism to avoid desiccation and intracellular ice nucleation. Using the confocal microscope in combination with several fluorescent stains, we were able to visualize the exquisite architecture of the biofilm matrix.

CBE Poster #470

Date: 01/2009
Title: **Physiological state of *Pseudomonas aeruginosa* in biofilms revealed by transcriptional profiling**
Authors: ***Phil Stewart*** and J. Folsom
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Pseudomonas aeruginosa PAO1 biofilms were grown *in vitro* for three days in drip-flow reactors using a glucose-minimal medium. The RNA was harvested from six replicate biofilms and the transcriptome was determined using Affymetrix microarrays. To gain insight into the priorities of the biofilm population, the MAS5 scaled signal intensity of each transcript was ranked. Similar rankings were obtained from data sets published in the GEO database: (www.ncbi.nlm.nih.gov/geo). By comparing the rank of genes selected as

markers for particular physiological responses between the biofilm and comparator data sets, it was possible to infer qualitative features of the physiological state of the biofilm bacteria. These biofilms appeared, from their transcriptome, to be glucose nourished, iron replete, oxygen limited, and growing slowly or exhibiting stationary phase character. The biofilm population did not indicate oxidative stress, but did exhibit copper stress. Of seven indicator genes for homoserine lactone mediated quorum sensing, only one (*rsaL*) was highly expressed in these biofilms. Efflux pumps were not up-regulated in the biofilm. Of potential extracellular polysaccharide synthetic loci, only the *pel* genes were moderately more highly ranked than in the comparator data sets. Genes associated with the elaboration of pili were strongly expressed by the biofilm cells. Genes associated with bacteriophage Pf1 were much higher ranked in the biofilm transcriptome than in all comparators. As the database of published transcriptomes grows, comparisons based on internally ranked sets can provide insight into the activities of a given specimen. The transcriptome of drip-flow biofilm underscores the oxygen-limited, slow-growing nature of the population.

CBE Poster #472

Date: 02/2009
Title: **Testing wound dressings using a new *in vitro* wound model**
Authors: ***Chelsea Lipp***, A. Agostinho, K. Kirker, P. Stewart, G. James
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsor: NIH Wound Care

Many modern wound dressings possess a variety of attributes designed to create a supportive wound healing environment. These attributes include absorbing exudates, providing optimum moisture balance at the wound surface, and preventing maceration of surrounding tissue. However, bacteria are often present in wounds as well, and heavy bacterial colonization and infection can interfere with the wound healing process. Thus, many wound dressings are also designed to control bacterial colonization. In this study, the effect of wound dressings on bacterial bioburden is investigated using a novel wound model. Wound dressings of various types were used in a new model system based on characteristics of both the colony biofilm and drip-flow models. The colony biofilm model is designed to grow biofilms on semi-porous membranes placed on top of a nutrient source (agar). The drip-flow reactor is designed for the study of biofilms grown under low shear conditions, where nutrient medium is pumped into the top of the reactor and allowed to drip down a sloped, inoculated surface and out the effluent. In this study, a semi-porous membrane was inoculated with *Staphylococcus aureus* and placed on top of an absorbent pad sitting on the declining surface of the drip-flow reactor. The absorbent pad wicked the nutrient medium upward, feeding the bacteria from below, and thus mimicked a wound-like condition. A wound dressing was then cut into a sterile 2.5 cm x 2.5 cm piece and placed on top of the inoculated membrane. After three days of growth, both the membrane and dressing were evaluated by plate counts, scanning electron microscopy (SEM), and fluorescent microscopy. Plate counts revealed that the samples with antimicrobial agents (silver or polyhexamethylene biguanide) had significantly fewer bacteria than those without antimicrobial agents ($p \leq 0.012$). Both the SEM and fluorescence microscopy evaluation supported the plate count results.

CBE Poster #476

Revised: 07/2009
Title: **Identification of bismuth thiols with activity against biofilms of wound isolated bacteria**
Authors: ***James P. Folsom***[1], P.S. Stewart[1], B. Baker[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Microbion BioSciences Corporation, Bozeman, MT and Vancouver, B.C., Canada
Sponsor: Montana Board of Research and Commercialization Technology

Chronic wounds develop when the host immune system has been overwhelmed by bacterial infection of an acute wound, and bacteria begin to invade and further destroy tissue. The three main types are venous leg ulcers, diabetic ulcers, and pressure ulcers. A key factor in the development of chronic wounds is loss or restriction of circulation in an extremity. Bacteria that infect chronic wounds persist in a biofilm state, and often are not controlled by antibiotics. A wide variety of antiseptics are also employed, but there are many

recalcitrant wounds that do not respond to current therapies. Bismuth thiols (BT) are the result of the combination of bismuth nitrate with a lipophilic thiol containing compound. This complex of bismuth with a thiol compound has been described as increasing the solubility and antimicrobial properties of bismuth. Using established in vitro biofilm methods, several bismuth thiols were evaluated for potential effectiveness in the treatment of chronic wounds. Wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used in the assays.

A few BT compounds with exceptional biofilm killing ability were identified. These have been further tested and compared to other treatments that may be used in chronic wound therapies using the drip flow biofilm model. This model confirmed these BTs are capable of killing preformed biofilms and are competitive with or superior to some other common treatments. Results from the colony biofilm model also demonstrated synergy when rifampicin and amikacin were combined with several BTs. Currently the two most promising BTs are being tested for their ability to promote wound healing in the presence of biofilms using the kerotinocyte scratch model. Using this model these BTs will also be compared with other treatments already in use.

