# PROCEEDINGS

CENTER FOR BIOFILM ENGINEERING

# Winter 2003 CBE Technical Advisory Conference

February 6–7, 2003
Montana State University–Bozeman
Bozeman, Montana

Sponsored by the
Center for Biofilm Engineering
a National Science Foundation
Engineering Research Center
at Montana State University–Bozeman

## GENERAL INFORMATION

### CBE LEADERSHIP

***Bill Costerton**, CBE Director and Professor, Microbiology*
***Phil Stewart**, CBE Deputy Director and Professor, Chemical Engineering*
***Anne Camper**, Associate Professor, Civil Engineering & Associate Dean for Research, COE*
***Al Cunningham**, Professor, Civil Engineering*
***Marty Hamilton**, Professor, Statistics*
***Paul Stoodley,** Assistant Research Professor, CBE*
***Paul Sturman**, CBE Coordinator of Industrial Development*

### A BRIEF HISTORY OF THE CBE

The CBE was established in 1990 with a grant from the National Science Foundation's Engineering Research Centers program. The NSF-ERC program was created to increase U.S. industrial competitiveness and to re-invent science and engineering education in U.S. universities. Under the leadership of its founding director, Bill Characklis, the new engineering center also drew support from the state of Montana, MSU–Bozeman, and industrial partners gathered during its pre-1990 work as the Institute for Biological and Chemical Process Analysis. Since the beginning, CBE researchers have been recognized nationally and internationally for leading-edge biofilm research, and for taking an interdisciplinary approach to the study of microbial growth on surfaces.

In the spring of 2001, the CBE's 11-year period of NSF-ERC program support drew to a close. The CBE's continued success is built on the foundation of many years of productive government-university-industry collaboration in pursuit of its vision to be a world leader in fundamental research, science and engineering education, and industrially relevant technology.

### MISSION AND GOALS OF THE CBE

***The mission of the Center for Biofilm Engineering is to advance the basic knowledge, technology and education required to understand, control and exploit biofilm processes.***

The CBE has identified goals in three areas of activity. In the area of research, the CBE's goal is to do leading edge fundamental research to elucidate mechanisms at work in bacterial biofilms. The CBE has been a leader in defining the structure and function of biofilms on surfaces, in understanding antimicrobial resistance mechanisms of biofilm, and in identifying the role of signal molecules in controlling bacterial behavior. Researchers at the CBE have demonstrated that biofilms are multicellular attached communities with primitive circulatory systems and a measure of cellular specialization. Understanding these "biofilm basics" presents opportunities for developing more effective strategies to control biofilms in industrial settings.

The second goal of the CBE is to make its research relevant to real systems, where the information can be useful. Industrial concerns shape and focus the research efforts. Technology transfer at the CBE involves not only information, but methods and technology development.

Key to the center's success is the CBE's third goal: to develop an interdisciplinary undergraduate and graduate education program, involving team research on industrially relevant projects.

## THE INDUSTRIAL ASSOCIATES PROGRAM

In addition to governmental funding sources, the CBE is funded through its diverse group of Industrial Associate members.

Benefits of membership include:

- **Attendance at Industrial Meetings**. The semi-annual meetings are exclusive to Industrial Associate members and CBE research collaborators (non-member companies may visit once to preview the Industrial Associates program). At each meeting, exclusive workshops are provided to give Industrial Associates hands-on training on the latest biofilm analytical techniques.
- **One vote on the CBE Technical Advisory Committee** to guide CBE research and policy.
- **Two days of consultation**.
- **Long-term visits** to conduct collaborative research.
- **Research sponsored by one company or a consortium of companies**.
- **Specialized workshops**.
- **Access to students trained in interdisciplinary, team research**.
- **Early access to publications**.
- **Access to the CBE's Biofilm Systems Training Laboratory (BSTL)**.

## CBE WEB SITE

More information about the Center for Biofilm Engineering is available at its website:
**http://www.erc.montana.edu/**

# Presentation and Poster Abstracts
# Technical Advisory Conference
# February 6–7, 2003


**Session 1: Medical (Chair: Mark Shirtliff)**

- Bacteria live in the darndest places! ..... 6
- Essential oil mouthrinse treatment of dental biofilms ..... 7
- Combatting biofilm-associated antibiotic resistance ..... 7
- A review of ultrasound-mediated drug delivery ..... 8
- Novel approaches to prosthetic joint infection diagnosis ..... 8
- Evaluation of *Staphylococcus aureus* gene expression in biofilms through microarray analysis ..... 9

**Session 2: Biofilm Methods (Chair: Darla Goeres)**

- Hot tub method development: A progress report ..... 9
- CDC biofilm reactor: Ruggedness test results ..... 10
- NMR microscopy of the impact of biofilms on transport phenomena in bioreactor flows and porous media ..... 10
- The biofilm imaging facility at CBE: A grant funded by the Murdock Charitable Trust ..... 11

**Session 3: Biofilm Structure and Function (Chair: Phil Stewart)**

- Characterization of the extracellular matrix of *Pseudomonas aeruginosa* biofilms ..... 11
- Heterogeneous growth in biofilms ..... 12
- Modeling biofilm antibiotic resistance: persisters ..... 12
- Biofilm fuel cells ..... 12

**Session 4: Distribution System Biofilms (Chair: Anne Camper)**

- Regulatory update: Heterotrophic plate counts ..... 13
- Assessing microbial contamination risks for small water systems ..... 13
- Rechargeable biocidal coating for biofilm control ..... 14
- Bioterrorism and water distribution systems ..... 14

**Session 5: Mechanical Disruption of Biofilm (Chair: Paul Stoodley)**

- Disruption of dental plaque biofilm via fluid forces ..... 15
- Effect of power brushing on biofilm removal using an interproximal model ..... 15
- Viscoelasticity of dental and other biofilms ..... 15
- Modeling biofilms as viscoelastic fluids ..... 16

**Session 6: Industry Interaction**

- Cooperation between industry, academics and institutes (1&2) ..... 16

## Posters


- Field study: Biofilm accumulation in hot tubs ..... 17
- Use of high performance liquid chromatography-diode array detection for the improved analysis of 2,4,6-trinitrotoluene and its reduced metabolites ..... 17
- Direct and Fe(II)-mediated microbial chromate reduction by *Cellulomonas spp.* ..... 18
- Imaging the influence of biofilm growth on bulk flow using nuclear magnetic resonance microscopy ..... 18
- Workshops offered by the biofilm structure and function research group ..... 19
- Siderophore mediated metal transport ..... 19
- Proteomic comparison of biofilm vs. detached methicillin resistant *Staphylococcus aureus* ..... 20
- Microbial process research for the commercialization of subsurface biofilm barrier technology ..... 20
- Bacterial biofilms in sinusitis ..... 20
- Control of acid rock drainage through stimulation of selected microbial populations in mine tailings ..... 20
- Detachment and antimicrobial resistance of single cells and cell clusters from *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms ..... 21
- Green adhesive and microbial polymer products ..... 22

# SPEAKER ABSTRACTS

## SESSION 1: Biomedical

### W03-S01
### Session Introduction

***Mark Shirtliff,*** *Assistant Research Professor, CBE*

### W03-S02
### Bacteria Live in the Darndest Places!

***Bill Costerton****, Director, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Some human organ systems are known to be colonized by bacteria; these include all of the digestive tract—including the stomach—now that we know about *Helicobacter pylori* and the outermost organs of several other systems. The outer organs (vagina and cervix) of the female reproductive tract are colonized, as are the outer parts (distal urethra) of the urinary tract in both sexes. These commensal bacterial populations grow as biofilms. The biofilms in the oropharynx shed both planktonic bacteria and biofilm fragments that may lead to transitory colonization of all parts of the pulmonary "tree". These tissue surface biofilms have been studied in some detail, and many papers have been published concerning the biofilms on both hard and soft surfaces in the mouth. One of the techniques on which we have relied is the use of scrapings or swabs of tissue surfaces to inoculate enrichment cultures, or for direct plating onto agar to yield colonies that can be identified as containing certain species of bacteria.

