Montana State University

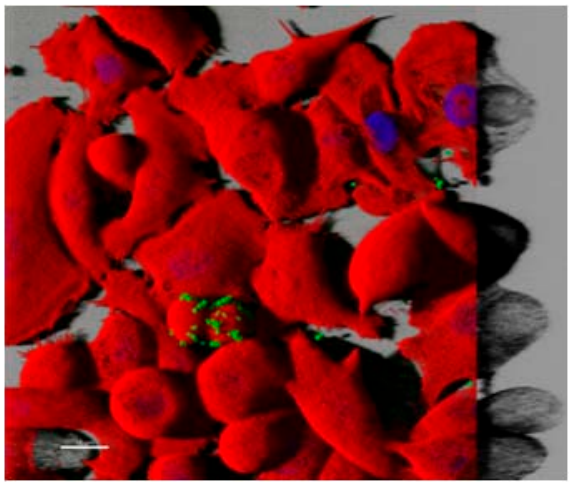
Center for Biofilm Engineering

Bozeman

montana biofilm SCIENCE & TECHNOLOGY meeting

February 8–10, 2010

proceedings



MSU-CBE, P. Secor

Montana Biofilm Science & Technology Meeting: February 8–10, 2010

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Speaker Abstracts

SESSION 1: Industrial Water Treatment

Biofilm control in industrial processes

Presenter: Laura E. Rice, Research Scientist
Affiliation: Nalco Company, Naperville, IL

Biofilm formation in industrial processes can lead to reduced manufacturing efficiency, reduced energy efficiency, corrosion, and poor product quality. Biocides are routinely used to control microbial growth that can lead to biofilm formation. The global market for active biocides is reportedly $5 billion annually. Each application presents specific technical, regulatory, and economic challenges that must be addressed appropriately for effective control and compliance. Biofilm-related problems specific to several industrial applications will be reviewed along with standard treatment strategies and industry best practices. Recent trends aimed at reducing raw material and energy costs in industrial applications and the potential impact of these changes on biofilm formation and control strategies will also be discussed.

Recycled/reclaimed water: Synopsis of workshops

Presenter: Anne Camper, Professor, Civil Engineering and Associate Dean, College of Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Recycled/reclaimed water is of growing importance because sources of fresh water are becoming increasingly compromised and scarce. Many of the same issues facing other water users are being examined by those responsible for the quality of distributed reclaimed/recycled water. Because most reclaimed/recycled water is generated from municipal wastewater, a great deal of concern revolves around water quality in distribution systems that transmit recycled/reclaimed water. There is particular interest in biofilm formation because these waters are usually high in organic carbon, nitrogen, and phosphate, which support microbial growth. Adequate disinfection of recycled/reclaimed water is also a significant issue. The industry is also aware that these environments may be conducive to pathogen survival, particularly in the biofilms. This presentation will be based on the content of two workshops held on existing and emerging challenges in systems that use recycled/reclaimed water. The first workshop was held on October 11, 2009, at WEFTEC (sponsored by the Water Environment Federation) in Orlando, Florida, and the second workshop, sponsored by the US EPA and the University of North Carolina, Chapel Hill, and held February 2–4, 2010. Major points from these conferences will be summarized, with emphasis on the regulatory environment and biofilms.

The influence of biofilms in the drinking water treatment industry

Presenter: Ben Klayman, PhD, PE; Process Engineer
Affiliation: Black & Veatch Corporation

It is widely understood that microbial biofilms are present everywhere that water comes into contact with a solid surface. This opportunity for microbial biofilm development exists in numerous locations throughout the drinking water treatment process, from the source water intake all the way to the customer's tap. Many drinking water treatment practices have been established specifically to address the presence of microbial biofilms.

This presentation will outline the major processes involved in drinking water treatment, with an emphasis on the influence of microbial biofilms. A review of the major chemicals and techniques used for microbial control will be covered, including how their implementation is oftentimes restricted by federal and state regulations and/or water quality outcomes. Case studies will be presented illustrating various examples of how treatment techniques are focused on dealing with microbial biofilms, including combating fouling of intake screens, channels, and water treatment equipment; controlling microbially induced corrosion and degradation of water quality through careful operation of the distribution system; and the intentional beneficial use of biofilms in water treatment.

Extremophile survival in industrial water

Presenter: Abbie Richards, Assistant Professor, Chemical and Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Many industrial processes operate under harsh conditions including high temperature, high pressure, high or low pH, and high osmotic strength. Such conditions often inhibit the growth of biofilm-forming microorganisms such as *Escherichia coli* and *Pseudomonas aeruginosa*. Certain microbes, however, generally classified as extremophiles, have adapted so they can thrive at temperatures over 100°C, with pH as low as 1 and as high as 11, and salinity up to saturation. If process conditions mimic terrestrial environments that are home to extremophiles, it is likely that these organisms could contaminate and grow within industrial processes. While the elimination of biofilm of non-extremophiles is difficult in itself, the eradication or even detection of contaminating extremophiles can present unique challenges. Adaptations of standard biofilm methods to permit biofilm growth of halophiles (salt loving microbes) and isolation/detection techniques will be discussed.

SESSION 2: Enzymes and biofilms

Biofilms: If you can't beat 'em, join 'em

Presenter: Sarah McHatton, R&D Group Leader, Institutional & Household Products
Affiliation: Novozymes Biologicals, Inc.

Bacteria have evolved impressive resistance to many antibiotics and biocides and biofilms can be particularly recalcitrant to such chemical control measures. A new approach (sometimes called bioaugmentation, bacteriotherapy, probiotic treatments, etc.) involves introducing beneficial microorganisms to an environment that is otherwise susceptible to undesirable bacteria and the disease, odor, fouling or other problems they can cause. These beneficial inoculants have a variety of modes of action, including formation of protective biofilms that prevent attachment of other microbes, competition for nutrients, and secretion of molecules that suppress growth or inhibit quorum sensing of undesirable bacteria. Beneficial bacteria/biofilm studies conducted by Novozymes Biologicals will be discussed in this presentation, and industrial and medical applications of beneficial bacteria from the literature will be reviewed.

Dispersin B antibiofilm enzyme: Scientific and commercial perspectives

Presenter: Sri Madhyastha, Chief Scientific Officer
Affiliation: Kane Biotech, Inc., Winnipeg, MB, Canada

The unique ability of dispersin B enzyme to specifically inhibit biofilm formation without affecting bacterial growth and to disperse preformed biofilms makes it a first of its kind antibiofilm enzyme. Dispersin B is

effective against biofilms of major bacterial pathogens. Enzymes such as dispersin B that degrade matrix polymers and destroy the physical integrity of the biofilm matrix have been shown to sensitize biofilm cells to killing by antimicrobial agents, bacteriophages, and macrophages *in vitro*. This talk will focus on some of the scientific and commercial aspects of the dispersin B enzyme. This enzyme has proven to be a useful tool for identifying the structural components of the biofilm matrix, for determining the physical and chemical properties of the matrix, and for elucidating the role of matrix polymers in biofilm-mediated antibiotic/antimicrobial resistance in a variety of bacterial species. Furthermore, dispersin B has been shown to be compatible and synergistic with most of the antimicrobials and enzymes, including deoxyribonuclease 1. Clinically, the use of dispersin B could be an attractive anti-biofilm strategy for the treatment and prophylaxis of bacterial infections involving biofilms. Commercially, dispersin B as an anti-biofilm component has applications in antimicrobial coatings of medical devices, wound care products, and for treating cystic fibrosis.