CBE Poster #478

Date: 02/2009

Title: **High copper concentrations decrease the toxicity and sorption of lead and zinc to the important biomining bacterium *Acidithiobacillus caldus***

Authors: ***John E. Aston***[1], W.A. Apel[2], B.D. Lee[2], and B.M. Peyton[1]

Affiliation: [1]Department of Chemical and Biological Engineering and Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Biological Systems Department, Idaho National Laboratory, Idaho Falls, ID

Sponsors: Idaho National Laboratory, Biological Systems Division; MSU IGERT (Integrated Graduate Education and Research Training); Montana EPSCoR

Acidithiobacillus caldus is found in acidic environments (pH 1–4) at temperatures between 32 and 50°C, and is believed to play a role in the biomining of metals by removing inhibitory sulfur layers from mineral surfaces. In the current study, the toxicity and sorption of lead, zinc, and copper to *At. caldus* strain BC13 were examined. In each case, metals decreased the overall cell yield and specific growth rate during batch cultivation. Lead, zinc, and copper IC50 values (± 95% confidence intervals) were calculated to be 39 ± 4.5 μM, 180 ± 36 μM, and 2,370 ± 630 μM, respectively. When lead and zinc were mixed, their toxicity appeared to be additive. When copper was mixed with lead or zinc, the observed toxicity was significantly less than expected. Langmuir sorption isotherms show a relatively high affinity for copper, with maximum specific loading capacities of 253 ± 60 μmol g^{-1}, 753 ± 164 μmol g^{-1}, and 1,582 ± 277 μmol g^{-1} calculated for lead, zinc, and copper, respectively. In addition, the presence of copper in metal mixtures decreased lead and zinc sorption significantly.

CBE Poster #482

Date: 02/2009

Title: **Rapid taxonomic classification and analysis of complex microbial communities using the PhyloChip microarray**

Authors: ***Seth D'Imperio***[1], J.G. Moberly[1], Ari Staven[1], E. Field[1], M.R. VanEnglen[1], S. Žižek2, D. Žagar[2], B.M. Peyton[1]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]University of Ljubljana, Ljubljana Slovenia

Sponsors: U.S. Department of Energy, Office of Science, Environmental Remediation Science Program

The comprehensive characterization of mixed microbial communities presents problems of scale, cost, and time to researchers. A 16S-based microarray (PhyloChip) was utilized with the goal of rapidly cataloging the diversity of complex environmental microbial communities. This technology was applied to sediment samples from Lake Coeur d'Alene in Idaho impacted by upstream metal mining on the Coeur d'Alene River.

A comparison of the microbial communities from this site and a non-metal-contaminated site in the same lake showed distinct differences at the Family level, in particular among the Enterobacteriales, Bacteroidetes, and Spirochetes. The technique was also applied to regions both up- and downstream of a mercury mine on the River Idrijca, Slovenia, and displayed significant phylogenetic differences between the sites sampled that were most pronounced in the Enterobacteriales and Pseudomonadales populations. Additionally, four strata of a simulated low level waste site at Idaho National Laboratory were investigated using the PhyloChip and revealed several significant variations within the Actinobacteria and Bacteroidetes populations. Furthermore, when combined with a dilution series prior to analysis on the PhyloChip, the method can be used to estimate relative cell densities for the OTUs that inhabit mixed communities.

CBE Poster #484

Date: 05/2009

Title: **Bacterial community structure from alkaline springs along a thermal gradient in Yellowstone National Park**

Authors: ***Kara Bowen De Leon***[1], S.E. Dowd[2], R.D. Wolcott[2], B.D. Ramsay[1], P. Gardner[1], B.M. Peyton[1], C. Hwang[1], and M.W. Fields[1]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Medical Biofilm Research Institute, Lubbock, TX

Sponsors: Thermal Biology Institute, Montana State University;
IGERT Program in Geobiological Systems at Montana State University;
Molecular Biosciences Program, Montana State University;
Medical Biofilm Research Institute, Lubbock, Texas

The Heart Lake Geyser Basin (HLGB) is located along Witch Creek and the northwestern shore of Heart Lake at the base of Mount Sheridan in Yellowstone National Park. The HLGB contains three major thermal groups that are mostly fumaroles and hot springs associated with fissures. Because this area is secluded many of the thermal features have not been studied and remain unnamed. Three springs were selected for characterization and were located in close proximity along a common stream. The three springs had pH values that were between 8.5 and 8.6 and the temperatures were 44°C, 63°C, and 75°C. DNA was extracted from sediment/slurry samples, and PCR amplicons were produced using universal bacterial primers of the 530-1100 region of the of the SSU rDNA gene sequences. Sequences were determined via bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP) and compared to conventional clonal libraries. Criteria were established to screen the bTEFAP libraries, and after screening the three libraries contained over 12,000 sequences with average lengths of approximately 258. Sequences were clustered at 98% similarity and sequences that did not fall into a group (singletons) were not further considered. Consensus and representative sequences from each cluster were blasted against NCBI's non-redundant database using BLASTN and the top cultured organism was used for phylogenetic comparisons. Interestingly, though the top cultured hits varied between bTEFAP and the clonal libraries, the distribution of sequences into phyla was the same. The 44°C spring was predominated by the groups *Chloroflexi* and *Cyanobacteria*, the 63°C spring was predominated by *Chloroflexi* and *Proteobacteria*, and the 75°C spring was predominated by the groups *Deinococcus*/*Thermus* and *Firmicutes*. The data suggested that population distributions shifted as temperature and geochemical factors affected carbon metabolism. Many of the OTUs were grouped as unclassified.

