CENTER FOR BIOFILM ENGINEERING

PROCEEDINGS

Winter 2005 CBE Technical Advisory Conference

February 2–4, 2005
Montana State University–Bozeman
Bozeman, Montana

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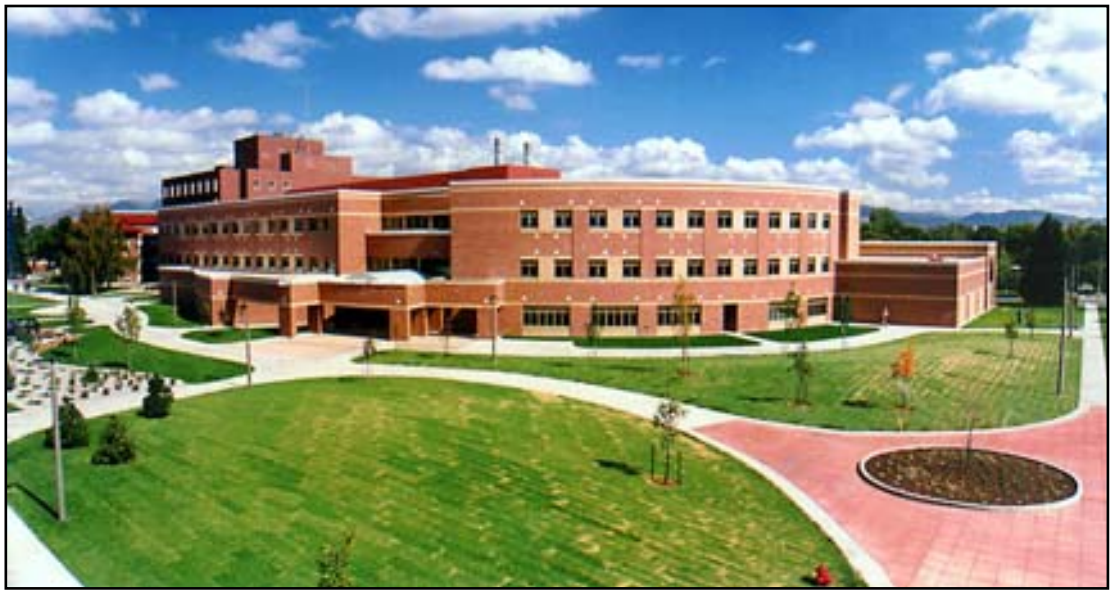

GENERAL INFORMATION

CBE LEADERSHIP

***Phil Stewart**, CBE Interim Director and Professor, Chemical & Biological Engineering*
***Anne Camper**, Associate Professor, Civil Engineering & Associate Dean for Research, COE*
***Al Cunningham**, Professor, Civil Engineering*
***Marty Hamilton**, Professor Emeritus, 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'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 university-industry-government 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 applied. 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 and maintain interdisciplinary undergraduate and graduate education programs involving team research on industrially relevant projects.

Industrial Associates Program Benefits

The CBE Industrial Associates Program provides support to help fund industrially relevant research and allows close interaction between industry representatives and CBE researchers and students. Some specific benefits of membership in this program are detailed below.

CBE Technical Advisory Conferences

Twice each year, CBE members convene in Bozeman for a Technical Advisory Conference (TAC)—an exposition of what's new in CBE research and a review of what's happening around the world in biofilm science. The TAC is a great way to keep up on the science as well as to interact with other industry and government representatives and CBE researchers. Meetings are open only to CBE members and invited guests.

Education and Training Workshops

CBE members are entitled to attend either basic or advanced biofilm methods workshops free of charge. Workshops are held the day before TAC meetings in both summer and winter, and feature the latest techniques in growing and assessing biofilms. In addition, the CBE offers specialty workshops (either in our laboratory or at your facility), tailored to your individual company needs. These can range from covering the latest microscopy techniques for your R/D department to assisting the education of your sales force in general biofilm understanding.