The whole field of tissue colonization has been revolutionized by new techniques, most of which are based on molecular analysis, rather than on recovery and culture. Our first firm proof that recovery and culture techniques fail to detect important groups of biofilm bacteria came in CBE studies sponsored by a member company. The object of the study was to define the bacterial population of the human vagina. Swabs of 3,000 volunteers showed that 10.8% of these people carried *Staphylococcus aureus,* in addition to the usual strains of Lactobacillus. When we used molecular techniques, such as 16 S r-RNA matching by Fluorescence In Situ Hybridization (FISH) and PCR analysis of c-DNA to analyze the vaginal populations of a subset of these volunteers, we found that *all* of them carried *S. aureus* on their vaginal epithelia. What was especially worrying was that the individuals who yielded positive swabs and cultures were not amongst the most heavily colonized when examined by the molecular techniques, and positive swabs and cultures seemed to be almost random.

The extensive use of molecular techniques has shown that children with middle ear infections (otitis media) have many species of bacteria in their middle ears, even though cultures of the liquid recovered from their ears and from inserted tubes are largely negative. Now that middle ear infections are known to be caused by bacteria and not viruses, as was suggested when bacterial cultures were negative, we must assume that the middle ear is colonized in normal people because of its direct connection with the oropharynx via the eustacian tubes.

We have known that the distal urethra of male humans is colonized by bacteria, but we have always assumed that more proximal organs, like the prostate gland, are essentially sterile in normal people. Curtis Nickel, a urologist from Queens University, has recently used molecular techniques to show that the prostates of all individuals in a 100-subject study of men between 55 and 60 years of age were colonized by a rich mixture of bacterial species. He found no difference in the degree of colonization or in the species involved, between people with symptoms of prostatitis and people with no symptoms or history of this disease. These findings raise the fascinating possibility that the two diseases that cause the greatest expenditure on antibiotics in the modern western world occur in organs that are colonized in all individuals in certain age ranges, and that disease symptoms are actually produced by aggressive host responses to the presence of these organisms. Generally a type II immune response causes symptoms, while a type I response does not; these diseases may best be treated by the induction of a more appropriate host response, instead of attempts to kill all the bacteria in an organ that is naturally colonized.

## W03-S03
## Essential Oil Mouthrinse Treatment of Dental Biofilms

***Mark Pasmore**, Assistant Research Professor, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Essential oil-containing mouthrinse (Listerine) has been shown to kill bacteria and improve dental health. This study used a biofilm model to assess the extent and pattern of biocidal activity through viability staining and confocal laser scanning microscope analyses. Two studies were performed in this work. The first study evaluated one-day-old *Streptococcus mutans* UA159 biofilms that were treated for 1–4 min with either the essential oil-containing mouthrinse or sterile water (negative control), neutralized with Letheen broth, and stained with a commercially available viability stain (BacLight™, Molecular Probes). The second study was a 4-day experiment in which the biofilm was treated twice daily with 60 seconds of Listerine. A sample was collected and analyzed on each of the days of operation using both CFU and staining techniques. The stained biofilms were viewed with a confocal laser scanning microscope to estimate thickness and viability. The percent of viable cells in biofilms treated with the essential oil-containing mouthrinse ranged from approximately 38% after a 1-minute exposure to 26% after 4 minutes. In comparison, the percent of viable cells in the negative control biofilms ranged from 84% to 91%. The average thickness of the biofilms after a 1-minute exposure to either treatment was approximately 69 μm; there was no marked change in biofilm thickness with increased exposure times. The confocal images showed the biofilm architecture to consist of mushroom and tower formations, with apparent channels within the structures. In the 4-day study, other mouthrinses were tested in comparison to Listerine. Some of these mouthrinses showed higher rates of killing while others were more effective at biofilm removal. Although Listerine did prove effective, this work suggests that alternative formulations targeted at biofilms may be more effective than the original.

## W03-S04
## Combatting Biofilm-Associated Antibiotic Resistance

***Brian Cali**, VP for Development, Microbia, Inc. Cambridge, MA 02141*

The most clinically significant trait of biofilm bacteria is their ability to resist killing by antibiotics, antiseptics and human host defense mechanisms. Surface-associated bacteria can be up to 1000 times more resistant to antibiotics than their free-floating counterparts. Microbia is developing compounds that address the problem of biofilm bacteria and related resistance mechanisms by eliciting one of two effects on target bacteria: (1) inhibiting surface attachment and, (2) inactivating resistance mechanisms to potentiate antibiotic killing. Successful development of such compounds will enable the treatment of systemic infections and prevention of medical device infections.

An emerging body of evidence suggests that biofilms play a significant role in difficult-to-treat and recurrent infections such as osteomyelitis, otitis media, endocarditis, and cystic fibrosis. In addition, recent reports indicate that biofilm bacteria may contribute to the spread of acquired resistance in bacterial populations. We will describe work to identify genes that contribute to the antibiotic tolerance of biofilm bacteria, and present data demonstrating that *fmtC*, a gene previously shown to be required for high-level resistance in methicillin-resistant *S. aureus* (MRSA) strains, is also important for biofilm-associated antibiotic resistance in methicillin sensitive *S. aureus*. These data indicate that biofilm-mediated and acquired antibiotic resistance mechanisms share common elements. Results demonstrating the effect of compounds that restore antibiotic effectiveness against biofilm bacteria in a mouse model of infection will also be presented. Such compounds could be used in combination with existing antibiotics to treat both infections with a biofilm component and those caused by organisms exhibiting acquired resistance.

Biofilm-mediated medical device infections cause substantial morbidity and mortality and are associated with significant cost to the health care system. Infected devices often have to be removed due to the inability of available antibiotics to overcome biofilm-mediated resistance. For orthopedic applications this

is a significant procedure requiring both surgery and weeks of antibiotic treatment prior to reimplantation. For catheters, removal is less problematic, but resulting sepsis infection is estimated to cause 60,000 deaths in the US each year and an estimated $8 billion spent on increased hospital costs. To provide new options to prevent such infections, Microbia is developing compounds that can block bacterial biofilm formation. We will show data about one compound class that substantially reduces the ability of bacteria to adhere to medical device surfaces and form a biofilm. A medicinal chemistry program has yielded compounds in this class with enhanced potency and spectrum coverage. The biofilm inhibitory effect is not surface specific, indicating that this class of compounds could be applied to a variety of medical devices.

**W03-S05**

### A Review of Ultrasound-Mediated Drug Delivery

***Pierre D. Mourad**, Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, Department of Neurological Surgery, University of Washington–Seattle, 98195*

Ultrasound with sufficient strength has been shown to facilitate and/or enhance the activity or delivery of therapeutic drugs and DNA in vitro and in vivo. In this talk I briefly review this literature, focusing on recent technology that may allow the enhanced delivery of antibiotics into sites of potential or actual infection associated with the implantation of prosthetic devices.

