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CBE Technical Advisory
Conference

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Sponsored by the
Center for Biofilm Engineering
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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 Sturman, CBE Coordinator of Industrial
Development*

A BRIEF HISTORY OF THE CBE

The CBE was established in 1990 through a grant from the National Science Foundation as one of fewer than two dozen Engineering Research Centers nationwide. The NSF-ERC program was created to increase U.S. industrial competitiveness and to reinvent 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 the mechanisms at work in bacterial biofilms. The CBE has been a leader in defining the structure and function of biofilms on surfaces, in understanding the antimicrobial resistance mechanisms of biofilm, and 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.

GENERAL INFORMATION

The second goal of the CBE is to make its research relevant to real systems, where the information can be useful. Industrial partnerships keep the CBE from being a traditional university “ivory tower,” collecting information that has no practical application. 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 the following:

- **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 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/>

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GUEST SPEAKER

W02-S01

Role of AI-2 signaling in cystic fibrosis biofilm formation

Jeff Stein, Chief Scientific Officer, Quorex Pharmaceuticals
M. Surette, University of Calgary

An increasing body of evidence indicates that cell-cell signaling systems play key roles in the induction of virulence factor expression in a broad array of bacterial pathogens. To date, studies of such systems have focused largely on the role of species specific signals: most prominently the homoserine lactone autoinducer signals produced by *Pseudomonas aeruginosa* which, at threshold concentrations, induce the formation of biofilms that play a central role in a large number of diseases, including cystic fibrosis. Recent studies have shown that bacterial inter-species signals may also play important, if not central roles, in the etiology of biofilm related disease. A recently discovered yet highly conserved bacterial inter-species signaling system, the AI-2 autoinducer system, also appears to be intimately involved in the regulation of biofilm by *Pseudomonas aeruginosa* and is present in high concentrations in the sputum of CF patients. Our studies suggest that components of the AI-2 system represent new, highly conserved bacterial specific targets for therapeutic intervention in this, and possibly other, bacterial diseases.

REGULATORY UPDATE

W02-S02

Biofilms and the FDA approval process

Bill Costerton, CBE Director, Center for Biofilm Engineering at Montana State University-Bozeman, 59717

In December of 2001 the FDA convened a conference on the Evaluation of Antibacterial Agents and Materials. Bill Costerton (CBE) and Guy Cook (Bacterin) were invited to make keynote presentations to this two-day meeting, which was held at the Devices Branch of the FDA, and attended by approximately 100 FDA staff members from the Devices Branch and five other units. Dennis Maki and Rabi Darouiche also made presentations, as did several medical microbiologists with interests and backgrounds in device-related infections. It rapidly became obvious that everyone concerned favored the CBE's direct methods of evaluation of the antibacterial efficacy of materials. Guy Cook's method of challenging putative antibacterial materials with wild strains of bacteria in flow cells using natural body fluids found particular favor, as did Guy's procedure of expressing antibacterial efficacy in terms of the number of days that biofilm formation was delayed in the case of each organism used in the challenge. Following a full day of presentations by researchers in this field, the organizers of this conference conducted a morning session in which written questions from the audience were presented to the speakers, and a very lively discussion ensued. This discussion centered on recent negative experiences with the mechanical heart valve that was approved by the FDA, and the acetabular cups that have failed very recently. The conclusion of the discussion was that *in vitro* data was a valuable indicator of effective antibacterial properties, and that devices could be characterized on the basis of the length of time that a particular material delays biofilm formation, on a species-specific basis. The FDA organizers of this conference declared their intent to re-write the standards and protocols that will be used for the evaluation of antibacterial properties of both agents and materials, based on their "take" from this conference. In addition to discussing this FDA standard-setting conference, Bill Costerton will also discuss recent developments in the ELISA test to detect incipient biofilm infections associated with vascular grafts and orthopedic devices.

SPEAKER ABSTRACTS

SESSION 1: Biofilm Control

W02-S04

Patterns of growth in *Pseudomonas aeruginosa* colony biofilms

Erin Werner, Keck Undergraduate Fellow, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Pseudomonas aeruginosa biofilms are resistant to killing by antibiotics. Mapping the patterns of growth in the biofilms will allow researchers to investigate the possible connection between areas of slow growth and antibiotic resistance. This project explores the patterns of growth in *P. aeruginosa* colony biofilms by using a green fluorescent reporter gene construct in a strain of *Pseudomonas aeruginosa*. The green fluorescent protein is active when the cell is growing. The intensity of the green fluorescence can be used to indicate growth in the biofilm down to the individual cell level. Colony biofilms of *P. aeruginosa* were grown for 48 hours, then frozen, sectioned and examined microscopically. These biofilms were 150 to 200 microns thick on average. A distinct band of fluorescence, the putative zone of growth, was evident along the air interface of the colony. This zone was approximately 63 microns thick. Oxygen concentration profiles are also being measured, since oxygen is likely to be limiting nutrient for these bacteria. The depth of oxygen penetration was about 48 microns. These results suggest that nutrient availability and growth are highly stratified in these biofilms. Such physiological heterogeneity could explain resistance to killing by antibiotics and other antimicrobial agents.

W02-S05

Innovative biofilm prevention strategies

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

The purpose of the Innovative Biofilm Prevention Strategies research project is to find novel methods of preventing biofilm development and/or eliminating biofilms in water delivery systems. A biofilm may be defined as a modular community of microbes embedded within a host- and/or microbe-derived hydrated matrix (usually exopolysaccharide) that

exists at a phase or density interface. The biofilm mode of growth enables resistance to antimicrobial-based removal strategies. In fact, previous research indicates that in order for sterilants and antibiotics to be effective against biofilm bacteria, concentrations 500 to 5000 times greater than those required for killing planktonic strains of the same bacterial species are necessary. One objective of this research project was to screen potential technologies in the laboratory using annular reactors and mixed population biofilms of drinking water origin. The technologies investigated so far are: cell-to-cell signaling molecules, the bioelectric effect, and a biocidal Halosource coating.

Three potential cell-to-cell signaling molecules were evaluated for their ability to promote biofilm detachment. The first two act as non-specific antagonists to bacterial signaling molecules. The last signaling molecule evaluated was not an antagonist, but a native autoinducing signaling molecule from the *P. aeruginosa* quorum sensing system: butyryl homoserine lactone (BHL). None of the signaling molecules showed significant efficacy on biofilm detachment from mixed species biofilms of drinking water origin.