Disruption of *Pseudomonas aeruginosa* biofilms by enzymes

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

The matrix polymers of a biofilm are vulnerable to degradation by enzymes. The interaction of enzymes with biofilm can provide some basic insight into the biochemical nature of the matrix and has potential to lead to strategies for controlling detrimental biofilms. This project investigates the susceptibility to enzyme attack of biofilms formed by the model organism *P. aeruginosa*. Literature reports and unpublished data from other work suggest that the matrix of *P. aeruginosa* biofilms could contain extracellular DNA, *pel* and *psl* polysaccharides, and proteinaceous components pili and CdrA. Enzymes used in this work include DNAase, protease, amylase, and cellulase. Measurements made include: 1) biofilm removal effected by static treatment with enzyme solutions for both drip-flow reactor and CDC reactor grown biofilms, 2) changes in biofilm material properties in response to enzyme treatment measured by atomic force microscopy, and 3) changes in apparent viscosity of a biofilm suspension in response to enzyme treatment. Collectively the preliminary results indicate that all four enzymes can reduce the cohesiveness of *P. aeruginosa* biofilms, with amylase having the most consistent and strongest effects. These results support a model of *P. aeruginosa* biofilm cohesion in which multiple extracellular polymeric substances interact to provide mechanical integrity of the biofilm.

Next generation sequencing: Implications for biofilm research

Presenter: Seth D'Imperio, Visiting Scientist, (former CBE Postdoctoral Researcher)
Affiliation: Novozymes Biologicals, Inc.

Molecular techniques have been an integral part of biofilm research since its inception. While many of these methods have enjoyed significant improvements over the last twenty years, DNA sequencing technology has remained relatively static until recently. The advent of so-called "Next Generation" sequencing platforms has changed the landscape of molecular research in the past five years and opened the door for new methods of analysis. Several of these new sequencing methods will be discussed and their attributes and pitfalls compared in the context of biofilm research.

Algal biofuels

Presenter: Brent Peyton, Professor, Chemical and Biological Engineering;
Associate Director, Thermal Biology Institute
Co-authors: R Gardner, S D'Imperio, K Cooksey, A Staven, M Fields, R Gerlach, K Moll
Affiliation: Thermal Biology Institute and Center for Biofilm Engineering,
Montana State University, Bozeman, MT

Economical production of fuels and other products from algae is limited by the ability to maintain sustained growth in pond-type reactors. In these open systems, long-term productivity of desired organisms may be limited by (1) contamination by competing microbial species or predators, (2) mass transfer of carbon dioxide, and (3) sufficient availability of quality water. Lower quality water can be used for growth and ponds can be built on non-arable land, so that issues of food-for-fuel are eliminated. Results will be presented on the isolation and characterization of lipid-producing microalgae isolated from a variety of environments. Data include culture optimization, algal growth kinetics, growth yields, and lipid production rates and yields. This bio/chemical engineering approach is critical to the optimization of lipid production and the future design and scale-up of large algae to fuel and chemical systems.

Highlights from recent biofilm meetings

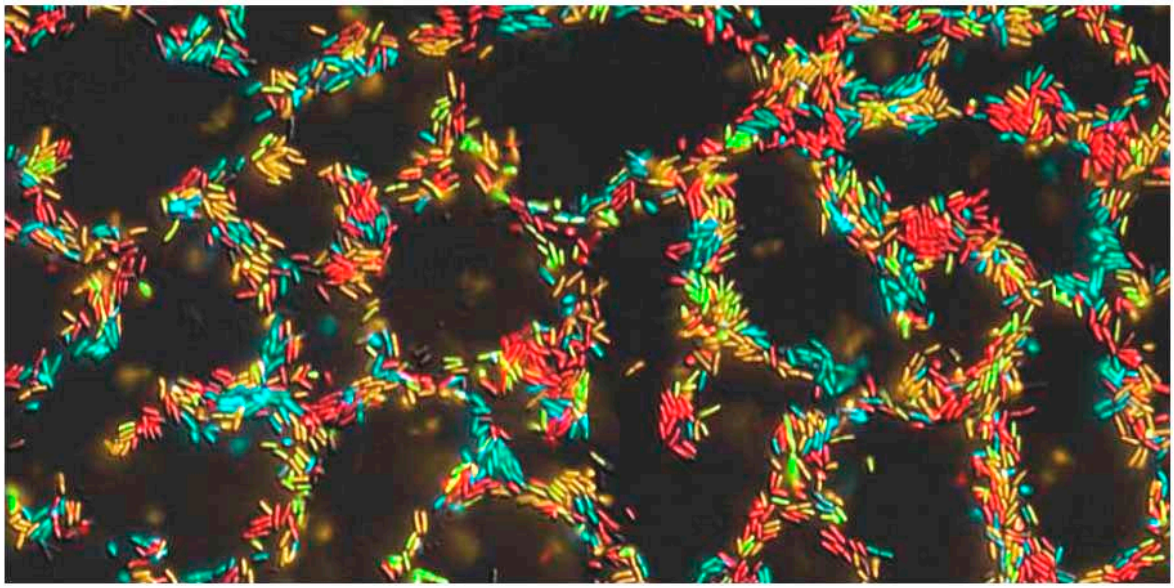
Presenter: Phil Stewart, CBE Director
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Highlights from two recent international biofilm-themed meetings will be briefly presented. The first meeting was the inaugural gathering of what looks to be a new series; it was dubbed EuroBiofilms 2009 and took place in Rome in early September. The American Society for Microbiology held its fifth special conference on biofilms in Cancun, Mexico, in November. Both meetings had over 400 attendees. Four recurrent themes and two methods were chosen for mention. The four themes are molecular ecology, the biofilm matrix, cyclic di-GMP, and differentiation in biofilms. Molecular ecology refers to the use of sequencing technology to identify microorganisms in mixed-population communities. Two examples from the dental world are shared here to illustrate ongoing efforts to relate community profiles to states of disease or function. Molecular ecology techniques are advancing rapidly, dropping in price, and seem likely to become widely used complements to traditional assays of colony formation. Many researchers reported finding extracellular DNA in the matrix of biofilms. In addition, new research suggests that multiple polymers interact in the matrix to provide cohesion. Cyclic di-GMP is an intracellular "secondary messenger" involved in a global switch between a dispersed, motile lifestyle and a sessile, biofilm lifestyle. This paradigm continues to gather momentum as the number of bacteria in which it operates grows. Now details of the pathways by which an environmental cue such as phosphate concentration or oxygen concentration is translated into a decision to make or break a biofilm are emerging, with cdiGMP figuring in the middle of the pathway. One example illustrates the transduction of a signal (nitric oxide concentration) into a response that results in active dispersal of the biofilm. Biofilms harbor cells in distinct physiological states, even biofilms formed by a single species. These special cell states can arise by mutation and selection, or by extracellular signaling and genetic regulatory pathways. Differentiation is a reality that has to be confronted both in laboratory research and practical applications. Finally, two methods are shared: a method for fluorescent illumination of active cells in a biofilm is illustrated with a stunning micrograph and a method for performing controlled experiments with well-defined packets of bacteria entrapped in microfabricated structures.

Social evolution theory and biofilms

Presenter: Joao Xavier, Assistant Professor, Computational Biology
Affiliation: Center for Systems Biology, Harvard University, Cambridge, MA, and Sloan-Kettering Institute of the Memorial Sloan-Kettering Cancer Center, New York, NY (starting December 1, 2009)

Darwin's theory of natural selection suggests that individuals will strive to selfishly increase their reproductive success. However, **cooperation**, where individuals perform apparently costly actions to benefit others, is observed at all levels of biological organization. This well known conundrum of evolutionary biology applies readily to biofilms: Bacteria come together to form elaborate biofilms, with striking structures and collective functions that suggest strong cooperation. Yet, bacteria are individual organisms subject to natural selection. What prevents them from exploiting their neighbors for a short-term evolutionary benefit that would harm the entire biofilm? I will show how we can analyze this problem under the lens of 'social evolution theory', a field that aims to explain the evolution of cooperation. Using computational models and experiments with *Pseudomonas aeruginosa*, we have shown that bacteria have figured out ways to solve the conflict between their individual and social interests. Our findings have potential applications to the control of unwanted biofilms but also demonstrate how biofilms are powerful model systems in evolutionary biology. In the end even we, as a society, can take lessons on how to live cooperatively from the social lives of bacteria.