CBE Poster #485

Date: 07/2009
Title: **Microbially enhanced carbonate mineralization and geologic containment of sequestered supercritical CO_2**
Authors: ***Alfred B. Cunningham***[1], L. Schultz[1], R. Gerlach[1], J.P. Kaszuba[2], L. Spangler[1], A.C. Mitchell[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]University of Wyoming, Laramie, WY
Sponsors: US Department of Energy EPSCoR program and ZERO Emissions Research and Technology Program

Geologic sequestration of CO_2 involves injection into deep underground formations including oil beds, unminable coal seams, and saline aquifers with temperature and pressure conditions such that CO_2 will likely be in the supercritical state. Supercritical CO_2 injection into the receiving formation will result in elevated pressure in the region surrounding the point of injection, and may result in an upward hydrodynamic pressure gradient and associated "leakage" of supercritical or gaseous CO_2. Therefore mechanisms to reduce leakage and to mineralize CO_2 in a solid form are extremely advantageous for the long-term geologic containment of CO_2.

This poster will focus on microbially based strategies for controlling leakage and sequestrating supercritical CO_2 during geologic injection. We will examine the concept of using engineered microbial barriers (Mitchell et al., 2008; Mitchell et al., 2009) which are capable of precipitating calcium carbonate under high-pressure subsurface conditions. These biomineralization barriers may provide a method for plugging preferential flow pathways in the vicinity of CO_2 injection, thereby reducing the potential for unwanted upward migration of CO_2, as well as mineralizing injected CO_2. A summary of experiments investigating 1) microbially enhanced carbonate mineralization and 2) biofilm and associated calcium carbonate formation and plugging using an etched-plate porous media reactor will be presented, and the potential for microbially enhanced CO_2 sequestration discussed.

Mitchell AC, Phillips A, Hiebert R, Gerlach R, and Cunningham AB, "Biofilm enhanced geologic sequestration of supercritical CO_2," *Inter. J. Greenhouse Gas Control*, 2009; 3(1):90-99.

Mitchell AC, Phillips A, Hamilton M, Gerlach R, Kuszuba J, and Cunningham AB, "Resilience of planktonic and biofilm cultures to supercritical CO_2," *J. Supercrit. Fluids*. 2008; 47(2):318-325.

CBE Poster #486

Date: 05/2009
Title: **Expression of a novel ncRNA in *Pseudomonas aeruginosa* during stationary-phase growth and iron starvation**
Authors: ***Laura K. Jennings***, A. Law, Y.N. Oh, M. Dlakic, M.J. Franklin
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Small non-coding regulatory RNAs (ncRNAs) are involved in many bacterial post-transcriptional regulatory processes. Recently, a number of ncRNAs have been identified from the intergenic regions of the *Pseudomonas aeruginosa* genome. Included in these are the prrFI and prrFII ncRNAs that are regulated by iron limitation, and are involved in iron metabolic processes. We used comparative genomics to identify conserved and structurally stable ncRNAs in intergenic regions of *P. aeruginosa*. Using this approach, we identified a third ncRNA (between the coding regions PA4634 and PA4635) that is induced by iron starvation. Expression of this ncRNA, designated PA4634.1, was tested using an oligonucleotide microarray. Similar to prrFI/FII, PA4634.1 was only expressed during iron starvation. However, unlike prrFI/FII, its expression was only detected during stationary phase growth. Northern blotting of RNA from *P. aeruginosa* PAO1 and a PA4634.1 deletion mutant confirmed expression of PA4634.1 only in iron depleted medium and in stationary phase. Sequence alignments suggest that the PA4634.1 promoter region may contain an iron-repressive Fur box as well as a $^{-}$10 consensus sequence for the RpoS sigma factor. To test the role of RpoS on PA4634.1 expression, Northern blotting was performed on a *P. aeruginosa rpoS*

mutant strain, and no expression of PA4634.1 was detected. The results indicate that PA4634.1 is expressed only in stationary phase under control of the RpoS sigma factor and under iron starvation, conditions likely to exist during biofilm infections.

CBE Poster #487

Date: 07/2009

Title: **Application of molecular techniques to elucidate the influence of cellulosic waste on the bacterial community structure at a simulated low level waste site**

Authors: ***Erin K. Field***[1], S. D'Imperio[1], M. VanEngelen[1], B.M. Peyton[1], R. Gerlach[1], B.D. Lee[2], A. Miller[2], W.A. Apel[2]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Idaho National Laboratory, Idaho Falls, ID

Sponsors: Department of Energy, Environmental Remediation Sciences Program

Low-level radioactive waste sites frequently contain cellulosic waste in the form of paper towels, cardboard boxes, or wood contaminated with heavy metals and radionuclides such as chromium and uranium. To understand how the soil microbial community is influenced by the presence of cellulosic waste products, multiple soil samples were obtained from a non-radioactive model low-level waste test pit at the Idaho National Laboratory. Samples were analyzed using 16S rDNA clone libraries and 16S rRNA gene microarray (PhyloChip) analyses. Both the clone library and PhyloChip results revealed changes in the bacterial community structure with depth. In all samples the PhyloChip detected significantly more unique Operational Taxonomic Units (OTUs), and therefore more relative diversity, than the clone libraries. Calculated diversity indices suggest that diversity is lowest in the Fill and Fill Waste layers and greater in the Wood Waste and Waste Clay layers. Principal coordinates analysis and lineage specific analysis determined that Bacteroidetes and Actinobacteria phyla account for most of the significant differences observed between the layers. Overall, these results suggest that the presence of the cellulosic material significantly influences the bacterial community structure in a stratified soil system. This study demonstrates the value of using PhyloChip and clone library analyses to complement each other to gain more information about the microbial community. Current and future studies include flow-through column studies in which the influence of metal mobility on the microbial community as metal contaminated cellulosic waste is broken down will be assessed through the use of PhyloChip and GeoChip (a functional gene microarray) analyses.