Another innovative technology, the bioelectric effect, was also tested. The efficacy of antibiotics has shown to be increased through the application of weak electric fields. This study was designed to evaluate the ability of the bioelectric effect to enhance the antimicrobial properties of free chlorine against mixed population biofilms of drinking water origin. In this study the application of current had no effect on planktonic bacterial kill rates when applied alone to the reactor system or in combination with free chlorine administration. In addition, electrical current application to the reactor systems did not result in a reduction in the numbers of biofilm bacteria when applied alone and did not have an additive or synergistic bactericidal action when coupled with free chlorine administration. When these results are coupled with the observation that electric current application resulted in high corrosion rates seen on the electrodes and metals that were in contact with the fluid component of electrically treated reactors, our conclusion is that the efficacy of the bioelectric effect in biofilm clearance strategies for water distribution systems is not indicated.

The final technology studied was the incorporation of a halogen-binding molecule into surface coatings. This technology is the product of the Halosource Corporation and has numerous applications in industry for halogen stabilization and biocidal surfaces. A polycarbonate substratum 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, and can be recharged by free chlorine within the surrounding bulk fluid. The ability of Halosource coatings to mediate the reduction of an established biofilm under typical drinking water free chlorine levels was investigated. The results suggest that the Halosource surfaces accumulate fewer attached bacteria than unamended polycarbonate surfaces once chlorine is applied to the system.

W02-S06

A microtiter plate screening method for biofilm disinfection and removal

Betsey Pitts, Research Associate, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Most existing biofilm testing methods are inadequate for the task of screening numerous chemistries. This is because these methods rely on viable plate counts, a slow and labor-intensive technique that measures only disinfection. The purpose of this study was to develop a rapid spectrophotometric method for screening biofilm control agents that could measure both killing and biofilm removal. Single-species biofilms were grown using the known biofilm forming organisms *Pseudomonas aeruginosa* (PA01) and *Staphylococcus epidermidis* (ATCC 35984). Biofilms were grown in polystyrene 96-well microtiter plates for 24 hours, the disinfection or removal agent to be tested was applied, then wells were filled with either the biomass indicator crystal violet (CV) or the respiratory stain 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Biofilm-associated stain was solubilized with ethanol, and the color of the ethanol solution was read in a plate reader at either 450 nm (CV) or 540 nm (CTC). Biofilms of both species were challenged with a variety of agents and assayed using both stains. The results of these experiments show that bacterial activity, as measured by CTC staining, decreased as the treatment concentration increased. The dose response relationship for removal, as measured by CV

staining, was very different for the two species. Removal of *P. aeruginosa* biofilm by chlorine was essentially independent of concentration. Removal of *S. epidermidis* biofilm increased strongly as the chlorine concentration increased. The *P. aeruginosa* response to chlorine, which indicates significant disinfection of bacteria at high chlorine concentrations (1000 mg/L) but not much physical removal, illustrates the value of being able to measure both disinfection and removal. A direct comparison of plate counts and optical staining intensities from chlorine-treated wells on the same plate showed that plate counts and the optical methods gave results that were positively correlated.

W02-07

Critical review of a recent controversial paper

Phil Stewart, Professor of Chemical Engineering and Deputy Director for Research, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

This presentation will provide a commentary on a recent paper by Amy Spoering and Kim Lewis entitled “Biofilms and Planktonic Cells of *Pseudomonas aeruginosa* Have Similar Resistance to Killing by Antimicrobials.” The inflammatory title and radical conclusion — that biofilm resistance to antimicrobial agents can be studied using free-floating bacteria — challenge a widespread perception that the biofilm state is unique. This review will comment on some of the limitations of the evidence presented in this article and present an alternative interpretation of the data.

SESSION 2: Material Properties of Biofilm

W02-S09

Biofilm mechanics and surface migration

Cory Rupp, Keck Undergraduate Fellow, Mechanical Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Biofilm contamination is a major issue in industry and medicine. Contamination of medical devices, such as urinary or venous catheters or ventilator tubes, often results in infection. In industry, problems occur in paper mills, food processing plants and heat exchange

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systems. Many biofilm contamination problems occur in flowing fluid systems. We used an in vitro flow system to study the influence of fluid shear on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. Digital time-lapse microscopy was used to record and analyze biofilm deformation and the movement of biofilm along the sidewalls of the flow cells when subjected to a range of fluid shear stresses. One type of movement was the stretching (strain) and relaxing of the biofilm. Both the shear stress and the strain were measured to produce stress-strain curves. From these data we determined that both biofilms could be described as viscoelastic fluids with the Elastic Modulus (E, a measure of the rigidity of a material) ranging between 10 and 100 N/m². These values are comparable to that of slug pedal mucus. We also analyzed the movement of biofilm along the flow cell wall. Time-lapse movies of *S. aureus* biofilm suggested that the cell clusters were moving by rolling along the walls. The rolling motion was verified by tracking points on the biofilm and comparing the position curve to that of a cycloid curve. This allowed us to determine the angular velocity and surface velocity. Cell clusters in biofilms were found to travel at velocities of up to 6-10 µm/min. Since the rolling occurs along the sidewall, it must be caused by a different mechanism than that of gravitated sediments rolling along the bottom of a streambed. In this case, the rolling represents a continuous detachment from the substratum at the upstream edge of the biofilm cluster and a reattachment at the downstream edge. Fundamental understanding of the material properties of biofilms may explain dynamic biofilm behavior such as shear-induced detachment and surface transport, two largely unstudied mechanisms which may be significant in the dissemination of biofilms.

W02-S10

Modeling biofilm behavior

Brett Towler, PhD Candidate, Civil Engineering, Center for Biofilm Engineering at Montana State University-Bozeman, 59717

Biofouling, the detrimental effect of biofilm accumulation, can have a significant hydrodynamic impact. The highly compliant nature of this viscoelastic growth can dramatically affect the drag force on a flowing fluid. Clearly then, biofilms are an important flow boundary condition. While extensive data exists on the nature of the interaction

between water and common boundaries such as steel, concrete and plastic, comparatively very little is known about the material properties and frictional resistance induced by biofilm. The mechanics of biofilm have been investigated using a rotating-disk rheometer at the Center for Biofilm Engineering (CBE) at Montana State University-Bozeman. Nine mixed-culture biofilm samples were grown in a flow-through reactor for a period of approximately 12 days. The samples, grown on 40 mm anodized aluminum disks, were subjected to a creep procedure at a constant shear stress of 0.1 N/m². Shear strain measurements were recorded during 3-minute relaxation and recovery periods. Linear viscoelastic theory was chosen as a means of determining a suitable constitutive relationship for this biofilm. This technique seeks to represent a viscoelastic material as a composite of 'dashpots' and 'springs' elements arranged in series and in parallel. In this way, these two elements, representing the viscous and elastic nature of the material, provide a mechanical analog of the materials behavior. Using non-linear regression techniques, the creep test data was fit to several constitutive equations and the quality of these regression curves was measured using the sum of squared residuals. A 3-element General Kelvin model provided the best fit to the experimental data with an average R² of approximately 0.95 for all 9 data sets. The opportunity now exists to incorporate this constitutive relationship into a computational fluid model. Such a model would allow for the prediction of hydrodynamic resistance and provide significant insight into mechanically induced detachment phenomena.