Patterns in a biofilm of *P. aeruginosa*. From Xavier, Martinez-Garcia, Foster, "Social evolution of spatial patterns in bacterial biofilms: when conflict drives disorder". Am Nat, 2009. 174(1): p. 1-12.

See also:

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Regulation of toxicity in *Pseudomonas aeruginosa* biofilms by the magnesium transporter MgtE

Presenter: Gregory Anderson, Assistant Professor, Biology
Affiliation: Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202

Pseudomonas aeruginosa establishes chronic biofilm infections in the lungs of individuals with cystic fibrosis (CF). During this process, the bacteria transition from an acute, virulent phenotype to the biofilm phenotype, which is characterized by a general decrease in toxicity. Recent studies have begun to elucidate the signals that influence this transition during biofilm formation on abiotic surfaces. To investigate chronic *P. aeruginosa* biofilm formation in the context of CF lung infection, we have created a novel co-culture model system wherein *P. aeruginosa* biofilms develop directly on CF-derived airway epithelial cells in culture. These co-culture biofilms display similar characteristics to *P. aeruginosa* biofilms formed on abiotic surfaces, including morphology, requirement of the same genetic factors, similar gene expression, similar nutritional requirements, and increased antibiotic resistance compared to planktonic bacteria. Using this co-culture model system, we have performed a microarray analysis of tobramycin-treated biofilms. One gene upregulated in this experiment (*mgtE*) encodes a putative magnesium transporter. Intriguingly, mutation of this gene led to increased cytotoxicity of the bacteria toward the epithelial cells, while antibiotic resistance remained unaffected. This cytotoxicity effect required a functional bacterial type III secretion system (T3SS). We found that *mgtE* mutation enhances transcription of T3SS, while *mgtE* overexpression inhibits T3SS transcription. This effect appeared to be mediated via direct or indirect interactions with the T3SS transcriptional activator ExsA. We further found that while *P. aeruginosa* MgtE can, in fact, transport magnesium, this function is dispensable for the T3SS-mediating function of the protein. T3SS is highly expressed during acute *P. aeruginosa* infections, but it is generally downregulated during chronic biofilm formation. Thus, using our novel biofilm co-culture model system, we have begun to dissect the genetic mechanisms involved in transitioning between acute and chronic infection lifestyles. Future investigations into the regulation of these factors may uncover the environmental signals that can manipulate the *P. aeruginosa* infection phenotype in the CF lung.

SESSION 3: Healthcare Associated Biofilms

Lessons from medical device testing

Presenter: Garth James, CBE Medical Biofilm Laboratory Manager
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

The Medical Biofilm Laboratory at the Center for Biofilm engineering has performed in vitro testing of a wide variety of medical devices in relation to microbial attachment and subsequent biofilm formation. Experimental design must balance model simplicity with simulating the clinical conditions of use. Furthermore, the design must enable the detection of significant differences between test and control devices. The most effective antimicrobial device strategies we have tested to date are surfaces that exude antimicrobial agents. However, this approach has limitations—such as the duration of efficacy—and also raises concerns about the development and spread of antibiotic resistant microorganisms. Establishing new approaches for preventing and treating infection related to medical devices is an extremely active area of medical research and development. Comparison of results of in vitro and in vivo testing, as well as clinical trials and use may help us validate and refine our test methods. Ultimately, the development of antimicrobial medical devices promises to help reduce the incidence and prevalence of healthcare-associated infections.

Making health care-associated infections *go away*: Embracing a biofilm strategy

Presenters: Deborah Burton, Vice President and Chief Nursing Officer, and
Marly Christenson, System Director, Clinical Transformation
Affiliation: Providence Health & Services, Renton, WA

This session will summarize work in progress aimed at eliminating CAUTI as part of Providence Health & Services' strategy to address all healthcare associated infections (HAI) across its five state health system. Despite decades of solid evidence, the CAUTI problem continues to thrive in health facilities. In its HAI journey, Providence identified pooled urine in Foley catheter drainage systems as a preventable source of equipment-related infection challenges in driving out CAUTI. In its quest to revolutionize and improve the nearly 80-year-old Foley drainage system design to prevent microbe and biofilm accumulation, Providence aligned with a urologics supply company and the Center for Biofilm Engineering. Providence's overall HAI and CAUTI strategy, as well as lessons learned and next steps in this Foley drainage system project will be highlighted.

Review of healthcare associated infections (HAI) on medical devices

Presenters: Elinor Pulcini, Research Scientist, and Diane Walker, Research Engineer
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

We will present a summary of a literature review conducted on antimicrobial medical devices, including in vitro test methods and modes of action of antimicrobial surfaces. The Health and Human Services Action Plan to Prevent Healthcare Associated Infections has indicated gaps in knowledge and practice. HHS identified biofilms and their role in infection as a crucial knowledge gap. The goal of this literature summary was to examine test methods that may help with making colonization claims with the FDA.

Vascular catheter infections. . . technical challenges for industry

Presenter: Marcia Ryder, RN, MS, PhD; Consultant
Affiliation: Ryder Science

Healthcare associated infections have escalated to the fourth leading cause of death in the United States. At the same time, antimicrobial resistance has heightened to the level of a global threat. A majority of these infections are associated with some type of implanted medical device. Despite historic efforts to prevent and treat these infections, the incidence, morbidity and mortality, and associated costs continue to rise to a level of intolerance by consumers, government agencies, and payors. The current demand for improvement is compelling and the healthcare system is forced to sort out why our current approaches to prevention, diagnosis, and treatment of these chronic infections are not working. The world of medical microbiology has been rocked by discoveries related to microbial biofilm survival strategies that enable them to persist on medical devices and biological tissue in protected communities that facilitate their profound recalcitrance to antimicrobial therapy. Our current clinical methodology for diagnosis and treatment remains erroneously centered around the single planktonic cell theory and so are the regulatory parameters, since there has been no standardization in surrogate or real-time measurement for biofilm as a precursor to infection.

This presents a unique challenge for industry in that new products developed for infection prevention or treatment must be bound to the precise understanding of pathogenesis of infection, clinically relevant testing models, biofilm analysis, and clinical diagnostic methodology.

Goal: The goal of this presentation is to provide insight on issues surrounding the invention and development of antimicrobial vascular access products from a clinical perspective.

Keratinocytes produce pro-inflammatory markers in *Staphylococcus aureus* biofilm infection

Presenter: Pat Secor, PhD candidate, Cell Biology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Chronic wounds are characterized by prolonged inflammation and elevated neutrophil levels. The keratinocyte is the major cell type in the epidermis, has the ability to produce several pro-inflammatory molecules, and is an active participant in the inflammatory process. Few studies have investigated the effects of bacterial biofilms on epidermal cells. Most studies involve either direct contact with washed planktonic bacteria or the application of known or unknown soluble products from overnight cultures grown with constant agitation to various mammalian cell cultures.

Changes in gene expression patterns of primary human keratinocytes in response to *Staphylococcus aureus* biofilm and planktonic *S. aureus* soluble products were analyzed using microarray technology. Host cells were exposed to soluble (bacteria free) planktonic bacterial products or cell free bacterial biofilm soluble products. It was found that soluble products from *S. aureus* biofilm induced higher expression of genes that encode for pro-inflammatory molecules such as cytokines (TNFα, IL-6) and chemokines (IL-8, CXCL1, CXCL2, and CXCL3) than planktonic *S. aureus*. IL-8 and the C-X-C ligand family members are strong chemoattractants for neutrophils and are a component of the innate immune response. *S. aureus* biofilms induce higher expression levels of chemokines *in vitro* and may induce abnormal levels of chemokine production in vivo, leading to unbalanced regulation of a complex chemokine network which may result in chronic activation of the inflammatory response and sustained neutrophil levels observed in chronic wounds.