Research/Testing Projects

CBE members can fund research and testing projects at a discounted rate. Advantages of directly funding project work at the CBE include complete confidentiality, negotiable intellectual property, and project direction by scientists and engineers at the top of biofilm investigation. In addition, the CBE offers Industrial Associate members no-cost participation in undergraduate research projects of their choosing. This is an opportunity to have a top-notch student work on a problem specific to your needs, at no additional cost.

Product/IP Development Consulting

CBE faculty and staff can assist members in evaluating commercial or product-related ideas as they relate to biofilms or biofilm control. Each member company is entitled to two free days of consulting annually. We can offer confidential feedback on R/D direction, marketing ideas or strategic decisions. Many of our members have found that this benefit alone is worth the annual membership fee.

Regulatory Interactions

Bringing a product to market today frequently requires registration with, or approval of, a regulatory agency. CBE staff maintain close ties with decision makers at FDA, EPA, and other US Government agencies concerned with biofilms. CBE scientists are on the forefront of biofilm methods development and assessment—areas of expertise frequently sought out by the regulatory community. In addition, it's our policy to feature regulatory agency speakers at our TAC meetings whenever possible. If you are seeking insight into the process of registration or approval, the CBE can help.

For More Information

To see if membership is a good fit for your company, please contact Paul Sturman, CBE Industrial Coordinator, at (406) 994-2102, or via email at paul_stu@erc.montana.edu. Or visit us on the web at www.erc.montana.edu.

Presentation and Poster Abstracts
Technical Advisory Conference: February 2–4, 2005

Posters

SPEAKER ABSTRACTS

Keynote Speaker:

W05-S01
Resistance, Persistence and Consistence in Biofilm Communities

Peter Gilbert, *Professor of Microbial Physiology, University of Manchester, United Kingdom*

It is my intention in this presentation to convey some of the flavour of biofilm research at Manchester by touching on several aspects of our ongoing research. Whilst the talk will draw as much as possible on unpublished data, my enthusiasm will be tempered by the need to protect Intellectual Property.

Resistance: We have for many years been concerned with generic mechanisms by which biofilm communities resist the actions of chemical agents. Together with work from the CBE, a consensus opinion would be that both reaction-diffusion limitation of drug access and physiological heterogeneity within biofilm communities are major determinants of recalcitrance, but that the effects of diffusion limitation alone are minimal. We have had cause to revisit the latter assumption for cationic biocides. Spatially engineered aggregates of various bacteria were constructed using lectins, such that aggregate size could be controlled and multi-lamellar constructs prepared where different species comprised the inner and outer shells. Such aggregates could be exposed to antimicrobials and then disassembled by addition of an appropriate sugar agonist prior to performing viability assessments. Target organisms were selected according to their binding affinity and susceptibility towards a range of cationic biocides. It became apparent that both aggregate size and the spatial arrangement of the different species substantially affected the degree of disinfection. Analyses of adsorption kinetics for the biocides into and onto such aggregates indicated that whilst the total consumptive loss of biocide was unaffected by aggregate composition or size, there were delays in achieving dynamic equilibrium for constructed aggregates over that of simple homogeneous suspension of cells. Cells within the centre of aggregates would therefore be exposed to gradual, rather than sudden, changes in biocide concentration. When these concentration-time profiles were replicated in cell suspensions using electrically driven syringe drivers, then the protection afforded by construction of aggregates could be duplicated. Results therefore suggest that for cationic antimicrobials, it is the rate of change in biocide concentration to which cells are exposed, rather than the final exposure level, that determines outcome, and that diffusional limitation in biofilms is sufficient to confer protection.