W02-S11

Basic principles of viscoelasticity applied to biofilms

Aleksandra Vinogradov, Professor, Mechanical and Industrial Engineering, Montana State University-Bozeman, 59717

This paper provides an overview of the basic principles of viscoelasticity theory and their application to biological materials, including biofilms. Experiments indicate that there are clear similarities between the mechanical properties of biological and common engineering materials. In particular, similarly to synthetic polymers, biological materials tend to exhibit strain rate dependent behavior; i.e., they appear to be stiffer when stretched

at high strain rates and softer if pulled at lower strain rates. Moreover, biological materials display such properties as *creep* and *stress relaxation* usually observed in polymers. When a biological tissue is suddenly stressed and the stress is maintained constant in time, the respective response is characterized by continuing deformation, a phenomenon defined as *creep*. Alternatively, when the tissue is suddenly strained and the strain is maintained constant afterward, the corresponding stresses tend to decrease with time. This behavior is defined as *stress relaxation*. Essentially, biological materials combine elements of both elastic and viscous behavior and can be characterized as viscoelastic.

W02-S12 **Biofilm image analysis toolbox**

Haluk Beyenal, Assistant Research Professor, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

To quantify biofilm heterogeneity we have developed and implemented methods of extracting morphological features from images of biofilm. This is a first step toward quantifying the relationship between biofilm heterogeneity and the underlying processes, such as mass-transport dynamics, substrate concentrations, and species variations. We have examined two categories of features: areal, which quantify the relative magnitude of the heterogeneity, and textural, which quantify the microscale structure of the heterogeneous elements. In our previous studies, we have integrated algorithms to calculate biofilm structural parameters to a computer program written in C++. However, the program was developed for the UNIX/Xwindow environment, and running the software was difficult for most biofilm researchers who were not experienced UNIX users. Additionally, the software did not allow any modifications or custom designed image operations. For these reasons, we have re-designed, improved, and re-written the software in MATLAB. Users can now load images manually into MATLAB and calculate the following parameters of the images: textural entropy, homogeneity, energy, contrast, correlation, maximum probability, areal porosity, run lengths, diffusion distances, and fractal dimension. This presentation shows (1) how to use the toolbox, (2) how to quantify

biofilm images, and (3) how to interpret the data. The software will be available on the CBE web site (<http://www.erc.montana.edu>).

SESSION 3: Biofilm Methods

W02-S14 **Recent advances in biofilm methods from the perspective of reactor development**

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

Biofilms exist in extremely diverse environments. For each environment, certain growth conditions will dominate in importance. Researchers interested in the exploitation and/or control of biofilms have the need to re-create a relevant biofilm in the laboratory where the dominant parameters may be manipulated and the results studied. Varieties of laboratory reactors exist that enable a researcher to re-create the relevant biofilm, although no one reactor is appropriate for every situation. This presentation will review a collection of the reactors currently in use or under development at the CBE. The advantages and disadvantages of each system will be discussed as well as its appropriate applications. The goal of the presentation is to update industry representatives on methods development occurring in reactor design so that they may make informed decisions in their own biofilm research.

W02-15 **Assessment of a new method for biofilm analysis: Solid state cytometry for counting suspended, disaggregated bacteria**

Marty Hamilton, Professor, Statistics, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Epifluorescent microscopy is a standard technique for estimating the number of fluorescently labeled bacteria in a suspension. Though this technique is relatively simple and easy to perform, the estimated cell density is sometimes plagued by large statistical uncertainty. Consider the use of membrane filtration to retain and concentrate bacteria. Because it is not feasible for a

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technician to count the whole filter, counts are taken in a few microscopic fields that altogether represent a small fraction of the filter area. Then the counts are scaled up to estimate the total number of cells on the filter. If the cell density is low, there will be few, even zero, cells per field; in this case the estimated cell density has a large standard error. For this reason, if it is anticipated that the concentration of bacteria in a suspension is low, a larger volume will be filtered. This approach will increase the number of cells on the filter so that each field of view will have enough (>20) counts, thereby reducing statistical uncertainty in the density estimate. However, it is not always practical to filter a large volume, even if one can anticipate a low density. For example, if a large volume is passed through the filter and the solution contains autofluorescent material, that material may coat the filter and overwhelm the fluorescent signal of individual cells.

A recently developed technology, solid state cytometry, is capable of counting the entire area of a 25 mm membrane filter and of detecting a *single* fluorescently labeled bacterium on that filter. In this study, counts were performed by an instrument that was designed and manufactured for cell counting purposes. The instrument scans the filter with a laser and sends images to a computer, where pattern and fluorescent signal recognition software identifies the cells. We compared the bias, repeatability and the overall performance of conventional epifluorescent microscopy with this new solid state cytometry. Bias was measured by counting known concentrations of fluorescently labeled beads. The repeatability and practicality were assessed using both bead suspensions and bacterial (*E. coli*) suspensions.

We also investigated sampling issues that arise with the conventional microscopy method. We performed experiments to determine whether random sampling of fields on the filter provided more reliable estimates than the conventional systematic sampling. We evaluated the variability between filters and assessed the potential advantage of counting five fields on each of three filters rather than counting 20 fields on a single filter.

W02-S16

Use of spectroscopy to investigate biofilms

Peter Suci, Assistant Research Professor, Microbiology, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

The existence of chemical gradients in biofilms is integral to their description as complex communities composed of specialized cells. These gradients are in general dynamic entities, shifting with environmental conditions, the stage of biofilm development, and changes in biofilm species composition. Spectroscopic techniques provide a means to obtain chemical information non-destructively. Vibration spectroscopies provide a means to determine the chemistry of non-fluorescent substances. The spectra are rich in structure and originate from atomic vibrations associated with molecular bonds. Therefore, in principle, they contain information about all the bonds in every molecule in a complex mixture. In biological systems, infrared and Raman spectroscopies have been used to analyze protein secondary structure, characterize enzyme reactions, visualize chromophore-protein interactions, follow changes in DNA conformation, measure phase transitions in lipid membranes and characterize cell surface polysaccharides. In this short talk, use of infrared Raman spectroscopies to analyze biofilm-antimicrobial agent interactions will be presented. The potential for using these spectroscopies for characterizing chemical gradients in biofilms will be discussed.