Pressure ulcers: The human element

Presenter: Karen Zulkowski, DNS, RN, CWS; Associate Professor, Nursing
Affiliation: Montana State University, Bozeman, MT

There is a human face to the people with wounds and many of the persons with pressure ulcers are older and sicker. Persons with pressure ulcers are more likely to go from hospital to nursing home than any other condition and 60,000 people die each year as a direct result of their pressure ulcers. In addition, the number of persons with both primary and secondary diagnosis of pressure ulcers is rising.

Because persons with pressure ulcers are older and sicker, their wounds often become chronic. Approximately 60% of chronic wounds have been found to contain biofilm. Yet translating bench to bedside testing is not possible. Lack of readily available testing limits the medical provider's ability to accurately diagnosis and treat the wound.

The purpose of this presentation is to present the "faces" of those with pressure ulcers, discuss current treatments and numbers of people affected. Future research needs with practice applications will be presented.

Targeting microbial biofilms with protein cage nanoplatforms

Presenter: Peter Suci, Associate Research Professor, Plant Sciences & Plant Pathology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

Biofilms that colonize surfaces in the oral cavity are responsible for both caries and periodontal disease. The emerging paradigm is that the complex microbial communities that comprise periodontal consortia consist of both beneficial and pathogenic species, and that the bone loss and tissue destruction that accompany periodontal disease are caused primarily by the response of the immune system to pathogenic species. The implication is that periodontal disease can be treated by selectively eliminating putative pathogens, while minimizing the destruction of the commensal members of the community. We are developing a family of protein cage nanoparticles to selectively target biofilm pathogens with combinations of drugs and photosensitizers. Advantages of these protein cage nanoplatforms for this task will be discussed, and results showing that they can be used to selectively target and kill a pathogenic oral biofilm will be presented.

Poster Abstracts

Industry poster

Date: 02/2010

Title: **Control of bacterial biofilms with marine alkaloid derivatives**

Authors: ***Christian Melander***[1,3], L Peng[2], J Cavanagh[1,3], SA Rogers[2], E Garland[1]

Affiliation: [1]Agile Sciences, 840 Main Campus Drive, Raleigh, NC, 27606;
[2]Department of Chemistry, North Carolina State University, Raleigh, NC, 27695;
[3]Department of Molecular and Structural Biochemistry, North Carolina State University, Raleigh, NC, 27695

Derived from the sea-sponge *Agelas conifera* the 2-aminoimidazoles (2-AIs) are an emerging class of small molecules that have the ability to inhibit and disperse both gram-positive and gram-negative bacterial and fungal biofilms as well as mixed species biofilms through a non-microbicidal mechanism. Data will be presented that provides a brief overview of lead 2-AI analogues, together with those results demonstrating the ability of 2-AIs to work synergistically with conventional antibiotics and microbicides to eliminate established biofilms. Moreover, preliminary toxicity experiments suggest that 2-AIs are non-toxic to mammalian systems. Studies demonstrating the potential utility of these compounds in a variety of the applications including healthcare, industry, and agriculture will be presented.

About the technology: The use of 2-aminoimidazoles for remediating biofilm colonization was developed at North Carolina State University in the labs of Dr. Christian Melander (Department of Chemistry) and Dr. John Cavanagh (Department of Biochemistry). Agile Sciences Inc., Raleigh, NC Inc., holds exclusive rights to develop this technology.

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Huigens RW, Richards JJ, Parise G, Ballard TE, Zeng W, Deora R, Melander C, "Inhibition of *Pseudomonas aeruginosa* biofilm formation with bromoageliferin analogues," Journal of the American Chemical Society. 2007**;** *129*, (22), 6966-6967.

Industry poster

Date: 02/2010
Title: **Proprietary treatments to prevent biofouling in capillary flow cells**
Authors: ***Emmalee Terpenning,*** *SM Dean, S Smith, and C Hunt*
Affiliation: Battelle Memorial Institute, Columbus, OH

Biological fouling is an ongoing problem in the marine setting. We have studied the formation of biofilms in capillary cells undergoing proprietary antifouling treatments via in situ microscopy. The benefit to in situ visualization is observation of organisms with minimal disruption to the biofilm, enabling quantitative comparisons of antifouling treatments through analysis of the biofilm structure. Capillary flow cells were provided after being subjected to filtered seawater (30 ppt salinity, 10 to 20 °C) at a rate of approximately 1 ml per minute (~6.1 psi pressure). Control cells received no treatment, and experimental flow cells received proprietary treatment A, B, or A+B. Flow cells were sacrificed at intervals and biofilm formation was visualized in situ using a Zeiss epifluorescent microscope under phase contrast. The cells were then sequentially stained with Cyto-9 fluorescent dye, followed by capsule stain. Biofilm images were analyzed using the Image Structure Analyzer (ISA)-2 software provided by H. Beyenal and C. Donavan (2005) to calculate 2-D parameters of textural entropy, homogeneity, energy, areal porosity, run lengths, diffusion distances, and fractal dimension. Additional control flow cells were included with each analysis set: a negative control prepared with artificial seawater media; a positive control colonized for one week with *Halomonas pacifica* and *Skeletonema costatum*; and a background control, consisting of an untreated capillary cell. The preliminary results are highly suggestive that our proprietary antifouling formulations will be effective antifouling treatments for the flow capillaries, and the possibility exists to explore other antifouling applications with these treatments.

Center for Biofilm Engineering posters

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: 11/2009
Title: **Physiological activities and growth rate 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

The physiological activities and growth status of *P. aeruginosa* in biofilms were investigated via transcriptional profiling. Biofilms of strain PAO1 were grown in vitro for 3 days in drip-flow reactors using a glucose-minimal medium. 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. In 11 published data sets, specific growth rate correlated with the difference in rank of the *rpoS* and *fis* transcripts. Using this measure, the average specific growth rate the biofilm cells was between 0.03 h^{-1} and 0.12 h^{-1}—much less than the maximum specific growth rate of the microorganism in this medium of 0.74 h^{-1}. The biofilm population did not indicate oxidative stress, but did exhibit copper stress. Six of seven indicator genes for homoserine lactone mediated quorum sensing were expressed only at low levels. Efflux pumps were not up-regulated in the biofilm. Of 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 #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 #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], BM Peyton[1], R Gerlach[1], BD Lee[2], A Miller[2], WA Apel[2]
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[2]Idaho National Laboratory, Idaho Falls, ID
Sponsored by: 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: **Zinc ion species toxicity: Inhibition of cell yields and growth of *Arthrobacter* sp.**
Authors: ***James G. Moberly***[1,2], A Staven[2], RK Sani[1,3], and BM Peyton[2]*
Affiliation: [1]Washington State University, School of Chemical and Bioengineering, Pullman, WA;
[2]Current address: Montana State University, Department of Chemical and Biological Engineering, Bozeman, MT;
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Sponsored by: National Science Foundation

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 re-evaluation 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 #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], JE Aston[1], K Brileya[1], Z Jay[2], CG Klatt[2], S McGlynn[2], N Mallette[1], S Montross[2], R Gerlach[1], WP Inskeep[2], and DM Ward[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]Thermal Biology Institute, Montana State University, Bozeman, MT
Sponsored by: National Science Foundation

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 #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]

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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 #499

Date: 07/2009
Title: **Growth effects of oxygen exposure on *Desulfovibrio vulgaris* planktonic and biofilm cells**
Authors: ***Anitha Sundararajan*** and MW Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: 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 US Department of Energy.