**W03-S06**

### Novel Approaches to Prosthetic Joint Infection Diagnosis

***Robin Patel,** Infectious Diseases Research Laboratory, Mayo Clinic, 200 First St. SW, Rochester, MN 55905*

The majority of patients who undergo prosthetic joint replacement experience dramatic relief of pain and restoration of satisfactory joint function. Less than 10% of such patients develop complications during their lifetime; complications are most commonly the result of "aseptic" failure, followed by prosthetic joint infection. The pathogenesis of prosthetic joint infection is related to the presence of bacteria in biofilms, within which bacteria are protected from antimicrobial killing and host responses, rendering prosthetic joint infection difficult to treat. Compounding therapeutic hurdles to prosthetic joint infection are diagnostic challenges. Currently there is no method for the microbiologic diagnosis of prosthetic joint infection which exhibits both ideal sensitivity and specificity. Molecular methods (e.g. detection of bacterial 16S ribosomal DNA) have been used in an attempt to improve the diagnosis of prosthetic joint infection. Strategies which disrupt adherent bacteria should also improve the diagnosis of prosthetic joint infection and may enable detection of previously unrecognized bacteria in some cases of "aseptic" loosening of prosthetic joints. In this presentation, molecular and biofilm-directed approaches (including combinations thereof) to the diagnosis of prosthetic joint infection will be presented.

**W03-07**

### Evaluating *Staphylococcus aureus* Gene Expression in Biofilms Through Microarray Analysis

***Mark Shirtliff,** Assistant Research Professor, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

*Staphylococcus aureus* is a gram positive, ubiquitous bacterial species, with the predominant reservoir in nature being humans. This microbe is able to cause a number of persistent infections including osteomyelitis, endocarditis, abscess, and implanted medical device infection through colonizing and synthesizing a "slime" layer, termed the glycocalyx or biofilm. This layer prevents infection resolution by antimicrobial agents and host phagocytic cells. Once an implant or tissue is colonized and chronic infection ensues, the only treatment option is surgical removal or débridement.

This research seeks to identify biofilm-specific gene products and key genetic regulators of *S. aureus* biofilm formation through two-dimensional gel electrophoresis and DNA microarray technology. Methicillin Resistant *S. aureus* (MRSA) biofilms were grown at 37$^{o}$C in a once-through reactor system using the internal lumens of silicon tubing as attachment surfaces. Biofilm samples were harvested at various times post-inoculation (8 hr, and 2, 7 and 14 days

post-inoculation) to represent early-to-late developmental stage biofilms. For *in vitro* planktonic cultures, aliquots were obtained at logarithmic, stationary, and post-stationary growth phases.

Samples were prepared for proteomic studies by suspending cells in cold phosphate buffer solution containing a protease inhibitor (0.3 mg PMSF/ml), bead beating, centrifuging, and collecting the supernatant. Protein concentration was quantified, and two dimensional electrophoresis was performed. Protein spot identification was accomplished by matrix assisted laser desorption/ionization time-of-flight mass spectrometry of trypsin digested fragments. For microarray experiments, samples were resuspended in RNA later (Ambion Inc.) and then RNA was isolated using bead beating and column extraction. RNA between samples were quantified, equalized, and labeled (CY3/CY5) during reverse transcription. Labeled cDNA was applied to DNA microarrays constructed by The Institute for Genomic Research, Pathogen Functional Genomics Resource Center (PFGRC) through a grant by The National Institute of Allergy and Infectious Diseases of the National Institutes of Health (N01-AI-15447). Triplicate arrays were read and compared in order to ensure accurate and reliable scientific technique. Microarray experiment results and biofilm specific proteins (and their immunogenic potential) will be presented.

Immunogenic gene products will be tested for their efficacy to prevent biofilm-mediated infections in an artificial abscess model and a medical implant infection model in New Zealand White rabbits. In addition, a more complete understanding of the bacterial factors involved in *S. aureus* biofilm formation and maturation will necessarily result. This understanding will enable one to create novel materials, surfaces, and/or disinfection strategies that resist or eliminate staphylococcal fouling and biofilm formation. Also, the global interrelation between gene expression and protein production will also be discerned for the first time in *S. aureus*, thereby allowing staphylococci to be understood at a new level. Lastly, the results obtained in the evaluation of *S. aureus* biofilm formation may be used as a model for the biofilm formation by other closely related gram positive bacterial species, including *Streptococcus spp*., *Listeria spp*., *Clostridium spp*. and *Bacillus spp*.

## SESSION 2: Biofilm Methods

### W03-S08
### Session Introduction and Progress Report: Developing a Method to Test the Efficacy of Chemical Hot Tub Disinfectants

***Darla Goeres**, Research Engineer, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Hot tubs are approximately 500-gallon pools of hot water used for recreational and therapeutic soaking. They are operated at 38–40 $^{o}$C and include a jet and bubble system to enhance the bathing experience. Water is recycled through a skimmer and filter system; chemicals, including a disinfectant, are added to maintain the water quality. The Centers for Disease Control and Prevention (CDC), the National Spa and Pool Institute (NSPI) and NSF International issue guidelines for ideal hot tub operating conditions. Ideal conditions are difficult for the owner/operator to maintain. Hot tub use is linked to respiratory, skin, eye, ear, urinary and gastrointestinal infections.

Hot tub disinfectants registered with the US Environmental Protection Agency (EPA) must meet the criteria listed in the DIS/TSS-12 EFFICACY REQUIREMENTS: Swimming Pool Water Disinfectants. The criteria include both a laboratory evaluation and a field test of the potential disinfectant. In each case, the efficacy is based upon the reduction or control of suspended bacteria. The EPA has recognized the need to address the issues of biofilm, water chemistry and relevant design when assessing the potential for a disinfectant to maintain a safe bathing environment in a hot tub. This talk presents the progress made on a five-year project to develop a standard method for testing the efficacy of hot tub disinfectants against biofilm and suspended bacteria, including results obtained from a laboratory system developed to model the key parameters associated with hot tub design and operation.

## SPEAKER ABSTRACTS

### W03-S09
### CDC Biofilm Reactor: Ruggedness Test Results

***Marion Osterud**, BSTL Intern, and **Marty Hamilton**, Professor of Statistics, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

In a collaborative effort with Biosurface Technologies Corporation, the CBE is both developing a biofilm reactor that can be efficiently manufactured and creating a standard operating procedure (SOP) for growing a biofilm in that reactor. Because researchers at the Centers for Disease Control and Prevention conceived of the first version of this reactor, it has been named the CDC Biofilm Reactor. In this presentation, we describe the results of a "ruggedness test" for determining the effect on biofilm growth measurements in the CDC Biofilm Reactor when physical or chemical conditions depart from the SOP. The response measurement was bacterial biofilm density (log10 cfu / $cm^2$) for a single species *Pseudomonas aeruginosa* biofilm. The operating conditions were varied according to a fractional factorial design, plus center points. Each of two technicians was assigned a reactor for purposes of conducting a series of 10 experiments with 5 or 6 biofilm density measurements per experiment. Based on a multiple regression analysis of the observations, we will describe quantitatively the ruggedness of this biofilm growth system.

There were two unanticipated results. The biofilm density was systematically higher in one reactor that in the other. Subsequent evaluation of the reactor design led to the conclusion that the baffle—a rotor in the middle of the reactor—was the cause of the difference. We have decided to modify the design of the baffle so it will create a shear stress on the biofilm that is more uniform from reactor to reactor. The other unanticipated result pertains to the two techniques, homogenization and sonication, for disaggregating biofilm bacteria after the biofilm sample had been scraped from the substratum into suspension. The difference between homogenization and sonication depended on the speed at which the baffle rotated while the biofilm was growing. Homogenization produced higher CFU counts for a biofilm grown at slow rotation, and sonication produced higher CFU counts for a biofilm grown at fast rotation. The biological and physical explanation for this result has not been elucidated.