CBE Poster #488

Date: 07/2009

Title: **Relationship between toxicity and zinc ion activity in *Arthrobacter* sp. isolated from heavy metal contaminated sediments**

Authors: ***James G. Moberly***[1,2], A. Staven[2], R.K. Sani[1,3], and B.M. Peyton[2]*

Affiliation: [1]Washington State University, School of Chemical and Bioengineering, Pullman, WA;
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[3]Current address: South Dakota School of Mines and Technology, Department of Chemical and Biological Engineering, Rapid City, SD

Sponsor: NSF

Due to its high solubility under a wide range of pH conditions, aqueous zinc is found in many natural and anthropogenic systems. However, the speciation of zinc is critical in assessing toxicity to microorganisms. Combined thermodynamic modeling, statistical analysis, and batch culture studies using *Arthrobacter* sp. JM018 suggest that the toxic species may not solely be the free ion but may also include $ZnHPO4^0$(aq). Cellular uptake of $ZnHPO_4^0$(aq) through inorganic phosphate transporter (*pit* family), which requires a neutral metal phosphate complex for phosphate transport, may explain the toxicity. At 100 μM total zinc, $ZnHPO_4^0$(aq) contributes 50, 82, and 87% of the neutral metal phosphate pool at pH 6, 7, and 8 respectively. At 50 μM total zinc, toxicity of zinc to cultures supplied with organic phosphate (glycerol-3-phosphate) show little significant response to pH (α=0.05, p=0.07) while toxicity of zinc in inorganic

phosphate supplemented cultures show significant pH dependence (α=0.05, p=0). These findings may suggest a reevaluation of models for toxicological studies and risk assessments and have wider implications for pH responsive cellular toxic heavy metal flux as *pit* inorganic phosphate transport system is a widely distributed in bacteria, archaea, and eukaryotes.

CBE Poster #489

Date: 07/2009
Title: **Efficacy of zosteric acid against *Candida albicans* biofilm**
Authors: ***Federica Villa***[1], B. Pitts[2], P.S. Stewart[2], B. Giussani[3], D. Albanese[4], and F. Cappitelli[1]
Affiliation: [1] Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy;
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA;
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The ubiquitous commensal yeast *Candida albicans* is frequently implicated in many invasive biofilm-associated infections, exhibiting resistance to traditional antifungal agents. As a consequence, a new approach to controlling fungal biofilm has become imperative. One of the most recent and fascinating trends in antibiofilm strategies is the employment of molecules able to interfere, in sub-lethal doses, with the formation of biofilms. To this end, according to the literature, the plant metabolite zosteric acid is potentially a good candidate as it combines low toxicity with antifouling properties. The aim of the present work was to test, for the first time, the efficacy of zosteric acid against *Candida albicans* biofilm.

The promising non-toxic antifoulant was synthesized using a novel method and the most effective concentration was obtained from microplate assays using a mathematical representation able to simulate cellular behaviors in complex scenarios. Then the performance of zosteric acid was tested under continuous flow conditions using the Centers for Disease Control biofilm reactor (CDC bioreactor). We demonstrated that zosteric acid was active at 10 mg/L and it reduced microbial adhesion by 75% on both hydrophilic and hydrophobic surfaces. Results from CDC biofilm reactor studies showed a reduction of biofilm formation by 80%. These findings were confirmed by confocal microscopy. The control sample showed a dense multilayer network of yeast and filamentous forms, whereas the treated sample exhibited an atypical biofilm architecture consisting of yeast cells and very few filamentous forms. In addition, FUN 1 viability staining and cryosections of biofilm samples indicated that zosteric acid induced a significant reduction in biofilm thickness (70%) while maintaining metabolically active cells.

These findings suggested that zosteric acid significantly reduced *C. albicans* adhesion and its dimorphic switching from yeast to the filamentous form. These preliminary results are encouraging for the use of zosteric acid as a preventive approach against deleterious *Candida albicans* biofilm.

CBE Poster #490

Date: 04/2009
Title: **Biofilm-induced alteration of radial fluid flow**
Authors: ***Robin Gerlach,*** R. Fortenberry, and A.B. Cunningham
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsors: Department of Energy–ZERT and ERSP; MSU Undergraduate Scholars Program

Subsurface remediation of carbon dioxide is a promising technology which espouses the principles of more eco-friendly energy and industrial productivity. The technology itself is still in its infant stages, however, and one potential problem area surrounds leakage of CO_2 from its subsurface aquifer back into the atmosphere, rendering the process useless and expensive. Biofilms—agglomerations of microbial cells within an extracellular polymeric matrix—are theorized to reduce fluid transport through porous media. *Cellulomonas* sp. strain ES6 was grown under constant flow conditions in a radial (circular) flow reactor,

whose flow induced a biofilm growth pattern. Tracer studies were used for quantitative and qualitative analysis of fluid dynamics in the reactor. The biofilm showed a propensity to affect dye transport through the reactor by reducing the void space of the reactor. This result indicates that biofilms are potentially efficacious in decreasing void space and permeability in their immediate surroundings, a characteristic which could be exploited by researchers involved in the development of subsurface biofilm technologies.