Although it is an obligate anaerobe, *Desulfovibrio vulgaris* Hildenborough 29579 is capable of surviving in environments 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 #502

Date: 11/2009
Title: **Evaluation of contaminated bulk soap dispensers for biofilm bacteria: Comparison of two methods of analysis and effectiveness of dispenser washing procedures**
Authors: ***Lindsey Lorenz***[1], B Ramsey[1], M Fields[1], D Goeres[1], C Zapka[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT
[2]GOJO Industries, Inc., Akron, OH
Sponsored by: GOJO Industries, Inc.

Previous research demonstrated that up to 25% of open refillable bulk hand soap dispensers are contaminated with approximately 6 LOG_{10}(CFU/mL) heterotrophic bacteria based upon samples collected from the bulk soap[1]. A research project was initiated to determine if biofilm growth within the dispensers contributed to bulk soap contamination. Additional questions posed were: 1) does dispenser type and/or construction material affect the level and type of contamination, 2) does the bacterial community profile change depending upon the analysis method, and 3) how does the presence of biofilm impact the ability to clean and sanitize the dispensers.

Plastic counter-mounted, plastic wall-mounted, and stainless steel wall-mounted dispensers collected from various locations in Ohio were analyzed for suspended and biofilm bacteria using two approaches: 1) heterotrophic and coliform viable plate counts and total cell counts, or 2) community analysis using PCR and gene sequencing of DNA recovered from the dispensers. Bacterial identifications from the plate counts were performed using biochemical profiling of isolated colonies. Three washing procedures were evaluated

for plastic wall-mounted dispensers: 1) a simple rinsing technique, 2) a rinse and scrubbing technique, and 3) a rinse, scrub, 5,000 mg/L bleach treatment, rinse combination.

Results indicated that the bulk soap was contaminated with 4–7 LOG_{10}(CFU/mL) bacteria and 4–7 LOG_{10}(CFU/cm^2) biofilm bacteria from the inside of the dispensers (n=6), independent of dispenser type or construction material. Overall, the biochemical profiling identified 14 unique bacterial species, and 11 different genera from all the dispensers tested, whereas the community analysis method identified 13 unique genera and possibly dozens of different species. All microorganisms observed are considered opportunistic pathogens. The results of the two approaches were comparable at the genus level but some differences were observed. The washing study results showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks of cleaning a dispenser, then rinsing it with 5,000 mg/L bleach.

These studies showed that dispensers contaminated with bacteria in the bulk soap also had high levels of biofilm bacteria that would be available to re-contaminate a dispenser, even if the old soap is emptied and the dispenser washed and treated with bleach before new soap is added. In addition, while the bacterial diversity was relatively low compared to other environments, detection of SSU rRNA gene sequences suggested the presence of organisms not detected via cultivation-based techniques (for some samples).

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

CBE Poster #503

Date: 11/2009
Title: **Visualizing the biofilm matrix using new and classic fluorescent stains**
Authors: ***Betsey Pitts***[1], D Gray[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
[2]Life Technologies, Molecular Probes, Eugene, OR

Biofilms are complex, adherent micro-ecosystems, potentially containing many types of organisms, architectural features, and structural building blocks. Direct visualization of biofilms via microscopy has demonstrated the presence of two dominant constituents of interest: cell bodies and extracellular polymeric substances (EPS), of which the EPS has been more challenging to visualize by fluorescence microscopy. Researchers have successfully used several fluorescent stains for cell bodies such as LIVE/DEAD BacLight Bacteria, acridine orange, and DAPI, but stains labeling the EPS have generally been limited to calcofluor white and specific fluorescently labeled plant lectins such as wheat germ agglutinin and concanavalin A. To identify new stains for labeling the EPS, we screened a variety of fluorescent stains on *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Escherichia coli* biofilms and observed the labeling patterns. The biofilms were grown on glass coupons in a CDC reactor and labeled with stains diluted in water. Each sample was imaged on either an epifluorescence or confocal microscope with water immersion objectives. We found a number of fluorescent stains, originally developed for other applications, capable of illuminating aspects of biofilm extracellular matrices in these organisms. These stains vary in chemical structure, excitation and emission spectra, and include reagents such as SYPRO® Ruby, BODIPY® 630/655-X, SE, Texas Red® C2-dichlorotriazine, m-dansylaminophenylboronic acid, and CellMask™ Orange plasma membrane stain. The variety of stains that labeled the EPS may allow for simple reagent multiplexing, and combinations are being further optimized to allow robust multifactoral analysis of biofilms within a single sample.

CBE Poster #504

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

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

CBE Poster #505

Date: 11/2009
Title: **Utility of biofilms in geological carbon sequestration**
Authors: ***Robin Gerlach,*** *AB Cunningham, AC Mitchell*
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US Department of Energy; Zero Emissions Research and Technology (ZERT)

Geologic sequestration of CO_2 involves injection into underground formations including oil beds, deep unminable coal seams, and deep saline aquifers with temperature and pressure conditions such that CO_2 will likely be in the supercritical state. Supercritical CO_2 ($scCO_2$) is only slightly soluble in water (approximately 4%) and it is therefore likely that two fluid phases will develop in the subsurface, an aqueous and a supercritical phase. Supercritical CO_2 is less dense and much less viscous than water therefore creating the potential for upward leakage of CO_2 through fractures, disturbed rock, or cement lining near injection wells. Our research focuses on microbially based strategies for controlling leakage of CO_2 during geologic sequestration.

We examine the concept of using engineered microbial biofilms capable of precipitating crystalline calcium carbonate and hypothesized that the combination of microbial biofilms and mineral deposits, if targeted in high permeability areas, fractures, and near injection wells can result in the long-term sealing of preferential leakage pathways.

Batch and flow experiments at atmospheric and high pressures (> 74 bar) have shown the ability of microbial biofilms to decrease the permeability of natural and artificial porous media, survive the exposure to $scCO_2$, and facilitate the conversion of gaseous or supercritical CO_2 into long-term stable carbonate phases.

Successful development of these biologically based concepts could result in a CO_2 leakage mitigation technology which can be applied either before CO_2 injection or as a remedial measure.

Acknowledgement: This work was funded by the Zero Emissions Research and Technology (ZERT) program (DOE Award No. DE-FC26-04NT42262). However any opinions, conclusions, findings or recommendations expressed herein are those of the authors and do not necessarily reflect those of DOE.

CBE Poster #506

Date: 11/2009
Title: **Bacterially induced calcite precipitation and strontium co-precipitation under flow conditions in a porous media system**
Authors: ***Robin Gerlach,*** *AC Mitchell, L Schultz, and AB Cunningham*
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US Department of Energy and the National Science Foundation

The process of *in situ* carbonate mineral formation has implications in many environmental applications including, but not limited to, aquifer decontamination, enhancement of soil stability, and carbon capture and storage (CCS). The high stability of carbonates and the potential for co-precipitation of contaminants within carbonates are attractive attributes for several potential engineering applications.

Ureolytic precipitation of calcium and strontium carbonates by *Sporosarcina pasteurii* was examined in two-dimensional flat plate porous media reactors. Complete reactor plugging due to biofilm formation and calcium carbonate precipitation was achieved in Sr-free systems after 14 hours and in Sr-inclusive systems after 15 hours. Comparison of the reactor influent and effluent after 11 hours indicated that Ca^{2+} concentrations in the Sr-free reactor effluent were reduced to approximately 0.48% of the influent concentration, while the Ca2+ and Sr2+ concentrations of the Sr-inclusive effluent were reduced to 0.64% and 2.34% of the influent concentration, indicating a slight inhibitory effect of strontium on calcium carbonate precipitation. Despite this slight inhibition, more than 98% of the Ca^{2+} entering the reactors was precipitated. Calcite was identified as the main mineral formed and larger mean crystal size and density were observed near the reactor influent. Homogenous partition coefficients calculated from extracted precipitates suggest higher Sr^{2+} partitioning near the inlet region, where higher precipitation kinetics exist. Results confirm the possibility of effective calcite-based co-precipitation of Sr^{2+} under flow conditions and contribute toward the development of field-scale calcium carbonate mineral-based immobilization strategies.