### W03-S10
### NMR Microscopy of the Impact of Biofilms on Transport Phenomena in Bioreactor Flows and Porous Media

***Joe Seymour**, Assistant Professor of Chemical Engineering, Montana State University–Bozeman, 59717*

Nuclear magnetic resonance (NMR) microscopy is magnetic resonance imaging (MRI) with resolution of 100 μm or smaller[1]. A primary advantage of the method is that it is completely non-invasive and probes the physical and chemical environment of the atoms (*e.g.* $^{1}H$, $F^{19}$, $P^{31}$) present in the molecular make-up of the sample. While spatial resolution in NMR microscopy is limited to 5–10 μm, the technique allows for the measurement of molecular dynamics and chemical state within that spatially resolved region of the sample. The ability to correlate structural and dynamic information has established the method as a preeminent technique for the study of transport phenomena in heterogeneous systems. The presentation will overview NMR microscopy methods applicable to biofilm systems with a focus on length- and time-scale dependent motions and localized spectroscopy. Data for the velocity distribution in 1 mm square duct bioreactors with and without *Staphylococcus epidermidis* biofilm will be presented. The initiation of non-axial secondary flows due to the presence of the biofilm indicates that models for transport need to account for three dimensional velocity fields. The presence of secondary flows leads to complex mass transfer that can enhance or inhibit mixing depending on the nature of the secondary flows[2]. The measurement of the impact of biofilm growth on transport processes in porous media is particularly suited to the use of NMR since the opaque nature of porous media prohibits the use of optical methods. NMR microscopy studies of homogeneous and heterogeneous porous media will be presented to demonstrate the measurement of velocity and dispersion in these systems and highlight the potential for studies with biofilms underway in the MSU–Bozeman NMR Microscopy Laboratory.

[1] P.T. Callaghan, Principles of Nuclear Magnetic Resonance Microscopy, Oxford University Press, New York, 1991.

[2] J.M. Ottino, The kinematics of mixing: stretching, chaos and transport, Cambridge University Press, Cambridge, 1989.

**W03-S11**
### The Biofilm Imaging Facility at CBE: A Grant Funded by the Murdock Charitable Trust

***Luanne Hall-Stoodley,*** *Assistant Research Professor of Microbiology, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

In a grant submitted to the Murdock Charitable Trust last year, the CBE requested funding to improve our imaging capability. The CBE was awarded $630K to purchase a new confocal microscope and a flow cytometer. The goal of this project is to establish a state-of-the-art imaging facility dedicated to the study of biofilms. The facility will combine confocal scanning laser microscopy and flow cytometry, and will improve the ability of the Center for Biofilm Engineering (CBE) to investigate dynamic, living biofilms *in situ* in three dimensions and in real time.

Imaging biofilms is crucial to biofilm research. High-resolution confocal images provided the first evidence for the structural three-dimensional complexity of biofilms. Imaging studies also showed that flow and nutrient conditions, and concentrations of cell signaling molecules, played key roles in biofilm development. Such studies are fundamental to a better understanding of the complex nature of biofilms, their relationship to the surface substratum, microbial physiology and species composition. Most recently the *dynamic nature* of biofilms is being investigated. These complex microbial communities change over time, moving, shedding and re-growing adherent colonies. The spatio-temporal aspect of biofilm development is a principal research priority at the CBE. This research objective underlies several key questions that await elucidation such as: 1) Why are biofilms less susceptible to many biocides than free-floating bacterial cells? 2) How do biofilm organisms respond to surfaces genetically and phenotypically? and 3) How does biofilm development proceed over time? These questions require state-of-the-art imaging capabilities in order to examine the spatio-temporal relationships between biofilm structure and function.

Confocal microscopy is an essential component of this imaging capability because other microscopic techniques require fixation and dehydration and therefore disruption of the three dimensional structure. Improvements in confocal imaging technology now allow the use of spectrally proximal molecular reporter probes to follow gene regulation during biofilm development. The flow cytometry instrumentation requested in this proposal directly complements confocal imaging data by dramatically enhancing our ability to quantitate microorganisms within a biofilm, and facilitating the isolation of new reporter strains to investigate the functional relationships between biofilm structure and development. Quantitative analysis is essential to the engineering systems approach that the CBE is known for, and flow cytometry will offer innovative improvements in biofilm quantification. This combination of imaging instrumentation will allow the CBE to continue as a preeminent and exceptional research facility.

## SESSION 3: Biofilm Structure and Function

**W03-S12**
### Session Introduction

***Phil Stewart****, Professor of Chemical Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

**W03-S13**
### Characterization of the Extracellular Matrix of *Pseudomonas aeruginosa* Biofilms

***Mike Franklin****, Associate Professor of Microbiology, Montana State University–Bozeman, 59717*

Individual bacterial cells, although often no more than one micrometer in length, are often found as far as one millimeter away from the substratum when growing in biofilms. This suggests that most of the bacteria are not directly attached to the surface by a specific adhesin, but are maintained in an extracellular matrix. Recent reports have examined the nature of the extracellular polymeric matrix, ultimately to identify novel biofilm disruption methods. Different studies have suggested that he matrix is composed of (i) DNA, (ii) protein, (iii) polysaccharide, or a combination of these components. In this study, we used defined media to manipulate the thickness of *P. aeruginosa* biofilms, and characterized the biofilm extracellular matrix material using Fourier Transform Infrared (FTIR) Spectroscopy. Using a mucoid cystic fibrosis pulmonary *P. aeruginosa* isolate, increased biofilm thickness correlated with increased amounts of the extracellular polysaccharide, alginate. Using an

*algD* reporter construct, we found that the increased alginate concentration was due to transcriptional activation of the *alg* biosynthetic operon. In addition, three extracellular proteases, AprA, LasB, and PrpL, were induced under conditions that resulted in thick biofilms. A deletion mutation in the *aprA* gene did not significantly affect the biofilm structure, suggesting that the AprA protein, although likely maintained in the biofilm matrix, is not essential for the three-dimensional structure of these biofilms. With the nonmucoid *P. aeruginosa* strain PAO1, no alginate was detected in the extracellular matrix by FTIR spectroscopy. The results suggest that the composition of the extracellular matrix varies with different bacterial strains, but that extracellular polysaccharides may be important for the production of thick, robust biofilms.

## W03-S14
### Heterogeneous Growth in Biofilms

***Erin Werner**, Undergraduate Researcher, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

*Pseudomonas aeruginosa* biofilms were shown to contain zones of active bacterial growth and also zones of little or no growth. In the colony biofilm system, the growing zone is located near the air interface. Oxygen microelectrodes were used to measure the depth of oxygen penetration, which was about 50 microns. Two different green fluorescent protein reporter gene constructs were used to visualize the region of growth. The most successful of these contained a stable gfp that could be induced by the chemical agent isopropylthio-beta-*D*-galactoside (IPTG). When 48 h old colony biofilms were transferred to plates containing IPTG, a bright fluorescent band developed in the biofilm near the air interface. This band was approximately 60 to 90 microns thick. As another test for growth, colony biofilms were exposed to the antibiotic carbenicillin. This agent causes growing cells to form long filaments. Filamentation was observed, using transmission electron microscopy, only in cells near the air interface of the colony. Preliminary experiments using medium supplements (arginine, nitrate) that enable anaerobic growth suggested that the gfp technique was not able to detect anaerobic growth. The inducible gfp construct was grown in a glass flow cell and observed by confocal scanning laser microscopy. This experiment revealed spatially heterogeneous patterns of growth in the biofilm.

## W03-S15
### Modeling Biofilm Antibiotic Resistance: Persisters

***Mark Roberts**, CBE Undergraduate Researcher, Chemical Engineering and **Phil Stewart**, Professor of Chemical Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

A mathematical model of biofilm dynamics was used to investigate the protection from antibiotic killing that can be afforded to microorganisms in biofilms based on a mechanism of "persister" cell formation. The persister state is a hypothetical, highly protected state adopted by a small fraction of the cells in a biofilm. Persisters were assumed to be generated at a fixed rate, independent of the presence of substrate or antibiotic. Cells were assumed to revert from the persister state when exposed to the dissolved substrate (oxygen). The model predicted that persister cells increased in numbers in the biofilm even if they were unable to grow. Persisters were predicted to accumulate in regions of substrate limitation. In these regions, normal cells fail to grow, but do occasionally convert to the persister state. Interestingly, this model is able to predict that biofilms become more resistant as they age, even if they are not growing thicker. The time scale for this gradually decreasing susceptibility was several to many days. When antibiotic treatment was simulated, bacteria near the biofilm surface were killed, but persisters in the depth of the biofilm were poorly killed. These cells quickly reverted and allowed the biofilm to regrow after antibiotic treatment ceased. This modeling study provides motivation for further investigation of the hypothetical persister cell state as an explanation for biofilm resistance to antimicrobial agents.