CBE Poster #491

Date: 04/2009
Title: **Isolation of anaerobic bacteria from chronic wounds**
Authors: ***Jeremy Woods*** and G. James
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsors: NIH (1 P20 GM078445-01) and the MSU Undergraduate Scholars Program

The goal of this project was to isolate a strict anaerobe from a chronic wound for subsequent studies to examine the role of strict anaerobes within the wound bed. The strict anaerobe *Clostridium perfringens* was isolated from a pressure ulcer sample using agars containing various antimicrobial agents under anaerobic incubation conditions. This isolate was subsequently used to inoculate a colony drip flow reactor along with the facultative anaerobe *Enterococcus faecalis*, also a chronic wound isolate. The colony drip flow was first inoculated with *E. faecalis* in order to form an initial biofilm which would create an anaerobic environment for the growth of *Clostridium perfringens*. Plate counts from the resulting biofilm revealed low levels of *Clostridium perfringens* but abundant *Enterococcus faecalis*. Future work with this model will focus on increasing the growth rate of *Clostridium perfringens* and microscopic analysis of the dual-species biofilm.

CBE Poster #492

Date: 04/2009
Title: **Lactoferrin, xylitol, and the inhibition of *Pseudomonas aeruginosa* biofilms**
Authors: ***Mary Cloud Ammons***[1], L.S. Ward[2], and G.A. James[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Glanbia Nutritionals
Sponsor: Glanbia Nutritionals Research and Development

The medical importance of bacterial biofilms has increased with the recognition that biofilms inhabit chronic wounds such as diabetic foot ulcers, venous leg ulcers, and pressure ulcers. Traditional methods of treatment have proven ineffective for biofilms; therefore our lab has described in vitro evidence to support the use of novel antimicrobials in the treatment of *Pseudomonas aeruginosa* biofilms. In an in vitro biofilm model with a clinical isolate of *P. aeruginosa*, a combined lactoferrin and xylitol treatment disrupted the structure of *P. aeruginosa* biofilms and resulted in a greater than two-log reduction in viability. These findings indicated that combined treatment with lactoferrin and xylitol significantly decreased ($P<0.001$) the viability of established *P. aeruginosa* biofilms in vitro, and that the antimicrobial mechanism of this treatment included both biofilm structural disruption and permeablization of bacterial membranes. Follow-up studies on these findings utilized both proteomic and transcriptomic analysis of lactoferrin and xylitol treatment, both independently and in combination. Two-dimensional gel electrophoresis (2-DE) indicated distinct changes in protein expression and post-translational modification associated with stress response and membrane integrity. Furthermore, microarray analysis indicated noteworthy changes in gene expression trends in motility, cell adhesion, adaptation, and secreted factors. Although many of the genetic elements whose expression changed with treatment are undescribed, over thirty-eight changed elements were biofilm associated. Taken together, these data indicate that lactoferrin and xylitol act as biofilm antimicrobials both independently and in combination, and that antimicrobial mechanisms are exerted both on the transcriptomic and proteomic levels.

CBE Poster #493

Date: 04/2009
Title: **Molecular level *in silico* analysis of mass and energy flows in microbial communities**
Authors: ***Ross P. Carlson***[1,2], R. Taffs[1], J.E. Aston[1], K. Brileya[1], Z. Jay[2], C.G. Klatt[2], S. McGlynn[2], N. Mallette[1], S. Montross[2], R. Gerlach[1], W.P. Inskeep[2], and D.M. Ward[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]Thermal Biology Institute, Montana State University, Bozeman, MT
Sponsor: NSF

Three methods were developed for the application of stoichiometry-based network analysis approaches to the study of mass and energy flows in microbial communities. Each has distinct advantages and disadvantages suitable for analyzing systems with different degrees of complexity and *a priori* knowledge. These approaches were tested and compared using data from the thermophilic, phototrophic mat communities from Octopus and Mushroom Springs in Yellowstone National Park (USA). The models were based on three distinct microbial guilds: oxygenic phototrophs, filamentous anoxygenicphototrophs (FAP), and sulfate-reducing bacteria (SRB). Two phases, day and night, were modeled to account for differences in the mass and energy sources and the routes available for their exchange.

The *in silico* models were used to explore fundamental questions in ecology including the prediction of, and explanation for, measured relative abundances of primary producers in the mat, theoretical tradeoffs between overall productivity, and the generation of toxic by-products, and the relative robustness of various guild interactions.

The three modeling approaches represent a flexible toolbox for creating cellular metabolic networks to study microbial communities on scales ranging from cells to ecosystems.