Session 4: Medical and Environmental Biofilms

W02-S18

Pathogenic mycobacteria: What biofilms may tell us about intracellular pathogens

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

Research on mycobacteria has concentrated largely on pathogenic species that are primarily intracellular pathogens with fastidious cultivation requirements. Most species, however, are saprophytic, living in water and soil habitats, and are able to survive in the

environment under variable and suboptimal conditions. *Mycobacterium paratuberculosis* causes paratuberculosis or Johne's disease in wild and domestic ruminants and is responsible for significant economic losses in the U.S., particularly in dairy herds. Although infection occurs via the fecal-oral route, little is known about the ability of this organism to survive in the environment and its resistance to control efforts. *M. paratuberculosis* within biofilms could serve as a potential reservoir of Johne's disease and facilitate persistence even after eradication efforts have been implemented. By investigating biofilm formation with *M. paratuberculosis*, we are investigating a possible mode of this pathogen's survival that has not been recognized previously. Three million people die each year from tuberculosis (TB) in spite of numerous anti-mycobacterial antibiotics and a vaccine. Clearly, better ways to treat TB are needed. We are investigating *Mycobacterium tuberculosis*-binding interactions with several cell types and pathogen recognition molecules. Even intracellular pathogens typically gain entry to a host tissue by using cell-to-cell recognition and attachment mechanisms. Bacilli-host binding interactions are increasingly being identified for *M. tuberculosis*. We have applied a novel experimental approach to functionally evaluate *M. tuberculosis* binding interactions with host cells using flow cells to more accurately simulate physiological conditions in the lung. By exploring extracellular adhesion events we anticipate the discovery of novel molecular targets that could be used to develop better therapies or an improved vaccine.

W02-S19

Human leukocytes adhere, penetrate, and respond to *Staphylococcus aureus* biofilms

Jeff Leid, Assistant Research Professor, Cell Biology and Neuroscience, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Biofilms are structured communities of bacterial cells enclosed in self-produced polymeric substrate that is adherent to either inanimate or animate objects. Biocides and antibiotics have been used as the principle weapons against biofilm infections, although in many cases these antibiotics are inadequate for the protection of the host. The underlying premise of my ongoing project is to revisit bacterial interactions in

the presence of mammalian leukocytes. This idea has been studied extensively with planktonic bacteria, but has not received as much attention in the world of biofilms. Although there have been important studies on leukocyte/biofilm interactions, we are still in the process of determining physiologically relevant leukocyte interactions with biofilms and what role, if any, the immune system plays in biofilm infections. However, it is clear from case studies of cystic fibrosis, endocarditis, and medically implanted catheters, that biofilms are important components in the pathology of a variety of human diseases. One of the commonly proposed mechanisms of biofilm resistance to attack from the immune system's sentinel leukocytes is through the formation of a physical barrier, termed the glycocalyx, leaving the leukocytes unable to penetrate the biofilm and exert their respective cytotoxic effects. Here, we have investigated the interactions of human leukocytes with *Staphylococcus aureus* biofilms under conditions that mimic physiological shear. Our data demonstrate that under shear forces, leukocytes are able to adhere to and penetrate a 7-day-old *S. aureus* biofilm grown under shear. Further studies with 2-day-old MRSA *S. aureus* biofilms showed that human leukocytes secreted the cytokines IL-1 β , IL-12, and IFN- γ suggesting the leukocytes were trying to mount a Th1-type response. Together, these data suggest that the interactions of human leukocytes with *S. aureus* biofilms are more complex than originally thought.

W02-S20

Proteomic evaluation of *Staphylococcus aureus* biofilms

Mark Shirliff, Post-Doctoral Researcher, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Staphylococcus spp. have been shown to attach to surfaces, producing a multilayered biofilm embedded within a glycocalyx. This glycocalyx, or slime layer, protects the bacteria from the host immune system and antimicrobial removal strategies. The initial attachment and the subsequent formation of the biofilm phenotype have long been implicated in the development of persistent infections including osteomyelitis, endocarditis, and contamination of indwelling medical devices. In addition, *S. aureus* is responsible for the *in situ* fouling of a many surfaces,

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including saliva ejectors and rinse applicators in dental clinics, flexible endoscopes, dialysis machine tubing, peritoneal catheters, surfaces in dairies, and industrial food packaging surfaces.

The goal of this project was to evaluate the structural characteristics and protein production of early and fully mature biofilms produced by methicillin resistant *S. aureus* (MRSA). The results from various growth conditions were compared in order to elucidate the *S. aureus* biofilm phenotype. When the morphologic properties of *S. aureus* biofilms were microscopically evaluated, growth occurred quickly post-inoculation. After 8 hours of biofilm growth (37°C, CY broth with Oxacillin (40 µg/ml), 0.7 ml/min flow rate, and 7.5 min residence time) using the internal surfaces of silicon tubing as attachment sites, 1.0×10^7 CFU/cm² of *S. aureus* and 0.62 µg protein/cm² were present on the silicon tubing lumen. After 4 days post inoculation these numbers increased to 4.4×10^8 CFU/cm² and 83 µg protein/cm². When the flow rate was increased to 2.1 ml/min, the 4-day biofilm did not detach, thereby demonstrating a significant ability to resist the higher rate of shear. When the mature biofilm was observed using confocal microscopy, an interesting morphology was observed. Microcolonies existed in discrete structures (30 – 80 µm high) and these structures had an intricate channel network between them that presumably provided access to environmental nutrients. While not previously reported for *S. aureus*, these are much like the structures reported in mature *P. aeruginosa* biofilms. When Live/Dead staining was performed on the biofilm structures, a homogenous mixture of live and dead *S. aureus* cells within microcolony structures was observed.

The frozen samples from the batch and biofilm cultures were resuspended in TE containing 0.3 mg PMSF/ml. Glass beads were added to the cell suspension and disrupted by a FastPrep® Instrument. Disrupted cells were centrifuged, the resulting supernatant was isolated, and the protein concentration was determined by the modified method of Bradford. A minimum of 500 µg of total protein for each condition was used for the 2-D electrophoresis (conducted according to the principles of O'Farrell and as outlined by Gorg, et al.). After 2D-gel electrophoresis, the gels were stained, compared for differences, and proteins of interest were excised from the gel. Following in-gel trypsin

digest, the amino acid sequence of the resulting peptides was determined via matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) analysis. The data was acquired through the XACQ (instrument control and data acquisition interface software for the Biflex III) and XMASS (data processing and manipulation). Based upon the molecular weight of the detected peptides, we extrapolated the amino acid composition and the identity of the original protein. All studies (from culture to 2D-gel electrophoresis and MALDI analysis) were performed in triplicate. The detected classes of proteins will be discussed.