## W03-S16
### Biofilm Fuel Cells

***Zbigniew Lewandowski**, Professor of Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

In chemical fuel cells, oxygen reduction is usually the rate-limiting reaction, and has to be enhanced by elevated temperature, pressure, or both. Using biomineralized manganese oxides as redox mediators of oxygen reduction, the reaction runs at ambient temperature and pressure, and the rate-limiting

reaction will be the rate of manganese turnover on the surface of the cathode.

Biofilms composed of manganese oxidizing bacteria are grown on the cathodic members of microbial fuel cells, on which they deposit manganese oxides. The biomineralized manganese oxides are then electrochemically reduced by the electrons derived from suitable anodic reactions, e.g. oxidation of hydrogen or a sacrificial anode. Biomineralized manganese oxides act as recyclable redox mediators of oxygen reduction in this process; manganese oxidizing micoorganisms oxidize manganese and reduce oxygen. As the process progresses, the biomineralized oxides are electrochemically reduced to manganese ions. However, since these ions are released within the biofilm of manganese oxidizing microorganisms, they will be microbially re-oxidized to manganese oxides, re-deposited on the surface of the cathode, and the cycle will continue.

Microbial fuel cells will be used to power chemical and biological sensors deployed at inaccessible locations.

## SESSION 4: Distribution System Biofilms

### W03-S17
### Regulatory Update: Heterotrophic Plate Counts

***Anne Camper**, Associate Professor of Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

For over 100 years, the number of bacteria in water has been used to provide information on the quality of that water. It is useful to understand the history associated with this analysis along with the myriad methods that have evolved. There are differences in terminology, guideline/regulatory values, and the interpretation of what the numbers mean depending on the country and industry. The temptation has been to establish standards or guidelines for the protection of public health associated with these methods, but data are lacking to substantiate this approach. This is true even with the recognition that opportunistic pathogens may be part of the HPC. The emerging view is that these counts should be used to provide a comparative evaluation of treatment, storage, and distribution rather than as absolute standards.

### W03-18
### Assessing Microbial Contamination Risks for Small Water Systems

***Phil Butterfield**, Assistant Research Professor of Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Small water systems account for the majority of waterborne disease outbreaks recorded in the United States each year. Small water systems typically serve a population less than 3,300 people, and include transient water systems such as one that serves a roadside convenience store. The goal of this project was to develop a comprehensive self-assessment toolbox that could be used by small water system managers and operators to determine where their system has the greatest potential risks from microbial contamination.

The toolbox consists of the following components:

- Microsoft Excel based ranking tool application
- Ranking Tool Instructions and Survey Answer Sheets
- Guidance Document

The user of the toolbox first answers questions in a series of surveys covering each major category of the water system. Answers from the survey are then placed into the ranking tool application. The ranking tool application provides immediate feedback to the user based on answers to the survey questions. The feedback is intended to point out those areas of the water system that may need attention or repair. The ranking tool application also provides the user with numerical ranking scores for each component of the water system so that the user can determine where priorities should be established for purposes of reducing possible contamination risks. Results are provided both in tables and bar charts. The user can also use the ranking tool application to evaluate "what-if" scenarios where the user changes answers to see the effect on numerical scores. Small water system users can also use the self-assessment tool in preparation for sanitary surveys by state agencies.

The ranking tool application uses the answers from survey questions to compute a score for each component of a water system category. Internal weighting (or importance) factors are used to compute scores for a category. A unique feature of the ranking tool is the ability to input expert opinion in the form of

scores for each answer and importance factors. A technique derived from decision making theory can be used to determine importance factors for each category. Ranked pairwise comparisons are used to determine the importance factors. Spreadsheets were developed that allow the person performing the pairwise comparison to determine if any reverse ranking has occurred. Small water system users do not have access to the importance factors; they can only be modified by qualified persons.

The presentation will provide a brief look at the background that went into the development of the toolbox. An explanation of how the ranking tool computes numerical scores will be reviewed along with the ranked pairwise comparison methodology. Finally, a demonstration will be presented showing some of the features and application of the ranking tool.

## S02-S19
## Rechargeable Biocidal Coating for Biofilm Control

***Alex Bargmeyer**, MS Candidate, Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

The drinking water industry is interested in finding new methods for preventing biofilm development and/or eliminating biofilms in the water delivery system. Concern for reducing biofilms in public water distribution systems is due to problems such as undesirable taste and odor, corrosion, and harboring opportunistic pathogens. Current methods employed to combat these problems include the use of disinfectants, reduction of organic material in the water, and pipe materials that reduce the amount of biofilm accumulation. One objective of this research project was to screen new and novel technologies in the laboratory using annular reactors and mixed population biofilms of drinking water origin. The most promising strategy employs a surface coating containing halogen-binding technology that renders the surface biocidal.

This technology is the product of the Halosource Corporation and has numerous applications in industry for halogen stabilization and biocidal surfaces. A polycarbonate substratum in the aforementioned reactor system is coated with polyurethane that has the Halosource compound incorporated into it. This compound is a derivative of an N-halamine that has the ability to bind oxidative chlorine. The first studies using Halosource coatings were designed to see if the surface had the ability to mediate the reduction of an established biofilm under typical drinking water free chlorine levels (1.0 mg/l residual). Results showed that the Halosource surfaces are able to significantly reduce the numbers of attached microbes in established biofilms when compared to polycarbonate surfaces once chlorine is applied to the system.

The latest experiments have tested the coating's ability to resist biofilm accumulation when the surfaces are continuously exposed to low chlorine and monochloramine residuals. The results thus far from these experiments are not as promising as those from the earlier experiment. It is hypothesized that the concentrations of chlorine, microbes (planktonic and biofilm), and other organic material influence the rate of charging and depletion, which influences the ability of the surface to be continuously biocidal. A mechanistic explanation of this behavior will be the focus of continuing research. In addition, field testing is currently underway at three drinking water utilities to provide a more realistic assessment of the coating's performance.

## W03-S20
## Bioterrorism and Drinking Water Distribution Systems

***Anne Camper,** Associate Professor of Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

An emerging area of concern for personnel responsible for domestic and military water systems is the intentional introduction of organisms or toxins that will result in a threat to public health. Virtually all of the research and data to date has dealt with the fate and transport of these bioterrorist agents in the water, with little recognition of the potential interaction with biofilm. Biofilms may act as a reservoir for these agents for periods of time exceeding that for their transport and disinfection/inactivation in the water. Consequently, the biofilm may be a reservoir for further public health threat, or it may be viewed as a tool to help determine if contamination has occurred. In the first case, basic knowledge about the ability of bioterrorist agents to persist and the technologies needed to ensure a clean system are vital for decision makers. In the latter case, there is potential for

developing a technology that, coupled with state-of-the-art sensor technologies, will provide enhanced detection and attribution of bioterrorist agents. Work is currently underway at the CBE to address both these scenarios.