CBE Poster #507

Date: 11/2009
Title: **Magnetic resonance approaches to investigate the correlation between biological function and transport phenomena in biofilms**
Authors: SL Codd, JA Hornemann, JD Seymour. Presented by ***Sarah Vogt***
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US Department of Energy: EPSCoR and ZERT; National Science Foundation

Our research group has been using magnetic resonance techniques to look at transport in biofouled capillaries and biofouled porous media and within the biofilm matrix. Recent results of pulsed gradient spin echo(PGSE) NMR measurements of the impact of environmental and chemical challenges on the biomacromolecular dynamics in medically relevant *Staphylococcus epidermidis* biofilm material will be presented. The data clearly demonstrate the ability of PGSE NMR to characterize molecular dynamics in biofilms, and the potential for such techniques to form a basis for sensors which can indicate the state of the biofilm after thermal or chemical treatment or to provide information to further understand the molecular level mechanisms of such treatments.

Aging of the biofilm biomass was seen to cause a fairly slow degradation of molecular weight and physical cross-linking of biomacromolecules, including those within cell membranes. The impact of chemical antimicrobial agents on the biomacromolecular diffusion was more complicated and showed significant differences in the molecular mechanisms of action on the biomass.

Recently it has been shown that *T2-T2* correlation experiments can illuminate pore distribution and exchange between pores in rocks, concrete, and other porous media. Our research group has recently used *T2-T2* experiments to probe changes in pore connectivity in porous beadpacks due to biofilm growth. The growth of biofilm in a porous media adds an additional gel phase to the pores, changing pore connectivity, effective pore size, surface and bulk *T*2, as well as introducing additional salts and paramagnetic impurities. Research compared *T2-T2* distributions in porous media before and after biofilm growth; results indicated that by varying exchange times, *T*2-*T*2 measurements may be able to determine the extent of biofilm growth in an opaque porous media such as geological formations. Only recently have researchers discussed the potential to yield the same information about such processes in heterogeneous gel materials. These techniques may be very informative in describing the nature of the EPS as a porous material, and in explaining the impact of antimicrobial challenges and genetic modifications on this porous gel matrix.

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CBE Poster #508

Date: 10/2009
Title: **Media pH, nitrate utilization, and accumulation of TAG: Two members of the Chlorophyta**
Authors: ***Rob Gardner***, P Peters, K Cooksey, and B Peyton
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US DoD-AFOSR; US DOE-Office of Biomass Production; NSF-IGERT

One important subtask during the former DOE Aquatic Species Program—researching algae capable of biofuel production—was finding a so-called "lipid trigger." While no individual molecule has been identified, circumstances that influence accumulation of triacylglyceride (TAG) have been investigated. Our group and others have shown that nitrogen limitation and—for a single species of *Chlorella*—a rise in culture medium pH, triggers TAG accumulation.

Due to the potential commercial use of pH and nitrogen levels to influence lipid production, the interplay between these two "environmental factors" was further investigated on *Chlorophyceae scenedesmus* sp. and *Coelastrella* sp. Growth was monitored optically and TAG accumulation was routinely monitored by Nile Red fluorescence and confirmed by Gas Chromatography. Both organisms grew in all medium treatments and TAG accumulation was observed by two distinct control points, medium pH and nitrogen limitation.

CBE Poster #509

Date: 11/2009
Title: **Counterintuitive effects of quorum sensing on biofilm antibiotic tolerance**
Authors: ***Trevor Zuroff***, J Lloyd-Randolfi, H Bernstein, L Jimenez Taracido, and R Carlson
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: NIH-National Center for Research Resources (NCRR)

Biofilms plague both medical and industrial surfaces and have inspired intensive antifouling efforts using technologies like antibiotic impregnated coatings and quorum sensing inhibitors. This study systematically examines the effect of AI-2 quorum sensing, nutritional environment, temperature, and culture growth

stage on *Escherichia coli* biofilm antibiotic tolerance. These fundamental parameters were found to strongly influence biofilm antibiotic tolerance, causing up to ten-million fold differences in viable cell counts over a twenty-four hour treatment period. For instance, interrupting AI-2 quorum sensing via gene knock outs in either ΔluxS, ΔlsrR, or ΔlsrF at 37°C on LB medium supplemented with glucose resulted in approximately 10 million more viable cells than wild-type or ΔlsrK gene knock out cultures when treated with the antibiotic ampicillin. However, this effect was negated when the cultures were grown on LB medium in the absence of glucose or grown at room temperature (21°C). The findings were tested at different culturing phases and also compared with planktonic culture behavior. Both had significant effects on cell viability after antibiotic treatment. This study highlights the dynamic nature of biofilm antibiotic tolerance. The results are critical for rationally designing and testing antibiofilm strategies.

Acknowlegments: Grant Number P20 RR16455-08 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and by NIH Grant Number EB006532

CBE Poster #510

Date: 01/2010
Title: **A collaborative effort for standardization of the (minimum biofilm eradication concentration) MBEC™ Assay**
Authors: ***Myles Perkins***, DK Walker, and DM Goeres
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Innovotech

The Standardized Biofilm Methods Laboratory at the CBE is working with Innovotech, Inc. to create another ASTM Standard Method to add to the biofilm repertoire. The current biofilm standards were designed for biofilm growth under high, medium, and low shear conditions and are as follows:

- **A.** ASTM E2562-07 Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor
- **B.** ASTM E2196-07 Standard Test Method for the Quantification of a *Pseudomonas aeruginosa* Biofilm Grown with Shear and Continuous Flow using Rotating Disk Reactor
- **C.** ASTM E2647-08 Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown Using a Drip Flow Reactor with Low Shear and Continuous Flow

In 1996, Innovotech developed the MBEC™ Assay. It is a simple batch culture technique that not only grows up to 96 individual biofilms at a time under very low shear, which complements the existing growth reactors, but is also a disinfectant efficacy screening tool to determine the minimum biofilm eradication concentration and is widely used in many medical and industrial fields. A variety of bacterial species have been grown and tested using this method, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus* spp. This poster will outline the MBEC™ Assay and describe the futures steps to be taken along its path to standardization.

CBE Poster #511

Date: 01/2010
Title: **The impacts of *Staphylococcus aureus* biofilm on primary human keratinocytes**
Authors: ***Pat Secor***[1], K Kirker[1], G James[1], P Fleckman[2], J Olerud[2], and P Stewart[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT;
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Sponsored by: National Institute for General Medical Sciences (NIGMS)

Chronic wounds are characterized by prolonged inflammation and failure to re-epithelialize, and do not respond well to conventional treatment. Many factors have been implicated in the delayed healing of these wounds, including microbial infection. It has been speculated for several years that chronic wound infection may be biofilm related. *Staphylococcus aureus* has been implicated in several infectious diseases including acute and chronic skin infections. An *in vitro* model was developed to study host/pathogen interactions along with the role biofilm formation plays in pathogenesis. *S. aureus* biofilms were grown on 0.2 µm culture inserts and placed on top of a monolayer of human keratinocytes. Use of the culture inserts allowed for the removal of the biofilm from the keratinocytes with minimal disruption of the biofilm or keratinocytes, creating a convenient method for the study of host/pathogen interactions. Keratinocytes exposed to either planktonic or biofilm *S. aureus* secretions were examined via laser scanning confocal microscopy and bright field transmission microscopy. *S. aureus* biofilm secretions induced significant disruption of the cytoskeleton in the keratinocytes followed by induction of widespread apoptosis as revealed by terminal deoxynucleotidyl transferase dUTP nick end labeling. The disruption of cytoskeletal proteins and induction of apoptosis in keratinocytes may impact the natural healing process by inhibiting the re-epithelialization of the wound bed, leading to the chronic state of the wound. Planktonic *S. aureus* studied in the same manner were not found to induce these effects. Microarray analysis of keratinocytes exposed to either biofilm or planktonic secretions showed upregulation of several inflammatory genes. The level of expression of inflammatory genes in keratinocytes exposed to *S. aureus* biofilm was expressed an order of magnitude higher when compared to keratinocytes exposed to planktonic *S. aureus.* Here we demonstrate that *S. aureus* biofilm formation is critical for the disruption of the keratinocyte cytoskeleton, induction of apoptosis, and induction of a strong inflammatory response in vitro.