CBE Poster #494

Date: 05/2009
Title: **Microbial conversion of biodiesel byproducts to biofuel**
Authors: ***Kelly O'Shea*** and M.W. Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Biodiesel, an alternative to fossil fuels, is derived from carbon-fixing biological sources. One of the major byproducts from biodiesel production is crude glycerin. *Desulfovibrio vietnamensis* and *Desulfovibrio alcoholvorans* 6133 can oxidize lactate with sulfate as the electron acceptor. Both SRBs can grow syntrophically with methanogens, which replace sulfate as the terminal electron acceptors in a co-culture. Cultures of *D. vietnamensis* and *D. alcoholvorans* 6133 were investigated for their ability to utilize glycerol, cleaned glycerin, and crude glycerin as a carbon and energy source. Growth rates were measured via optical density (600nm). Lactate displayed the fastest growth rates for both strains. *D. vietnamensis* outgrew *D. alcoholvorans* 6133 on all four different carbon sources. Both *D. vietnamensis* and *D. alcoholvorans* experienced a lag in growth when transferred from lactate to glycerol, and another lag when transferred from glycerol to cleaned glycerin. The crude glycerin was a complex mixture of glycerol, salts, and methanol. Due to precipitation, it was not possible to measure the optical density of bacteria in the glycerin media. Different amounts of co-culture inoculations of *D. vietnamensis* and *Methanococcus maripaludis*, a hydrotropic methanogen; *D. alcoholvorans* 6133 and *M. maripaludis*; and *D. alcoholvorans* 6133 and *Methanoculleus marisnigri*, an acetoclastic methanogen, were also tested for their ability to convert varying concentrations of glycerol, cleaned glycerin, and crude glycerin into methane. Methane concentration was measured using gas chromatography. There was a lag in growth for each co-culture transferred from glycerol to cleaned glycerin and glycerin, and higher inoculations produced more methane in a shorter time period. The co-culture *D. alcoholvorans* 6133 and *M. marisnigri* produced the highest concentration of methane, while *D. vietnamensis* and *M. maripaludis* produced the lowest. The ability to utilize glycerin as a feedstock for microbial conversions will circumvent industrial purification processes and will possibly alleviate price constraints for the biodiesel market.

CBE Poster #495

Date: 04/2009
Title: **Quorum sensing, nutrient availability and temperature affect *Escherichia coli* K-12 biofilm antibiotic tolerance**
Authors: ***Trevor R. Zuroff***, J.D. Lloyd-Randolfi, H. Bernstein, and R.P. Carlson
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsor: NIH

Biofilms are thought to be involved in more than half of all medical infections and cost the U.S. healthcare system billions of dollars every year. Interrupting bacterial communication is thought to have great potential as an effective biofilm treatment strategy. This cell-cell communication, known as quorum sensing, has been found to play a critical role in the regulation of many gene functions, including those associated with virulence factors. This study focuses on the role of quorum sensing in *Escherichia coli* K-12 biofilm formation and antibiotic resistance. Four experimental strains were studied—each with a different whole gene knockout in the AI-2 quorum sensing circuit. The role quorum sensing plays in antibiotic susceptibility was tested using the shear-free colony biofilm system. Contrary to conventional wisdom, disrupting the quorum sensing circuit actually made the cells more than ten million times more tolerant to the antibiotic ampicillin as compared to the wild type cells. This effect was studied under different nutritional environments and found to vary with different antibiotics, with the presence or absence of different carbon and energy sources and with temperature. Examination of these variables gives a great deal of insight into the realm of bacterial communication and its role in biofilm antibacterial tolerance. The conclusions from this work could potentially lead to highly tailored, environment-specific antimicrobial treatments for controlling problematic biofilms.

CBE Poster #496

Date: 07/2009
Title: **Community based risk assessment on the Crow Reservation**
Authors: ***Mari J. Eggers***[1,2], C. Cummins[2,3], C. Richards[1], S. Plaggemeyer[1,2], the Crow Environmental Health Steering Committee[3], S. Hamner[1], S. Broadaway[1], T. Ford[4] and A. Camper[5]
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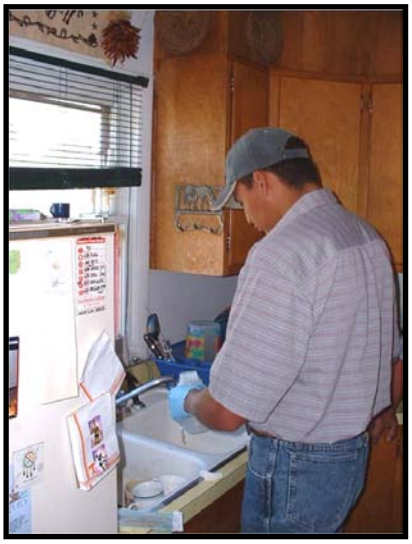
Sponsors: INBRE, NCRR-NIH grant No. P20 RR-16455-04; Center for Native Health Partnerships, NCMHHD-NIH grant No. P20MD002317; and an EPA STAR graduate fellowship #FP91674401.



Our project is a community based risk assessment of exposure to chemical and bacterial contaminants via domestic and cultural water sources on the Crow Reservation in south central Montana. Our hypotheses are that (a) shallow wells, traditional uses of river water, subsistence foods, land leasing practices, and additional aspects of Crow Reservation communities place residents at an increased risk of exposure to environmental contaminants and pathogens via water sources, local foods, and home environments; (b) following Community Based Participatory Research (CBPR) principles in conducting risk assessment, risk communication, and risk mitigation will be an effective way to reduce health disparities in our community and potentially in other Reservation communities, and (c) when Tribal College science majors are the research interns, community capacity to address environmental health issues will be strengthened. Our project is a partnership among Little Big Horn College (LBHC, the local Tribal

College), the Crow Tribe, the Indian Health Service Hospital, the Apsaalooke [Crow] Water and Wastewater Authority, Montana State University and the University of New England. Representatives of all partners meet monthly as the Crow Environmental Health Steering Committee to guide our work.

Family surveys to assess routes of exposure, home radon tests, bacterial and comprehensive chemical analyses of domestic and cultural water sources, and mercury analyses of local fish are being conducted, and along with existing data, will provide the basis for our risk assessment. Data gathered to date have identified several concerns: substantial fecal contamination of and high conductivity levels (a pollution indicator) in all three culturally important rivers, as well as high mercury levels in edible fish species. Initial well testing data indicates that bacterial contamination of wells may be common. Data are being used by the community to seek funding to upgrade the water and wastewater treatment systems.