Persistence: There has been much speculation about the persister state. This is defined as a phenotype, present at low frequency within all bacterial populations, that is especially resistant to treatment with a diverse range of antimicrobial drugs. Persister status has been suggested to relate to over-expression of RelE toxin and HipA in a hypothesis relating death by antimicrobials treatment to apoptosis, and persistence to the adoption of an apoptosis-deficient phenotype. Such hypotheses stem from studies of HipA mutants and DNA expression arrays of the survivors of antimicrobials treatments (Keren et al 2004, J. Bact. 186: 8172-80). At Manchester we have been able to separate persister cells from standard cultures using a fluorescent reporter gene, cytometry and FACS. In the absence of antimicrobial stress, patterns of gene expression were substantially different from those in the Keren et al (2004) study, suggesting that RelE and HipA are responses to antimicrobial treatments rather than pre-determinants of persister status.

Consistence: Molecular ecological approaches to the characterisation of complex biofilm communities, such as denaturing gradient gel electrophoresis (DGGE) of PCR products based on 16srDNA, enable community fingerprints to be generated that reflect the total population of culturable and unculturable bacteria. We have used such approaches to characterise biofilm materials associated not only with drain outlets, but also biofilms associated with the skin, mouth and gastrointestinal tract. A surprising outcome of such studies is that the community fingerprints obtained from different volunteers are highly conserved within that volunteer (>5 years in some examples) yet are distinct between individuals. Clearly the individual microbiome is not only colonisation-resistant but is also highly conserved. Studies currently underway are intended to elucidate those host factors (genetic, environmental, and developmental) that contribute to such individualised homeostasis.

SESSION 1: Biofilm Methods

W05-S03
Patterns of DNA Synthesis in *Staphylococcus epidermidis* Biofilms

Suriani Abdul Rani, *MSU-CBE MS Candidate, Chemical & Biological Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Growth activity and patterns of DNA synthesis in *Staphylococcus epidermidis* colony biofilms were visualized and quantified using 5-bromo-2-deoxyuridine (BrdU) labeling. Incorporation of the thymidine analog BrdU into DNA during DNA synthesis in place of the native nucleotide provides a marker for proliferating cells. Labeled DNA was subsequently illuminated with a fluorescently labeled monoclonal antibody that specifically recognizes brominated DNA. Distinct zones of DNA synthetic activity were observed close to the nutrient and air interfaces. Growth zones averaged between 16 and 30 μm. Slow-growing or non-growing cells were observed in the interior of the biofilms. Patterns of DNA synthetic activity changed in response to changes in the nutrient and oxygen conditions. When biofilm was subjected to anaerobic conditions, the growth zone was only observed at the nutrient interface. When glucose was added to the medium, variability in patterns was observed, probably due to non-growing or dead cells. When pure oxygen was introduced, a thicker growth zone was observed at the air interface which averaged 46 μm. Overall, the patterns observed suggest significant spatial heterogeneity in biofilm growth. This study suggests that BrdU labeling can be used to map patterns of DNA synthesis in biofilms. The presence of non-growing cells can be an explanation for reduced susceptibility of biofilm towards antimicrobial agents.

W05-S04
A Coupled Fluid-Biofilm Finite Element Model and Its Potential Applications

Brett Towler, Ph.D.*, Assistant Professor, Civil Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Fluid-induced detachment and hydrodynamic drag are two problems associated with microbial fouling. Biofilm accumulation on wetted interfaces can impair the hydrodynamic performance of pipe networks and ship hulls. The costs associated with reductions in conveyance and increased fuel consumption can be substantial. Biofouling is also relevant in the medical field where biofilm growth on medical devices can result in persistent infections and the detachment of dental biofilms has been linked to a range of systemic diseases.

The mechanics of this fluid-structure interaction are highly complex. Detachment and surface drag are due, in part, to the constitutive law governing biofilm. The flow regime is typically turbulent and, accordingly, solutions necessitate the use of a turbulence model. Finally, the equations governing the flow field and the attached biofilm must be coupled to reflect their interaction. Turbulence phenomena and geometric complexity prohibit the development of an analytical solution to this problem; however, using numerical methods to model the biofilm response to turbulent flow is feasible.