CBE Poster #512

Date: 11/2009
Title: **Differential protein expression in in-vitro biofilm models**
Authors: ***Elinor deLancey Pulcini***, D Samuelson, and G James
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT

In vitro model systems have become a standard procedure for biofilm research. It is well accepted that different model systems produce different biofilms, which can vary due to such parameters as shear force, flow rate, temperature, nutrient availability, or residence time. This can make comparisons between biofilm models somewhat problematic. We have noted, for example, that the effects of a treatment regimen on a biofilm can vary by the model in which it is tested. The goal of this work was to examine protein expression patterns in three different model systems compared with planktonic cultures. For this study, we chose to examine three model systems: the drip flow model, the colony model, and the 96-well microtiter plate model using a *Staphylococcus aureus* chronic wound isolate. These systems were chosen because they are all considered to be low to no shear and because there is published literature to indicate that biofilms formed in these systems show increased antimicrobial tolerance characteristic of biofilms. The drip flow model (DFR), an ASTM approved method (ASTM E2647–08), consists of four parallel test channels, each capable of holding one standard glass microscope slide sized coupon. The entire apparatus is maintained at a slant to allow culture medium to drip onto and flow down the slide surface. The colony biofilm model involves growing colonies on top of microporous membranes resting on an agar plate. These membranes, with the growing biofilm, can be transferred to fresh agar daily. The 96-well microtiter plate model

involves growing biofilms in the wells of a plate placed on a shaker. The medium is replaced every 24 hours. *Staphylococcus aureus* biofilms were grown in the three in vitro models using the Tryptic Soy Broth (or Tryptic Soy Agar in the case of the colony model) for 48 hours at 37°C. Proteins were extracted from samples collected from each model as well as from planktonic cultures and subjected to proteomic analysis using 2D gel electrophoresis. Protein gels were compared model to model and to planktonic culture, based on the presence or absence of protein spots in the gels. Selected differentially expressed proteins were identified using MALDI/TOF-TOF and overall protein expression patterns were compared. Protein expression patterns appeared to be the most similar between the colony model and the drip flow model and between planktonic culture and the 96-well microtiter plate model. The relative difference of the models compared to planktonic culture was calculated based on total number of proteins differentially expressed. The drip flow was 44%, the colony model was 37%, and the 96-well microtiter plate was 17% different from planktonic culture. These data indicate that biofilms grown in the 96-well microtiter plate are more similar to planktonic culture than biofilms grown using the colony or DFR models.

CBE Poster #513

Date: 11/2009
Title: **An in vitro model for the growth and analysis of chronic wound MRSA biofilms**
Authors: ***Alessandra Agostinho*** and GA James
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Introduction: Increasing evidence suggests that microorganisms inhabiting chronic wounds and responsible for delayed healing exist as biofilms. These microbial communities show extreme tolerance to antimicrobial agents as well host immune defenses; exacerbating this situation is the emergence of drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). However, traditional development and testing of antibacterial agents over the past century have focused on free-floating (planktonic) bacteria in culture that do not reproduce the clinical reality. The purpose of this research was to develop an in vitro model, the Colony/Drip Flow Reactor (DFR) model, for the growth and analysis of MRSA biofilms that could reproduce the way biofilms grow in chronic wounds. In this model the biofilms are grown on a black polycarbonate sterile filter membrane on top of an absorbent pad in a DFR and the biofilms are fed from below, reproducing the environment of the wound, in which the exudate perfuses to the surface of the ulcer, feeding the biofilm that stays in contact with air.

Methods: Membranes were inoculated with a wound clinical isolate of MRSA and the biofilms grown at room temperature with a flow rate of 5mls/hour per chamber of 10%-strengh tryptic soy broth. After 72 hours the biofilms were treated with saline solution (control), 1% silver sulfadiazine solution and 0.25% Dakin's solution. The treatments were conducted for 15 minutes by pipetting 0.5mls of the treatment solution on top of the biofilms. After 15 minutes the membranes/biofilms were transferred to DE broth for neutralization, dispersed, serially diluted, and plated on tryptic soy agar.

Results: Plate count results from four separate experiments showed an average bacterial count of 9.28 log Colony Forming Units/membrane (CFU/membrane) for the saline treated biofilms. Silver sulfadiazine solution presented a minimal effect on the biofilms, with an average log CFU/membrane of 9.23, while the Dakin's solution presented a 2.13 log reduction when compared to the saline control (7.15 log CFU/membrane).

Conclusions: The method here described allowed the growth of thick biofilms and is a repeatable technique for the growing of MRSA biofilms and the testing of antimicrobials against these resistant bacterial communities.

CBE Poster #514

Date: 01/2010
Title: **Analysis of morphological switch associated with viable but not culturable (VBNC) state in *Helicobacter pylori***
Authors: ***Crystal Richards***, K Williamson, and AK Camper
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: US EPA under the Science to Achieve Results (STAR), Center for Native Health Partnerships

Introduction: The gastrointestinal pathogen *Helicobacter pylori* infects approximately 50% of the world-wide population; however the exact route of transmission remains poorly understood. *Helicobacter pylori* is known to become viable but not culturable in most environments outside the mammalian stomach. It has been observed that the loss in culturability coincides with a change in cell morphology from a spiral, flagellated form to a coccoid, aflagellated form. The purpose of this research is to characterize the mechanism of cell morphology switching that is associated with loss of culturability in *H. pylori*.

Methods: The VBNC state was generated in *H. pylori* by prolonged exposure to atmospheric oxygen and by nutrient deprivation. *H. pylori* cells were grown on tryptic soy blood agar for 48 hours then transferred to a tryptic soy blood agar biphasic slant that contained a pool of tryptic soy broth with 5% fetal calf serum. Biphasic slants were incubated in a microaerophilic atmosphere for 24 hours and then were allowed to age naturally or were given one of two experimental treatments. *H. pylori* cells subjected to the experimental treatments were removed from the microaerophilic atmosphere and either incubated in the slant at 37 ˚C with atmospheric oxygen or the cells were transferred to ultra-pure milliQ water and then incubated at 37˚C. All three treatments were sampled regularly for viable and total cell counts. Additionally RNA was extracted from healthy and stressed *H. pylori* cells at 24 hours and after 7 days, purified and assessed for quality on the agilent bioanalyzer 2100.

Results: *Helicobacter pylori* converts to a non-culturable form within 24 hours of exposure to both experimental treatments. Healthy cells that were allowed to age in a microaerophilic atmosphere for 21 days showed a decrease in culturability accompanied by a concomitant increase in coccoid cells. After exposure to atmospheric oxygen, *H. pylori* switched from a helical form to a smaller, coccoid morphology (>95% of total cells were coccoid after 21 days). Nutrient deprivation also caused a complete loss of culturability; however the morphological conversion was significantly different, with approximately 40% converting to coccoid morphology after 21 days. Analysis of RNA quality showed intact 16s and 23s rRNA extracted from healthy cells and cells early in the oxygen exposure treatment. However, nutrient deprivation appeared to cause non-random fragmentation of the rRNA as early as 4 hours after exposure.