By fully understanding *S. aureus* biofilm formation and maturation, one may be able to engineer novel materials, surfaces, production line approaches, and/or antimicrobial strategies that resist or eliminate staphylococcal fouling and biofilm formation. In addition, biofilm-specific proteins may be used as vaccine candidates to prevent the formation of *S. aureus* persistent infections. 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.*

W02-S21

Molecular biology of bacterial extracellular polysaccharide biosynthesis

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When growing in biofilms, bacteria are often encased in extracellular polysaccharides. These polysaccharides affect the three-dimensional architecture of biofilms. Our previous results demonstrated that the structure of the polysaccharide plays an important role in the ability of these polymers to form the intercellular matrices of biofilms. As our model organism, we studied the opportunistic pathogen, *Pseudomonas aeruginosa*, which causes chronic infection in patients with cystic fibrosis. These infections grow as biofilms, in which the bacteria are encapsulated in the mucoid extracellular polysaccharide, alginate. Alginate is a polymer of β-1,4 linked mannuronic acid and guluronic acid that is often modified with O-acetyl side chains. The gene cluster *algI>algJ>algF* is required for alginate

modification and for the biofilm structure of *P. aeruginosa*. Our results have demonstrated that *algI* encodes a membrane protein and *algJ* and *algF* encoding proteins on the periplasmic face of the inner membrane. Sequence homology searches of microbial genomes revealed proteins with high similarity to AlgI from a variety of gram-negative and gram-positive bacteria that do not produce alginate. These *algI* homologs were often genetically linked to genes involved not only in polysaccharide synthesis, but for polymers other than alginate, such as cellulose and lipopolysaccharide biosynthetic genes. In this study, we performed a bioinformatics analysis of AlgI and gene cluster associated with *algI* homologs, to begin characterization of the diversity of extracellular polysaccharides from these other bacteria. Interestingly, AlgI homologs did not reflect the phylogeny of the host organisms, suggesting that the *algI* gene clusters may have evolved by lateral gene transfer. The *algI* clusters from some bacteria, including *Desulfovibrio vulgaris* and *Treponema pallidum*, contained genes for transposases near *algI*, suggesting that these genes may have been acquired recently by lateral transfer. The results presented here suggest that the genes required for alginate O-acetylation may have evolved from a family of genes whose products are involved in the modification of a variety of bacterial extracellular polysaccharides. Through lateral transfer, these genes were incorporated into polysaccharide biosynthetic operons resulting in polymer modification, and perhaps providing the bacteria with survival advantages in biofilms or in infectious diseases.

W02-S22

Culturability of medical biofilms

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The use of DNA analyses has expanded our understanding of bacterial diversity, suggesting that millions of different species of bacteria are present in the environment. Only a fraction of the extant species have been cultured, but it is this subset of organisms that the microbial community felt that we understood. However, the work of Rita Colwell and others has enlightened the science community that there are many commonly cultured organisms that

when collected under certain environmental conditions, cannot be persuaded to grow in the laboratory. These are often living in their native systems, but not in surviving culture, and have been termed "viable, but nonculturable." This concept lends itself to biofilm research where culture recoveries can be much lower than that which is present under the microscope despite careful scrape and disaggregation. Biofilms in some systems are much slower and less effectively cultured back to planktonic.

The work to be discussed involves two medical projects in which culture techniques were used and then compared to other techniques such as direct microscopy, fluorescence *in situ* hybridization and DNA analyses. In the initial research, *Staph aureus* was examined vaginally to determine the role of biofilms in toxic shock syndrome. Clinical samples were collected to identify the mode of growth of *Staph aureus* in this environment. The hospital involved performed culture analyses to determine presence or absence of *Staph aureus* within a clinical sample and then sent us twelve blind samples. In the second project, hip implants were examined for bacterial infection. Dr. Gerhard Maale, an orthopedic physician, has performed a number of hip implant revisions where the hip was culture-negative for bacteria, but during surgery he noted that the surrounding tissue resembled bacterially infected tissue. Dr. Maale removed a number of these hips, fixed them and sent them to the CBE for examination.

In both of these studies samples were handled aseptically so as not to introduce any new bacteria into the samples. The vaginal washes and tampons were examined by FISH analyses and all twelve were shown to be positive for *Staph aureus*, in comparison to the culture technique which, when the blind was lifted, turned out to have been 5 culture-positive and 7 culture-negative. These results have been repeated by Procter and Gamble, Inc., using PCR amplification followed by ribosomal DNA sequencing and they have shown similar results. The hips were examined with FISH and SEM, and the majority of the hips showed bacteria colonization. Bacteria were not found for some of the hips; however, only small sections of the hip were examined, and although these samples were negative for bacteria, the question is if the whole hip was bacteria-free.

SPEAKER ABSTRACTS

In both of these projects, culture technique grossly underestimated the bacteria in the patients examined. In both cases the bacteria were growing as biofilms, and therefore raises the question as to whether biofilms are causing false negatives in the culture technique, or whether the specialized environments caused the reduced culturability. In either case, the study raises questions for researchers and clinicians as to the reliability of culture technique in providing an acceptably accurate detection analyses for bacteria in some systems.

SESSION 5: Metal-Microbe Interactions

W02-S24

Passive film chemistry on stainless steel steel ennobled by biomineralized manganese

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The effect of ennoblement on chemistry of passive films on 316L stainless steel (SS) was quantified using surface-sensitive analytical techniques. Under well-defined laboratory conditions, SS coupons were ennobled to $\sim +350\text{mV}_{\text{SCE}}$ by biofilms of manganese-oxidizing bacterium *Leptothrix discophora* SP-6. Ennobled coupons were analyzed by x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectroscopy (ToF SIMS). From the XPS depth profiles of Fe, Cr, O, Ni, C and Mn, we evaluated thickness of the passive layers before and after ennoblement, while the ToF-SIMS depth profiles were used to evaluate spatial distribution of Mn, Cr, Fe and Ni on the surface. Because the ennobled coupons were covered with biomineralized deposits, sputtering was used to remove these deposits under ultrahigh vacuum conditions before probing the chemistry of the underlying passive layers. ToF-SIMS detected iron in the biomineralized deposits. Since we did not use iron in the growth media, the detected iron presumably originated from the passive layer. The main conclusion from this study is that ennobled coupons have significantly thinner oxide layers as compared with the pre-ennobled coupons, which may contribute to their susceptibility to localized corrosion.

W02-S25

Microbially deposited manganese and iron oxides on passive metals: Their chemistry, distribution, and consequences for material performance

Xianming Shi, MS Candidate, Mechanical Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

The open circuit potential (OCP) values of 316L stainless steel and Ti-6Al-4V corrosion coupons exposed to a fresh river were ennobled to as high as $+365\text{mV}_{\text{SCE}}$ and $+400\text{mV}_{\text{SCE}}$, respectively. With micro-chemical imaging capabilities and high detection sensitivity, a surface analysis technique based on ToF-SIMS was developed to identify the oxidation states and distribution of biominerals on the ennobled metal surfaces. ToF-SIMS spectra of the microbial deposits compared to spectra of different manganese and iron mineral standards indicated that the biominerals on the metal surfaces are a mixture of Fe_2O_3 , Mn_3O_4 , and MnOOH on fully ennobled coupons, while a mixture of Fe_3O_4 , Fe_2O_3 , Mn_3O_4 , and Mn_2O_3 on partially ennobled coupons (in terms of potential). Biomineralized manganese and iron oxides on the 316L stainless steel surfaces, regardless of the oxidation states, endanger the material integrity in a similar manner, as evidenced by the elevated OCP and increased cathodic current density upon mild polarization.