A dozen Tribal College science majors have gained meaningful research training and experience in environmental health over the past three years. Our interns work hard and are committed to gathering quality data that will be helpful to the community in addressing local environmental health issues.

The members of the Crow Environmental Health Steering Committee are: Urban Bear Don't Walk, Ada Bends, John Doyle, William Driftwood, Roberta Fitch, Brandon Goodluck, Larry Kindness, Myra Lefthand, Rose Morrison and Susette Nanto Spang.

CBE Poster #497

Date: 05/2009

Title: **Temporal and spatial organization within a syntrophic bacterial-archaeal biofilm**

Authors: ***Kristen Brileya***[1], C. Walker[2], S. Stolyar[2], D.A. Stahl[2], A.P. Arkin[3], T.C. Hazen[3], and M.W. Fields[1]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]University of Washington;
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Sponsors: Student funding provided by NSF-IGERT Program in Geobiological Systems (DGE 0654336) at Montana State University; ESPP2 (MDCASE) is part of the Virtual Institute for Microbial Stress and Survival (VIMSS) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program: GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

A syntrophic co-culture of the sulfate-reducing bacterium, *Desulfovibrio vulgaris* Hildenborough, and the methanogenic archaeon *Methanococcus maripaludis* was selected as a basal community that can directly and indirectly interact as a biofilm. It was hypothesized that hydrogen transfer would dictate co-culture biofilm formation in the absence of sulfate as terminal electron acceptor for *D. vulgaris* and without addition of hydrogen as electron donor for the methanogen. *M. maripaludis* did not form significant biofilms on a glass surface in batch and continuous mono-culture experiments, but *D. vulgaris* did. However, *M. maripaludis* did form a pellicle-like structure in batch, static cultures. A biofilm reactor was developed to co-culture *D. vulgaris* and *M. maripaludis* during syntrophic growth, and spatial and temporal organization was characterized using qPCR, epifluorescent microscopy, field emission scanning electron microscopy, methane production and protein and carbohydrate analysis. During early development, the biofilm initiated as a monolayer of *D. vulgaris* cells, and the mainly *D. vulgaris* biofilm contained extracellular filaments that have been previously described. Soon after the development of the *D. vulgaris* biofilm, *M. maripaludis* cells were observed, and the number of planktonic phase cells declined as the number of biofilm cells increased for both populations. Over time, the methanogenic biofilm stabilized, and the ratio of *D. vulgaris* to *M. maripaludis* cells was approximately 2.5, a similar ratio observed for cultures populated entirely by planktonic cells. However, at later time points, the planktonic populations had a ratio of approximately 0.2, and this ratio was significantly lower compared to biofilm. Both populations had 1- to 2-log more cells in the biofilm than the planktonic phase. As the methanogenic biofilm developed, extracellular structures continued to be observed. The results suggested that *D. vulgaris* initiated and

established a biofilm that then recruited *M. maripaludis*, and the biofilm grew and changed over time as the numbers of both populations increased.

CBE Poster #498

Date: 07/2009
Title: **Research support for designing a comprehensive biofilm efficacy test system**
Authors: ***Salman Adam, Steven Anderson***, and D.K. Walker
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsor: The Montana Board of Research and Commercialization Technology

Standard reactor systems exist for growing biofilm. These reactors house coupons on which the bacteria attach, forming the biofilm. The coupons containing the biofilm can then be removed from the reactor and used in efficacy testing. Biofilm efficacy testing includes numerous coupon manipulations, and the standard method for biofilm sampling allows for processing only one coupon at a time. The goal of this research project is to design a tool or device which minimizes manipulations and the amount of time required for sampling, thereby making the procedure more efficient. The successful design will adhere to all good laboratory practices (GLP) requirements.

Previous research resulted in the design of two prototype coupon manipulation tools to hold the coupon during efficacy testing. These coupon holders were constructed out of four materials: Teflon, polypropylene, polyethylene, and polysulfone. Other materials may also be favorable, such as acetyl, or plastics that are inexpensive and have high melting temperatures. Another requirement is that the device needs be able to handle pressure and extreme temperatures above 120°C. There are many materials out in the manufacturing world with such capabilities, but the added constraints of the material being chemically inert, economical, meeting statistical requirements, and yielding low manufacturing costs are very difficult to find in a particular material. This poster will describe the constraints placed upon materials used in biofilm research and selecting the best material for the construction.

CBE Poster #499

Date: 07/2009
Title: **Growth effects of oxygen exposure on *Desulfovibrio vulgaris* planktonic and biofilm cells**
Authors: ***Anitha Sundararajan*** and M.W. Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsor: ESPP2 (MDCASE), part of the Virtual Institute for Microbial Stress and Survival (VIMSS) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