## SESSION 5: Mechanical Disruption of Biofilms

### W03-S21
**Session Introduction**

***Paul Stoodley,*** *Assistant Research Professor, CBE*

### W03-S22
**Disruption of Dental Plaque Biofilm via Fluid Forces**

***Chris McInnes,*** *Principal Research Scientist, Philips Oral Healthcare, Inc. Snoqualmie, WA 98065*

The goal of oral hygiene is to maintain oral health. This is primarily accomplished through the prevention of dental plaque build-up, either by regular removal of biofilm deposits or by reducing their accumulation. The complexity of the oral cavity has lead to a diversity of oral hygiene products. Most consumers, at least in Western societies, utilize some form of a toothbrush to clean the exposed surfaces of their teeth. Cleaning areas less accessible to a toothbrush requires another set of tools (floss, pick, irrigator, professional cleaning, etc.) As these areas are harder to reach, many consumers ignore them. The result of this neglect often leads to gingivitis and, potentially, periodontal tissue loss as the tissue responds to the dental plaque biofilm build-up. Recent advances in oral care have led to products that utilize fluid activity to help reach beyond the bristle tips into areas such as the interproximal and subgingival spaces. The goal is the disruption of dental plaque biofilm via fluid forces. The belief is that fluids may more easily penetrate areas where a toothbrush bristle is unable to conveniently access. As research in this area has progressed over the last decade, the methodology used to understand and quantify this effect has evolved considerably. Laboratory research has shown that fluid flowing through the interproximal space can aid in the removal of biofilm in regions that a toothbrush's bristles do not access.

### W03-S23
**Effect of Power Brushing on Biofilm Removal Using an Interproximal Model**

***Heather Adams****, Undergraduate, Genetics/Molecular Cell Biology at Washington State University–Pullman, WA*

### W03-S24
**Viscoelasticity of Dental and Other Biofilms**

***Matt Winston,*** *Undergraduate Researcher, Mechanical Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Biofilms are found in almost all environments, ranging from the inner walls of water piping systems to the plaque on teeth that causes caries and gingivitis. The biofilm's unique internal structure allows it to resist common bacterial control mechanisms, both chemical and mechanical, making it difficult to remove. In dentistry, as in industry, it is desirable to remove these biofilms; a common strategy is to employ mechanical removal processes. In order to gain insight into how to optimize such removal processes, it is important to study the material properties of the biofilm. For the given study, biofilms were grown from the early dental plaque organism *Streptococcus mutans* in a rotating disk reactor with BHI media and 2% sucrose. The biofilms were subsequently tested using a TA Instruments AR1000 rheometer. Creep tests were performed on the biofilms, in which a constant shear stress was applied and held for a given time, and the resulting strain (amount of stretching) was measured. The stress was then removed, and the recovery of the biofilms was measured. Creep tests showed that the viscoelastic properties of the biofilms can be broken down into four separate parameters using a Burger model of springs (elastic elements) and dashpots (viscous elements). Also from this test the shear modulus of elasticity (G) can be found, along with viscosity and the linear elastic range of the biofilms. Shear modulus and viscosity describe the solid and fluid-like characteristics of the biofilms, while the linear elastic region shows at what point the biofilm's structure begins to break down. A wide range of viscosities and shear moduli were observed, and the linear elastic region was found to be between 3 ands 4 Pa. This is significant, as is gives greater insight into the general range of stresses need to break biofilms down. This viscoelastic phenomenon has also been seen in various

mucoid and non-mucoid *P. aeruginosa*, *S. aureus*, duck pond water, tap water and algal hot springs biofilms. By continuing this research we are hoping to gain further insight into optimizing the mechanical removal of biofilms.

**W03-S25**
### Modeling Biofilms as Viscoelastic Fluids

***Todd Shaw**, PhD Candidate, Mathematics, Montana State University–Bozeman, 59717*

A mathematical model is often used to better understand or to predict the behavior of a complex, dynamic process. The interaction between the bulk fluid and biofilm is such a process. Currently we are working on modeling this interaction and incorporating various material properties of biofilm into our modeling. Here we present a model for the 'flow' of the bulk fluid and biofilm. The model and flow is that of two immiscible fluids separated by an interface. The bulk fluid is assumed to be Newtonian and to evolve according to the Navier-Stokes equations. Experimental observations indicate that biofilm is most likely a viscoelastic fluid. Moreover, the polymer matrix (EPS) in which biofilm microorganisms enclose themselves is consistent with this idea. Viscoelastic fluids have complex constitutive equations (stress and deformation relationships), and this complicates the modeling and computing. In using computer simulations we hope to better understand the "mechanical" nature of biofilm as a material.

## SESSION 6: Industry Interaction

**W03-S25**
### Cooperation Between Industry, Academics, and Institutes

***Joel Berg**, Chair, Department of Pediatric Dentistry University of Washington, Box 357136*
***Bill Costerton**, Director, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

This presentation will discuss the various ways in which academic institutions, research institutes and industry can cooperate for mutual benefit. Over the past several years, the Center for Biofilm Engineering has maintained an excellent collaborative relationship with Philips Oral Healthcare in generating new science as part of product development efforts. This paper will review a variety of mechanisms by which academia and industry can pool resources toward achieving symbiotic objectives. Included in the review will be a discussion of the common missions of these two institutions, and how one can overlap these common grounds toward achieving targeted goals.

# POSTER ABSTRACTS

## W03-P288
### Field Study: Biofilm Accumulation in Hot Tubs

***Darla Goeres**, Linda Loetterle, Marty Hamilton, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

A literature survey showed that hot tub users may be at risk for respiratory, eye, ear, skin, gastrointestinal and urinary tract infections. Although little research exists that directly links biofilm in hot tubs to disease, data suggests that biofilm may serve as a reservoir. To document the presence of biofilm in hot tubs, a field study of four Bozeman-area hot tubs was conducted to test the accumulation of planktonic and biofilm bacteria over a four-week period. Sampling included bulk fluid, coupons attached to the wall, aerosolized bacteria, filters and general water chemistry (pH, temperature, alkalinity, hardness and disinfectant). Coupon biofilm accumulation ranged from 0.65 to 3.19 $\log_{10}$ cfu/cm$^2$ after 4 weeks. New filters were installed in two tubs, and after 4 weeks both had an approximate 2 $\log_{10}$ cfu/cm$^2$ accumulation of biofilm. Bulk fluid concentrations ranged from non-detectable to 3.85 $\log_{10}$ cfu/mL. These results document the need to include biofilm as part of a standard protocol for testing the efficacy of hot tub disinfectants.

## W03-P298
### Use of High Performance Liquid Chromatography–Diode Array Detection for the Improved Analysis of 2,4,6-trinitrotoluene and Its Reduced Metabolites

***Thomas Borch,** Ryan N. Jordan, Alfred B. Cunningham and Robin Gerlach. Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Explosives such as TNT are widely distributed environmental contaminants. Numerous laboratory and field scale studies addressing the biotic and abiotic reductive degradation of TNT in subsurface environments have been conducted. Many of the observed TNT metabolites are only metastable. Thus, fast and reliable analytical techniques have to be developed which will allow the complete separation of TNT and its reduced metabolites.

This poster presents the first Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method capable of separating all of the following nitroaromatic compounds in a single run:
TNT, 2-hydroxylaminodinitrotoluene (2-HADNT), 4-hydroxylaminodinitrotoluene (4-HADNT), 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'-Azoxy), 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2,2'-Azoxy), 2,2',6,6'-tetranitro-4,4'-azotoluene (4,4'-Azo), 4,4',6,6'-tetranitro-2,2'-azotoluene (2,2'-Azo), 2-aminodinitrotoluene (2-ADNT), 4-aminodinitrotoluene (4-ADNT), 2,4-diamino-6-nitrotoluene (2,4-DANT), 2,6-diamino-4-nitrotoluene (2,6-DANT), and triaminotoluene (TAT).
All peaks of interest were separated and detected (diode array detection) with a chromatographic resolution ($R_s$) better than 1.2 and with a retention factor (k') between 2 and 14.