W02-P232

Microelectrode facilities at the Center for Biofilm Engineering

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

Microsensors are becoming indispensable tools for studying biofilms and small biological samples. The Biofilm Structure and Function research group at the Center for Biofilm Engineering has developed several new microsensors and has been actively using microsensors in biofilm research. In this poster, we present dissolved oxygen, hydrogen sulfide, pH, local mass transfer, local flow velocity, local diffusivity, carbon dioxide, hydrogen peroxide, and chlorine microelectrodes. In addition, we present information about our upcoming Microsensors Workshop.

W02-P265

Biofilm mechanics and motion

Cory Rupp, Brett Towler, Suzanne Wilson, Laura Purevdorj, Paul Stoodley, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

Biofilm is a dynamic material that undergoes stretching, rolling, sliding, and detachment motions. By studying the material/mechanical properties of biofilms, it is possible to study and explain the motions biofilm may exhibit. This area of study is called biofilm mechanics.

Biofilm mechanics is a new concept in the study of biofilm, the importance of which has yet to be realized. Knowledge of the mechanical properties of biofilms will allow prediction of detachment, motion and other behaviors in response to an applied force.

Using time-lapse photography, the various modes of movement in biofilm have been catalogued. Knowing how something moves, however, is not as important as why it moves. To answer that question experimental tests must be performed. Using microscopy and rheology techniques, engineering values such as the elastic modulus, ultimate stress,

ultimate strain, and viscosity have been found for biofilms. The data and values obtained in these experiments is then used to explain the observations seen in the time-lapse photography.

W02-P275

Biofilm image analysis toolbox

Haluk Beyenal, Gary Harkin (Computer Science, MSU–Bozeman), Zbigniew Lewandowski, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

To quantify biofilm heterogeneity, the Biofilm Structure and Function research group has developed and implemented methods of extracting morphological features from images of biofilms. This is a first step toward quantifying the relationship between biofilm heterogeneity and the underlying processes, such as mass-transport dynamics, substrate concentrations, and species variations. In our previous studies, we integrated algorithms to calculate biofilm structural parameters to a computer program written in C++. However, the program was developed for the UNIX/Xwindow environment, and running the software was difficult for most biofilm researchers who were not experienced Unix users. Additionally, the software did not allow any modifications or custom designed image operations. For these reasons, we re-designed, improved, and re-wrote the software in MATLAB. The user can now load images manually to MATLAB and calculate the following parameters of the images: textural entropy, homogeneity, energy, contrast, correlation, maximum probability, areal porosity, run lengths, diffusion distances, and fractal dimension. This poster shows (1) how to use the toolbox, (2) how to quantify biofilm images, and (3) how to interpret the data. The software will be made available to public at the CBE web site: <http://www.erc.montana.edu>.

POSTER ABSTRACTS

W02-P276

Desorption kinetics of 2,4,6-trinitrotoluene (TNT) investigated in a well-defined soil system

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Soil contaminated with 2,4,6-trinitrotoluene (TNT) has been identified by the Department of Defense as one of its most critical environmental concerns. TNT contamination has occurred mostly through production, loading, and disposal of explosives at U.S. Army ammunition sites. The objective of this study was to find important chemical factors impacting desorption of TNT from a well-defined soil system. The effect of adding rhamnolipid biosurfactants, monovalent cations (NaCl), and divalent cations ($\text{Ca}(\text{ClO}_4)_2$) on 2,4,6-trinitrotoluene (^{14}C -TNT) desorption in an engineered soil slurry system was investigated.

An engineered soil was successfully developed by adsorbing α -FeOOH (Goethite) onto quartz sand (Figure 1), followed by adsorption of soil humic acid for use in column experiments. Goethite coated with humic acid was used for batch experiments. A significant increase in desorption of ^{14}C -labeled TNT was observed upon the addition of rhamnolipids at 25 and 250 times the critical micelle concentration. However, a decrease in desorption of ^{14}C -labeled TNT was observed in soil slurries upon addition of NaCl and $\text{Ca}(\text{ClO}_4)_2$, indicating an equilibrium controlled system (Figure 2).

Results of this study may aid in improving the design and operation of remediation technologies for explosive-contaminated soils.

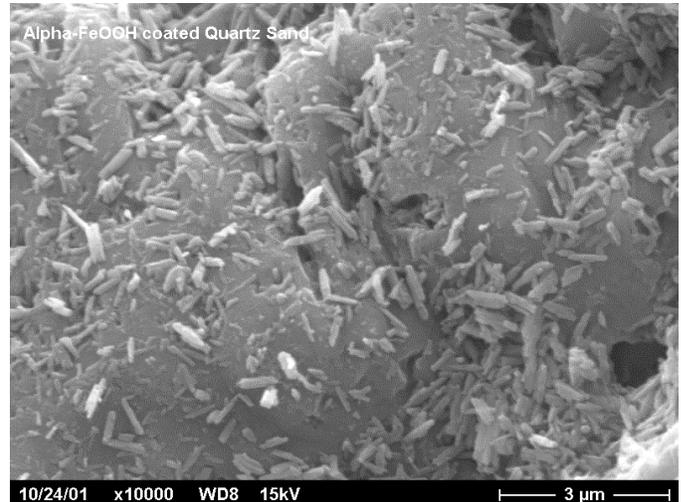


Figure 1. SEM image of α -FeOOH coated quartz sand. Goethite crystals were observed to maintain their characteristic needle-shaped structure after adsorption onto the quartz sand

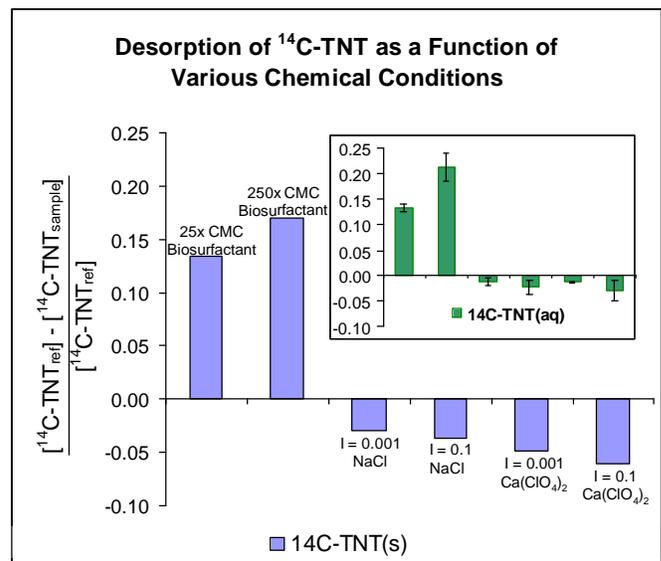


Figure 2. Desorption of α -FeOOH bonded ^{14}C -TNT as a function of added biosurfactants, NaCl, and $\text{Ca}(\text{ClO}_4)_2$ [The α -FeOOH bonded ^{14}C -TNT(s) was determined by use of a biological oxidizer followed by $^{14}\text{CO}_2$ analysis on a scintillation analyzer]. Desorption data are normalized to a TNT reference sample (added NANOpure water only). The insert shows desorption of ^{14}C -TNT based on aqueous TNT concentrations (14C-TNT(aq)). Error bars show +/- one standard deviation with $n = 3$. Note that the y-axis of insert was determined by the following equation: $([^{14}\text{C-TNT}_{\text{sample}}] - [^{14}\text{C-TNT}_{\text{ref}}]) / [^{14}\text{C-TNT}_{\text{ref}}]$.