Desulfovibrio vulgaris Hildenborough 29579, although an obligate anaerobe, is capable of surviving in environments that are exposed to millimolar levels of oxygen. Previous studies in *D. vulgaris* have shown that oxygen consumption and growth are decoupled when grown planktonically. *D. vulgaris* is capable of forming biofilms when grown anaerobically, and sulfate-reducing bacteria in natural environments such as subsurface sediments are more readily exposed to low concentrations of dissolved oxygen rather than air. In this study, planktonic and biofilm cells were exposed to different concentrations of dissolved oxygen to establish if exposure had any effects on cells and/or biofilm formation. Both planktonic and biofilm cells were washed anoxically to lower the sulfide levels upon re-inoculation in the presence of dissolved oxygen, and sulfide levels normalized to protein were similar between planktonic and biofilm cells. Results based on growth experiments revealed that the sensitivity of biofilm and planktonic cells was similar above 5 mg/l dissolved oxygen, and both cell types had almost a complete loss of cell viability. Biofilm cells appeared to be more sensitive compared to planktonic cells as indicated by lag periods and reduced growth rates when re-suspended as planktonic cells. However, biofilm cells still lagged when re-suspended in low levels of dissolved oxygen. In addition, biofilm formation was not hindered at up to 3 mg/l dissolved oxygen. The results suggested that both planktonic and biofilm *D. vulgaris* cells could tolerate exposures

under 5 mg/l dissolved oxygen, exposure over 5 mg/l dissolved oxygen caused significant cell death, and that biofilm cells lagged when transitioned to a planktonic growth mode.

CBE Poster #500

Date: 05/2009
Title: **Strain specific transcriptional responses of *Pseudomonas aeruginosa* to calcium during biofilm growth**
Authors: ***Kerry Williamson***, Marianna Patrauchan, and Michael J. Franklin
Affiliation: Department of Microbiology, Montana State University, Bozeman, MT
Sponsors: NIH-NIAID; J. Craig Venter Institute

Pseudomonas aeruginosa is an opportunistic pathogen that poses serious threats to the health of those with compromised immune systems, burn wounds, indwelling medical devices, and cystic fibrosis (CF). It has the ability to form biofilms, which exhibit increased tolerance of antibiotics and host immune responses.

Calcium plays a key role in bacterial signaling, induces extracellular virulence responses, and influences the structure of biofilms. Elevated calcium levels are present in the lungs of CF patients, due to the pathology of the disease. Therefore, a better understanding of the physiological effects of increased calcium levels on *P. aeruginosa* is needed.

Through microarray technology, transcriptomic expression profiles can be obtained to determine bacterial responses to elevated calcium levels, and perhaps illuminate mechanisms of interest.

CBE Poster #501

Date: 05/2009
Title: **Stratified growth rate, *rpoS* and *rhlR* expression levels in *Pseudomonas aeruginosa* biofilms**
Authors: Ailyn C. Perez-Osorio, Kerry S. Williamson, and ***Michael J. Franklin***
Affiliation: Department of Microbiology, Montana State University, Bozeman, MT

Nutrient and oxygen gradients form within biofilms and induce the bacteria to respond in a localized manner. Here, we combined laser capture microdissection microscopy (LCMM) with qPCR and qRT-PCR to characterize the gene expression patterns that emerge as a response to these gradients. Cell numbers were estimated by genome copies (qPCR of 16S rDNA) from discrete locations within biofilms. By using the 16S rRNA to 16S rDNA ratio at different biofilm depths, the growth status of cells isolated from discrete regions within biofilms was determined. Since 16S rDNA can be used as a normalizing factor by serving as a measure of cell counts, the copy number for individual genes on a per cell basis was estimated. This allowed the determination of localized ribosomal content as well as expression levels of *rpoS*, *rhlR*, and the housekeeping gene, *acpP* on a per cell basis in biofilms. The present work represents the development of alternative internal controls that are suitable for normalizing qRT-PCR data derived from limited cell numbers obtained from biofilms by LCMM. In addition, we demonstrate the stratification of growth rates among cells growing in biofilms, where the highest cellular rRNA levels were found near the air-biofilm interface.

Additional posters:

Optimization of a biofilm disinfectant test method for supporting efficacy claims

Author: Marc Rindal
Affiliation: EPA, Fort Meade, MD

Background: The EPA is responsible for registering antimicrobial products used to control biofilm. Antimicrobial products with claims to prevent, destroy, repel or mitigate biofilm require an EPA registration; product efficacy data supporting the claims are required for registration. Currently, the EPA does not recognize a specific method for evaluating disinfectant products against biofilm. As a result, the EPA is seeking to develop a quantitative method for measuring the efficacy of biofilm disinfectants that is relevant, robust, valid, rugged, and efficient. In this project, the CDC biofilm reactor method (ASTM # E2562-07) was used to grow *Pseudomonas aeruginosa* (*P.a*.) biofilm. A draft ASTM procedure (ASTM #WK Item #17314) was evaluated and modified as needed for efficacy data generation. Options for removal, disaggregation and enumeration of the biofilm from coupons for the purpose of performing product efficacy studies were explored. **Methods:** In ASTM #WK Item #17314, disinfectants are challenged with coupons (e.g., stainless steel discs) contaminated with a *P. a.* biofilm from the CDC reactor. The method calls for the transfer of coupons into multiple tubes (disinfectant and neutralizer), removal of the biofilm by scraping, disaggregation using homogenization, and enumeration by direct plating. Product efficacy is measured by comparing the log density of surviving cells to the number of cells from untreated controls. In this exercise, a single tube approach was evaluated and instead of scraping, combinations of sonication and vortexing of control and treated coupons were performed to assess removal and repeatability of results. **Results:** A log density ranging from 8.07 to 8.29 CFU/cm2 was consistently achieved from disaggregated biofilm recovered through sonication (with and without the addition of glass beads) and was equivalent to the log density achieved through scraping and homogenization (7.88 to 8.21 CFU/cm2). **Conclusions:** The ASTM Draft Procedure in conjunction with the CDC reactor can be successfully conducted in a simplified manner using a single tube approach with sonication.