A second method was developed for the combined analysis of explosives included in the EPA method 8330 and the above-mentioned TNT metabolites (23 chemicals in total). All chemicals were separated except the exception of Tetryl and 2-HADNT, which co-eluted.

The extremely unstable and oxygen sensitive TAT, produced during the reductive metabolism of TNT, was reliably detected, and a method was developed (based on a TAT stability study) to maintain TAT standards over extended periods of time.

To illustrate the applicability of the developed RP-HPLC method in a series of TNT degradation studies, experiments were set up in which the reductive transformation of TNT under biotic (Fe(III)-reducing) and abiotic conditions (in the presence of Fe(0)) was monitored.

The poster will describe the details of the chromatographic methods, the TAT study, and the TNT (bio)transformation experiments.

## W03-P299
## Direct and Indirect Microbial Reduction of Cr(VI) by *Cellulomonas spp.* in the Presence of Iron Minerals

***Sridhar Viamajala,*** *Robin Gerlach, Brent M. Peyton, Alfred B. Cunningham, William A. Apel*
*WSU/NSF IGERT Center for Multiphase Environmental Research, Washington State University; Center For Biofilm Engineering at Montana State University–Bozeman, 59717; Biotechnology Department, Idaho National Engineering and Environmental Laboratory*

We recently enriched and isolated a number of gram positive bacterial strains capable of Cr(VI), U(VI), and Fe(III) reduction from contaminated and uncontaminated areas at the Hanford Site (Washington). Using 16S rDNA and phospholipid analysis, most of the isolates were tentatively identified as Cellulomonas spp. The activity of Cellulomonas spp. in the Hanford subsurface could significantly influence the fate of contaminant mixtures consisting of heavy metals, radionuclides, and chlorinated aliphatics. Our current research is determining the kinetics of Cr(VI) and Fe(III) reduction by these isolates and the Cr(VI) reduction by microbially produced Fe(II). The current studies will form the basis for a mesoscale demonstration project at the INEEL which will begin late in 2002.

## W03-P300
## Imaging the Influence of Biofilm Growth on Bulk Flow Using Nuclear Magnetic Resonance Microscopy

***E.L. Gjersing****, S.L. Codd, P.S. Stewart, J.D. Seymour*
*Department of Chemical Engineering and Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Nuclear Magnetic Resonance (NMR) microscopy is an excellent method for imaging living systems since it is innocuous and non-invasive. In addition to imaging, NMR microscopy techniques can be used to obtain information about transport phenomena such as fluid velocities and diffusion. The current focus of this research is to map the flow patterns through biofilm fouled capillaries. Insight to how biofilm structures affect bulk flow will contribute to better computer models of biofilm behavior. NMR microscopy techniques were used to image the biofilms and the flow around them in1mm-square glass capillaries. Figure 1 contains five successive images through the cross section of the capillary that were generated from NMR microscopy data. Figure 2 shows velocity profiles in the x, y and z-directions for flow around a biofilm. For laminar flows in a clean capillary there are no x or y components of velocity while the z direction is both uniform and symmetrical. In contrast, a biofilm fouled capillary displays irregular flow patterns in the z direction along with distinct x and y flow perturbations. These results show that biofilm fouling has a significant impact on bulk flow which should not be ignored in behavior models. Future experiments will examine how flow patterns change over the course of biofilm growth and under different environmental conditions, in addition to characterizing the diffusion in this system.

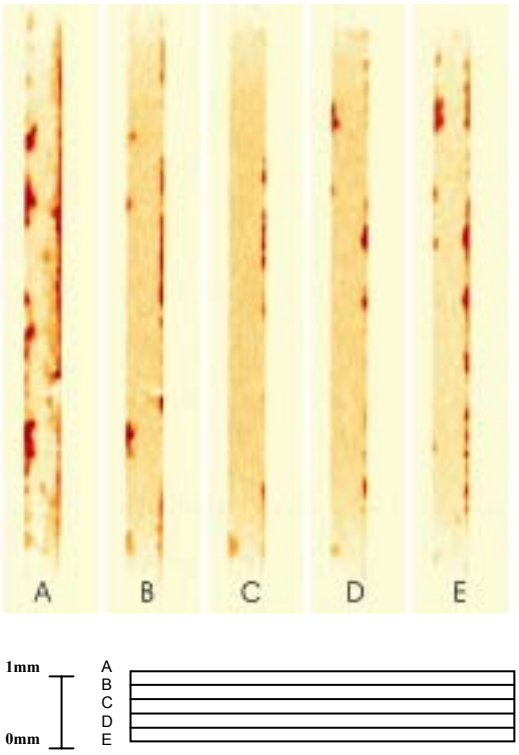


Figure 1. Successive images through cross sections of capillary. Dark spots indicate areas of biofilm growth. The field of view for each image is 20mm x 2.5mm with a slice thickness of 0.2mm.

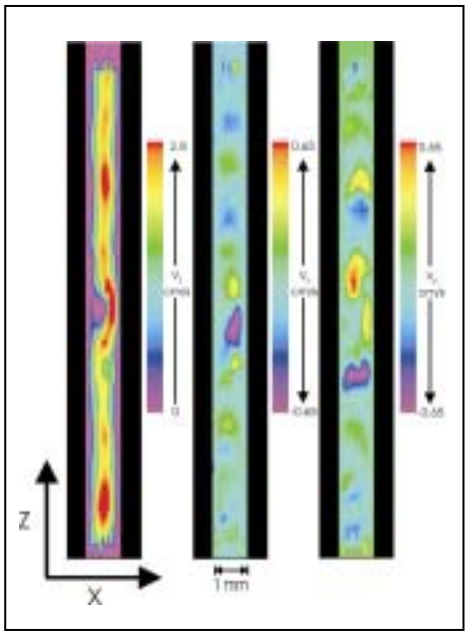


Figure 2. Longitudinal direction velocity maps in a biofilm fouled capillary.

## W03-P301
### Workshops Offered by the Biofilm Structure and Function Research Group

***Haluk Beyenal,*** *Zbigniew Lewandowski, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

The Biofilm Structure and Function Research Group organizes 1) Biofilm Image Analysis and, 2) Microsensors - manufacture and applications-workshops. In this poster we presented the contents of the workshops. The detailed information about the workshops can be found on the web pages below.

Biofilm Image Analysis
http://www.erc.montana.edu/CBEssentials-SW/MicrosensorWorkshop/Workshop-image_analysis_2003.pdf

Microsensors - manufacture and applications –
http://www.erc.montana.edu/CBEssentials-SW/MicrosensorWorkshop/Workshop-image_analysis_2003.pdf

## W03-P302
### Siderophore Mediated Metal Transport

***Abigail Aiken,*** *Anne Camper, William A. Apel, James N. Petersen, Brent M. Peyton, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Heavy metals and radionuclides are known to contaminate the soil and groundwater at more than half the DOE facilities in the United States, including the INEEL. Heavy metal and radionuclide contamination in soil and groundwater poses a severe threat to both human and environmental health. Because of this, it is essential to reach a thorough understanding of mechanisms of metal and radionuclide mobility, and how they are affected by microbial activity. A wide range of phenomena ranging from simple to complex interactions controls the behavior of toxic metals and radionuclides in the environment. Microorganisms can absorb, reduce, oxidize, solubilize or precipitate both toxic heavy metals and radionuclides, thus impacting the speciation, solubility and ultimately the mobility of these metals in the environment. In addition to the influence which whole cells can have on metal transport, microbes can also secrete compounds that facilitate or hinder metal mobility. Siderophores are strong iron-chelating agents, secreted by microorganisms under iron-starved condition, that have many potential applications, including both bioremediation and iron chelation therapies. Siderophores, although highly specific for iron, have been shown to bind other metals such as divalent heavy metals and actinides. Although highly specific for Fe(III), some metal siderophore complexes approach the stability of the Fe(III) complex, as seen for desferrioxamine B (DFO-B) complexed with Th(IV) and Pu(IV), which have stability constants of $10^{26.6}$ and $10^{30.8}$, respectively. Because of these high stability constants, siderophores have the potential to significantly alter the mobility of metal contaminants in subsurface environments. It is the goal of this work to determine the influence that siderophores have on the mobility of both radionuclides and heavy metals.