W02-P278

Quantifying biokinetic parameters in biofilms

Nurdan Yurt, Zbigniew Lewandowski, and John Sears, Center for Biofilm Engineering at Montana State University-Bozeman, 59717

The steady state diffusion-reaction equation for dissolved oxygen through biofilm in one dimension is given by the following nonlinear differential equation:

$$D_{eff} \frac{d^2 S}{dx^2} = \frac{m_{max} S X_f}{Y(K_s + S)} \quad (1)$$

Where D_{eff} is effective diffusion coefficient of substrate in biofilm; S is substrate concentration in biofilm; m_{max} is maximum specific growth rate; X_f is biofilm density; Y yield coefficient; K_s is half rate coefficient; x is position. The Equation 1 requires the following assumptions: uniform biofilm density, activity and diffusion coefficient. Although we are aware that biofilms are heterogeneous, for our application we assumed that the average values of the parameters (such as density, diffusivity etc..) are valid. Since the Equation (1) is a nonlinear second order differential equation, numerical methods should be applied for the solution. For stability and efficiency of numerical solution of Equation 1, it is necessary to rewrite in dimensionless form:

$$\frac{d^2 S^*}{dx^{*2}} = F^2 \frac{S^*}{(b + S^*)} \quad (2)$$

Where $\Phi = \sqrt{\frac{m_{max} S_b L_f^2 X_f}{Y D_{eff}}}$ is Thiele Modulus;

$\beta = \frac{K_s}{S_b}$ is dimensionless half rate Monod constant;

$S^* = \frac{S}{S_b}$ is dimensionless substrate

concentration; $x^* = \frac{x}{L_f}$ is dimensionless distance.

The boundary conditions for the solution of Equation 2 are as follows; @ $x^*=0, S^*=S^*_{measured}$; @ $x^*=0, dS^*/dx^*=0$; @ $x^*=1, S^*=1$.

Since the Equation 2 represents concentration distribution in a biofilm, the Φ and β can be calculated using experimentally measured concentration profiles.

As an example, we presented dissolved oxygen concentration profiles measured by microelectrode in our laboratory in Figure 1.

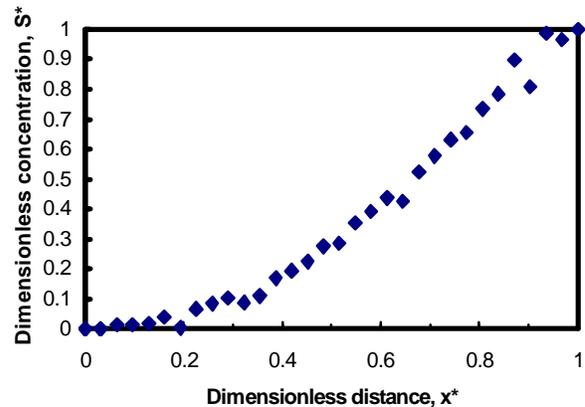


Figure 1. The variation of dimensionless dissolved oxygen concentration measured using microelectrode by distance through the thickness of the biofilm.

The dimensionless diffusion reaction model (Equation 2) was then solved for preassumed values of Φ and β using the explicit Runge-Kutta (4,5) formula, the Dormand-Prince pair. The error for these pre-assumed parameters was calculated as the sum of the squared differences between the experimentally measured oxygen concentrations and those predicted from the solution of the Equation 2 (SSD). We viewed the problem as a non-linear optimization, and searched the optimum values for the Φ and β to minimize the error (the calculated squared differences). We used a direct search method that did not employ the numerical or analytic gradients to calculate Φ and β values for SSD. The stability of the numerical solution was tested and repeated for different initial guess values. First, we calculated $\Phi = 3.47452$ and $\beta = 0.11238$ for experimental data presented in Figure 1. By using bootstrapping Monte Carlo we calculated average values and confidence intervals as $\Phi = 3.5635 \pm 0.7738$; $\beta = 0.1183 \pm 0.0547$ after 100 simulations.

We have developed a method of extracting biokinetic parameters from substrate concentration profiles measured in biofilms, and demonstrated its utility by calculating biokinetic parameters of microbial growth from experimentally measured oxygen concentration profiles. However, this method is only valid with the validation of the assumption stated in the paper.

POSTER ABSTRACTS

W02-P279

The effect of oxygen concentration on the degradation rate of MTBE

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Methyl tert-butyl ether (MTBE) is a fuel oxygenate added to gasoline to reduce carbon monoxide emissions. Widespread use of MTBE developed in response to the Clean Air Act of 1990. Although other fuel oxygenates exist, MTBE is the most commonly used. Leaking underground storage tanks are a large source of environmental MTBE contamination.

The chemical characteristics of MTBE make it a challenge to treat in the natural environment. MTBE is highly soluble in water, volatilizes easily, does not sorb well to soil matter, and tends to be less prone than the BTEX constituents to degradation.

In situ MTBE degradation by indigenous organisms often occurs too slowly for it to be a viable remediation mechanism. The addition of oxygen has been found to stimulate the degradation of MTBE, both by indigenous organisms as well as organisms used for bioaugmentation. The purpose of this study is to determine the effect of dissolved oxygen concentration on the degradation rate of MTBE by an organism known to survive on MTBE as a sole carbon source.

To date, one experiment has been completed. This experiment was run in batch microcosm format. Microcosms contained aqueous media, the microorganism PM1, and MTBE. Microcosms were treated with different headspace oxygen concentrations, and monitored for MTBE disappearance, tert-butyl alcohol (TBA) formation, oxygen consumption, and evolution of carbon dioxide. This initial experiment was inconclusive due to a lack of accounting for biomass. However, it is apparent from the presence of carbon dioxide and TBA that mineralization of MTBE is occurring. Future experiments will correlate degradation rate to biomass. Other experiments will investigate the effect of oxygen concentration on the degradation rate of an MTBE degrading culture isolated from an MTBE contaminated site in Montana.