Discussion: The conversion of *H. pylori* to a coccoid form is thought to be indicative of the switch to a VBNC state. Indeed, healthy cultures that age in a microaerophilic atmosphere seem to lose culturability in relation to an increase in coccoid cells. This morphological switch is also seen under exposure to atmospheric oxygen. However, this research has shown that *H. pylori* can become non-culturable without a majority of cells switching their morphology (seen in nutrient deprivation treatment). Nutrient deprivation also caused *H. pylori* to break down rRNAs, producing a reproducible fragmentation pattern. This fragmentation may be responsible for the inability of *H. pylori* to switch its morphology and may be indicative of an active mechanism for coping with environmental stresses.

CBE Poster #515

Date: 10/2009
Title: **Extraction, characterization and purification of bacterial siderophores**
Authors: ***Luis Serrano Figueroa*** and A Richards
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: Molecular Biosciences Program-MSU, MSU-CBE, Department of Microbiology, and Department of Chemical and Biological Engineering

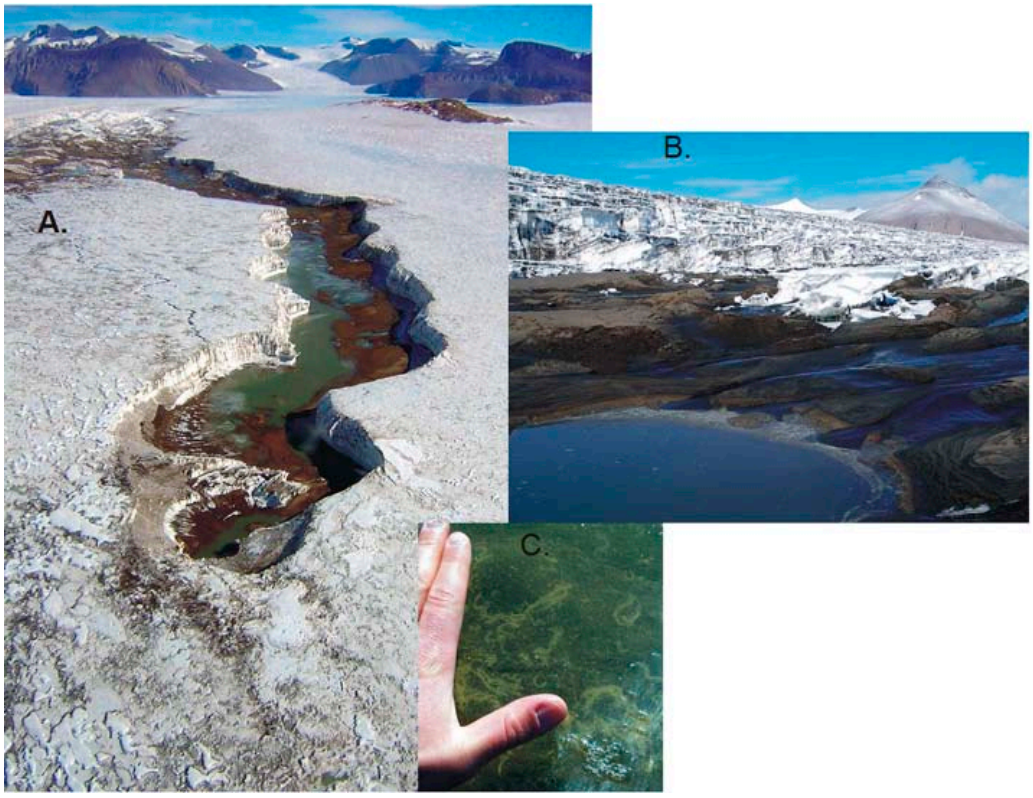
Siderophores are small organic compounds produced by many microorganisms under iron stress. Siderophores act as iron chelators and increase the amount of biologically available iron. Some siderophores are amphiphilic, behaving like lipid compounds in hydrophilic environments, and have the ability to form vesicles. In this project, we investigated siderophore production by a soda lake isolate (strain SL28) and purified siderophores by HPLC. Siderophore quantification was achieved by the Chrome Azurol Sulfonate assay (CAS; measuring absorbance at 630 nm). SL28 growth curves were obtained measuring optical densities at 600 nm. Siderophore extraction was accomplished using a Varian Solid Phase C2 cartridge. Additional purification of the siderophores was performed with a Dionex HPLC System (Dionex AD20 Absorbance Detector) using acetonitrile and nano-pure water as mobile phases. The siderophore maximum concentration in culture was 96.29 mM and 112.99 mM for Experiments 1 and 2, respectively. Control groups for CAS and growth curves were prepared and showed no siderophores and growth, respectively. As iron equivalents increase, siderophore vesicle size also increases. Siderophore production was confirmed with CAS assay, and their purification was achieved by HPLC.

CBE Poster #516

Date: 01/2010
Title: **Microbial growth in a humic-free environment on the Cotton Glacier, Antarctica**
Authors: ***Christine Foreman***, R Cory, J Lisle, P Miller, Y-P Chin, D McKnight, H Smith, M San Clements and C Ward
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT
Sponsored by: National Science Foundation

A supraglacial stream forms annually on the Cotton Glacier in the Transantarctic Mountains (see images below). Analysis by fluorescence spectroscopy of dissolved organic matter (DOM) from this stream in 2004-05 and again in 2009-10 showed that the concentration was low (44-48 μM C), and lacked humic signatures, unlike DOM typical of microbially based ecosystems. Analysis of the EEMs by PARAFAC indicated that amino-acid fluorophores dominate, which is consistent with DOM of microbial origin. These results indicate that DOM in this system is seasonally formed from soluble microbial products and that a reservoir of recalcitrant humified DOM does not pre-exist. After storage of the stream water in the dark for 7 days, humic peaks were detected in the EEMs, suggesting that the DOM can undergo humification. In most aquatic ecosystems, humic DOM acts as a natural sunscreen, and the absence of humics may represent an additional stressor influencing the microbial community. Nonetheless, the stream contained an active microbial assemblage with bacterial cell abundances from 2.94×10^4–4.97×10^5 cells ml^{-1}, and bacterial production ranging from 58.8-293.2 ng C l^{-1} d^{-1}. Chlorophyll-*a* concentrations ranged from 0.3 to 0.53 μg l^{-1} indicating that algal phototrophs were the probable source of the DOM. Microbial isolates produced a rainbow of pigment colors, suggesting adaptation to UV stress, and were similar to those from other cryogenic systems (*Cytophagales* and *β-Proteobacteria* lineages). Comparison of bacterial production rates to bacterial abundance and DOC concentrations confirmed that the slow microbial growth was sufficient to turn over the DOM pool rapidly, consistent with the absence of a recalcitrant humic DOM pool. Taken together, these results suggest that the occurrence of related phylotypes from diverse glacial environs is due to similar survival strategies and that UV stress due to the absence of humics is important in

supraglacial streams. Supraglacial streams provide an example of contemporary microbial processes on the glacier surface and a natural laboratory for studying the microbial adaptation to the absence of humics, as well as chemical processes controlling the eventual genesis of humic DOM.



CBE Poster #517

Date: 04/2009
Title: **Developing a three-species biofilm wound model**
Authors: ***Jeremy Woods*** and G James
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Sponsored by: NIH (1 P20 GM078445-01) and the MSU Undergraduate Scholars Program

As a response to this lack of information, a three-species biofilm wound model was developed at the Center for Biofilm Engineering Medical Biofilm Laboratory. The biofilm wound model utilized aerobic bacteria to create anaerobic areas within the biofilm that could support growth of anaerobic bacteria. This model approximated the wound bed while incorporating a strict anaerobe (*Clostridium perfringens*) as well as a facultative anaerobe (*Staphylococcus aureus*), and an aerobe (*Pseudomonas aeruginosa*). Microscopic imaging of the biofilm allowed for the location of each species within the biofilm to be determined. Plate counts also allowed for the determination of the number of each species within the biofilm. Current research is focused on testing the efficacy of antimicrobial treated gauzes on such multi-species biofilms.