## POSTER ABSTRACTS

### W03-P303
### Proteomic Comparison of Biofilm vs. Detached Methicillin Resistant *Staphylococcus aureus*

***Katherine Cooperstein**, Zachary Bell, Mark Shirtliff, Paul Stoodley, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a common cause of disease in humans. MRSA can form biofilms and detach as infectious emboli (detached particles) from the mass of biofilm in dynamic flow. Our goal was to determine if MRSA biofilm differs from the detached populations of MRSA by proteomic evaluation. A reactor system for simulated dynamic flow produced both detached populations and biofilm for collection after seven days of growth. Analysis of the proteomic structure was performed using 2-D gel electrophoresis (for isolation of proteins demonstrating altered expression upon comparison) and matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification of proteins.

### W03-P304
### Microbial Process Research for the Commercialization of Subsurface Biofilm Barrier Technology

***Al Cunningham,** Professor of Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

An engineered microbial biofilm barrier capable of reducing aquifer hydraulic conductivity while simultaneously biodegrading nitrate has been developed and tested at a field-relevant scale. The 22-month demonstration project was conducted at the MSE Technology Applications Inc. test facility in Butte, Montana, USA. It consisted of a 130 ft wide, 180 ft long, 21 ft deep PVC- lined test cell, with an initial hydraulic conductivity of $4.2x10^{-2}$ cm/s. A flow field was established across the test cell by injecting water up-gradient, while simultaneously pumping from an effluent well located approximately 50 ft down-gradient. A 30 ft wide biofilm barrier was developed along the centerline of the test cell by injecting a starved bacterial inoculum of *Pseudomonas fluorescens* strain CPC211a, followed by injection of a growth nutrient mixture composed of molasses, nitrate, and other additives. A 99% reduction of average hydraulic conductivity across the barrier was accomplished after 3 months of weekly or bi-weekly injections of growth nutrient. Reduced hydraulic conductivity was maintained by additional nutrient injections at intervals ranging from 3 to 10 months. After the barrier was in place a sustained concentration of 100 mg/l nitrate nitrogen, along with a 100 mg/l concentration of conservative (chloride) tracer, was added to the test cell influent over a 6-month period. At the test cell effluent the concentration of chloride increased to about 80 mg/l while the effluent nitrate concentration varied between 0.0 and 6.4 mg/l.

### W03-P305
### Bacterial Biofilms in Sinusitis

***Steve Fisher,** Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Abstract not available.

### W03-P306
### Control of Acid Rock Drainage Through Stimulation of Selected Microbial Populations in Mine Tailings

***Paul Sturman,** Coordinator for Industrial Development, Center for Biofilm Engineering at Montana State University–Bozeman, 59717; USEPA Mine Waste Technology Program; MSE Technology Applications, Inc.; USGS*

Acid rock drainage (ARD) from abandoned hard rock mine lands is a major environmental problem that impacts both ground- and surface-water throughout the Western United States and is a major contributor to loss of habitat for fisheries. Abandoned mine lands (AML) often contain unmined mineral deposits, waste rock, and mine tailings which contain high concentrations of metals in the form of metal sulfides. When oxygen-containing rainwater, streamwater, and/or groundwater comes into contact with these materials, chemical oxidation reactions occur that liberate the bound metals into solution and may radically lower the pH of the receiving water. The purpose of this research is to further develop an inexpensive and potentially widely applicable treatment technology to utilize indigenous microorganisms within mine tailings to abate ARD at its source.

The goal of this work was to control acid and dissolved metals production in pyritic mine tailings through the selective enhancement of microbial populations. The primary means of enhancement was the addition of easily assimilable organic carbon, particularly molasses and whey. Mine tailings were acquired from 2 sites: Fox Lake Mine in Manitoba, and the abandoned Mammoth Mine in the South Boulder River drainage, MT. The Fox Lake tailings are relatively new, with high pyrite content, while the Mammoth tailings were deposited over 100 years ago and represent a highly leached situation. Both sets of mine tailings were packed into laboratory columns and periodically fed dissolved molasses and whey.

Results indicated that both molasses and whey treatments were capable of reducing effluent oxidation-reduction potential (ORP) and increasing effluent pH, however, high variability between the columns was noted. Column effluent metals data indicates that the whey treatments were more effective than molasses in reducing dissolved Al, Fe and Zn from the columns. Microbial enumeration from column effluent indicates that the treatment did not reduce levels of iron and sulfur oxidizing bacteria, as it was expected to do, but it did significantly stimulate SRB. SRB reduce SO4 to H2S, which readily reacts with dissolved metals and precipitates as a metal sulfide. SRB also generate alkalinity (OH-) as a product of metabolism, increasing column pH.

These experiments indicate that microbial populations in tailings respond inconsistently to organic carbon treatment. Ongoing research suggests that populations of iron oxidizing hetertrophic bacteria (of the genus Sulfobacillus) are stimulated by organic carbon treatment, particularly molasses. SRB growth is also stimulated by organic carbon treatment, but may not be able to overcome the acidifying effects of iron oxidizing heterotrophs in all cases. For field application, further understanding of the responses of these populations to various organic carbon treatments is necessary to insure beneficial outcome.

**W03-P307**

## Detachment and Antimicrobial Resistance of Single Cells and Cell Clusters from *Pseudomonas aeruginosa* and *Staphylococcus aureus* Biofilms

***Suzanne Wilson,*** *Research Assistant, Center for Biofilm Engineering at Montana State University–Bozeman, MT 59717*

The detachment of cell clusters from biofilms enables them to disseminate, flow downstream, and reattach, establishing another biofilm event. In this study detached biomass from biofilms grown under a constant flow rate were filtered from the effluent and microscopically examined to determine the size distribution (number of cells per particulate) and detachment frequency from biofilms composed of gram positive and gram negative bacteria. Biofilms were grown from three individual strains of *Pseudomonas aeruginosa,* and also from *Staphylococcus aureus* to compare with a gram positive non-motile species. Biofilms were grown in glass flow cells for 7 days under laminar flow. Effluent samples were taken on various days during the course of the experiment; data from day 5 were selected for statistical analysis. The detachment distribution from each of the *Pseudomonas* strains was similar (PAO1 averaged 3.5 cells/cluster; JP1, 3.1; and FRD, 2.9 respectively). Most of the detached particulates from the *Pseudomonas* biofilms occurred predominantly as single cells (c.a. 75%), ranging from 55% for PAO1 to 90% for FRD1. Detaching particulates from the *S. aureus* biofilm were larger on average (11.2 cells/cluster) and more evenly distributed among cluster size.

Detached particles were also evaluated for increased antibiotic resistance over planktonic cells. Sonicated particulates were exposed to a range of Oxacillin between 0.05 µg/ml and 20 mg/ml, and the MBC values were compared to that found using either stationary or exponential planktonic cells as inoculum. There was no significant difference ($P < .01$) between the antibiotic resistance of the clump and the stationary planktonic inoculum, while the exponentially growing cells expressed normal sensitivity. This would indicate that the low metabolic rate of the cells within the detached particles would account for the high MBCs.

## POSTER ABSTRACTS

**W03-P308**
### Green Adhesive and Microbial Polymer Products

***Tony Haag**, Gill Geesey Montana State University–Bozeman, 59717*
*Joan Combie, Montana Biotech Corporation, Belgrade, MT 59714*

Abstract not available.