W02-P280

Lectin staining of gram-positive and gram-negative bacterial glycoconjugates

Jessica Janzen, Luanne Hall-Stoodley, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

In situ lectin staining of biofilms was studied using *Pseudomonas aeruginosa* ERC-1 and FRD 1 (Gram-negative organisms) and *Staphylococcus epidermidis* and *S. aureus* (Gram-positive organisms). Planktonic solutions were initially stained with a panel of 7 lectins, and a full titration of the range of concentrations suggested by the manufacturer was performed with each lectin for each type of bacteria. The lectins examined were **Con A, Coral Tree, PHA-L, PNA, SBA, UEA-1, and WGA**. Texas red conjugated SYTO 59 was used to stain bacterial nucleic acid in conjunction with the FITC or Alexa 488- lectin stains.

Con A gave the best glycoconjugate staining results for planktonic *P. aeruginosa* ERC-1 and FRD 1. Conversely planktonic *S. aureus* and *S. epidermidis* were positively stained with several lectins. Con A, WGA, Coral Tree, and SBA, which gave the best staining, were then tested with a *S. aureus* biofilm and visualized under a confocal microscope.

W02-P281

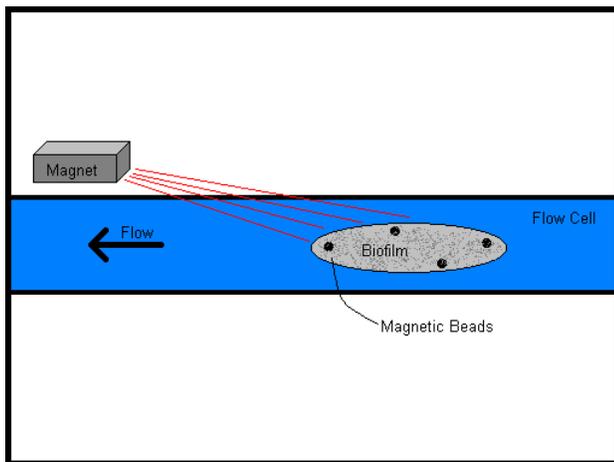
Using electromagnetism to investigate the material properties of biofilm

Ryan Cargo, Cory Rupp, Paul Stoodley, Center for Biofilm Engineering at Montana State University–Bozeman, 59717

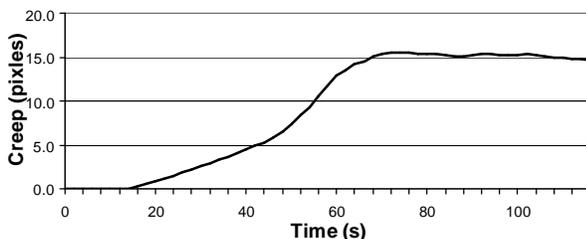
Treating biofilm as a material with specific mechanical properties is becoming increasingly important in predicting and manipulating biofilm behavior in response to fluid shear. Detachment of biomass into the surrounding bulk fluid can cause spreading of infection and obstruct fluid flow through blood vessels. Understanding detachment and other material properties of biofilm will assist in finding ways to control detachment and excessive biofilm growth. Previous methods of testing involved growing biofilm in glass flow cells and then altering the fluid flow conditions over the biofilm to create

fluid shear. This shear force induced visco-elastic responses in the biofilm that could be recorded and analyzed through microscopic imagery. However, in this method the control over the shear stress was relatively crude. A new way of testing biofilm at high oscillatory frequencies with precise applied forces was needed to investigate dynamic rheological parameters. The basic design idea was to be able to manipulate the biofilm without altering flow conditions around it. This was accomplished by injecting iron beads into the fluid flow, which would then settle and embed themselves into the biofilm's surface. An outside magnet (see Figure 1), and later electromagnet, could apply precise forces on the biofilm, pushing and pulling it in ways to specifically measure stress, strain, and creep. This method has worked thus far although it is still in its preliminary stage. Data so far has been consistent with that taken from the fluid-shear method, and will be presented.

Keywords: biofilm, mechanical/material properties, stress, strain, creep, electromagnetic, in-vitro, shear, detachment



Creep Induced by Electromagnetic Force



W02-P283

A plate assay for assessing mycobacterial attachment

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Plate assays using 24-well cluster plates or 96-well microtitre plates have been used to screen for initial attachment and biofilm formation for several organisms (Kolter and O’Toole). This is a useful assay to screen for genetic mutants, culture conditions, inhibitory treatments or, treatments that promote attachment. Because mycobacteria are slow growing, we were interested in developing this method as a preliminary screen in order to conserve media and save time before growing mycobacteria in flow cells.

However, mycobacteria do not stain well with crystal violet, the stain commonly used in evaluating attachment in these assays. Furthermore, standard solvents did not extract stains from the unique mycobacterial cell wall.

In spite of these potential pitfalls, an assay was developed that can preliminarily assess mycobacterial attachment under shear conditions.

POSTER ABSTRACTS

W02-P284

Modeling of biofilm growth and detachment: Initial computer results pictorial

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Biofilm computer models are currently being developed by a number of research groups to expand knowledge about the self-organization of biofilm bacteria. We have created a quantitative three-dimensional, dynamic, stochastic, discrete/differential computer model to simulate biofilm growth and detachment. The model mimics the behavior of a biofilm system with a simple set of experimentally-based stochastic “rules” applied locally to each individual bacteria cell within the biofilm. These local “rules” lead to repeatable patterns on a large scale. Based on the premise that bacterial cells organize themselves within a biofilm as a response to their local environment, the computer model’s structure is created as a process of self-organization. Initial results indicate qualitatively good results when compared to laboratory biofilms. The model adequately captures the inherent variability of real biofilms. The first set of computer experiments showed how biofilm growth dynamics were affected by a bacterially derived detachment factor. The experiments were designed to investigate the effects of two mechanisms by which bacterial masses could detach from the biofilm.

Eradicating biofilms with atmospheric plasma

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Atmospheric Glow Technologies has developed a novel method of biofilm destruction on medical and dental instruments using the patented One Atmosphere Uniform Glow Discharge Plasma (OAUGDP™). The OAUGDP™ is a non-thermal plasma that produces reactive oxidative species at ambient temperatures and standard pressure. *Pseudomonas aeruginosa* biofilms were formed over 24 h or 7 days and exposed to the OAUGDP™-produced oxidative species. This exposure was accomplished either through direct exposure to the plasma or through remote exposure to the oxidative species that were convected over the surface of the sample. Standard plate counts and microscopic analyses demonstrated destruction of the biofilm in 1-4 minutes. Variable Pressure Scanning Electron Microscopy revealed no substantial damage to surfaces following exposure. Experiments confirm the efficacy, reliability, and commercial feasibility of this technology for rapidly destroying tenacious biofilms that form on medical and dental devices.