A computational model that describes the response of individual biofilm structures to turbulent flow has been developed at MSU's Center for Biofilm Engineering. The model employs a sequentially coupled finite element technique to resolve biofilm deformation and internal stress distributions due to changes in the surrounding flow field. Based on previous work, a linear viscoelastic constitutive law is employed to define the stress-strain relation.

The next step in the development of this model is to calibrate model results with experimental measurements. In doing so, this computational tool may: 1) allow for the estimation of species-specific viscoelastic material parameters; 2) allow for the calculation of failure stresses (i.e. the internal stresses at which detachment occurs); 3) lead to the development of detachment prediction methods based on these calculations; and 4) catalyze the development of a design equation for estimating the biofilm contribution to turbulent energy losses in pipeline systems.

SPEAKER ABSTRACTS

W05-S05
Adaptive Responses to Antimicrobial Agents in Biofilms

Phil Stewart, *MSU-CBE Interim Director, Center for Biofilm Engineering, and* ***Barbara Szomolay****, MSU PhD Candidate, Mathematical Sciences****,*** *Montana State University–Bozeman, 59717*

Microorganisms in biofilms can respond to antimicrobial treatments and adapt to become less susceptible to these treatments. Reports in the literature suggest that cells in a biofilm demonstrate adaptive resistance more effectively than corresponding planktonic cells. We propose here that, in biofilms, reaction-diffusion limited penetration may result in only low levels of antimicrobial exposure to deeper regions of the biofilm. Sheltered cells are then able to enter an adapted resistant state if the local time scale for adaptation is faster than that for disinfection. This mechanism is not available to the planktonic population. A mathematical model is presented to illustrate. Results indicate that, for a sufficiently thick biofilm, cells in a biofilm implement adaptive responses more effectively than do freely suspended cells. Effective disinfection of such a biofilm requires applied biocide concentrations that increase quadratically or exponentially with biofilm thickness.

SESSION 2: Environmental Biofilms

W05-S08
Biofilms: Regulatory Challenges

Melba Morrow, *Special Assistant to the Director, Antimicrobials Division, Office of Pesticide Programs, US EPA*

The documented existence of biofilms, their microbial composition and the expressed desire of industry to develop products to prevent, control or eliminate biofilms have caused the Antimicrobials Division (AD) to develop novel approaches to facilitate product development. AD recognizes that the approaches used in generating efficacy data for planktonic organisms may not be fully applicable when generating data for products designed to combat biofilms, thus some modification of existing approaches is required. In our efforts to be both progressive and creative in the area of product development, AD has resorted to reclassifying some products that remove biofilms, conducting literature searches to keep abreast of the evolving scientific issues, convening expert panels and working closely with the Center for Biofilm Engineering and other facilities in the research arena. Once AD stepped into the realm of the development of products to be used to combat biofilms, we further realized that the scope of our efforts was not limited to problems encountered with the development of products for use on hard surfaces only. Dental unit waterlines, and spas and hot tubs, and the peculiar problems associated with determining product efficacy under these scenarios had to also be considered.

W05-S09
Multi-Species Oral Biofilm Model

Garth James, *CBE Medical Projects Manager, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

During this project a multi-species biofilm model for the evaluation of anti-plaque treatments was developed utilizing the Drip Flow Biofilm Reactor (DFR). The DFR was initially developed by the CBE, with additional development in collaboration with Biosurface Technologies Corporation. For use as a dental model, biofilms were grown on hydroxyapatite-coated glass coupons using an inoculum of 9 oral bacteria. The reactors were fed a growth medium (Bradshaw Marsh Medium) containing sucrose, glucose, and hog gastric mucin. The biofilms were initiated by adding the facultative anaerobes (*Actinobacillus actinomycetemcomitans, Actinomyces naeslundii*, and three Streptococcal species: *gordonii*, *mutans*, and *orallis*). After three days of growth the obligate anaerobic species (*Tannerella forsythensis*, *Fusobacterium nucleatum, Prevotella intermedians, Porphyromonas gingivallis*) were added to the system. After seven days of total growth, the biofilms were removed from the coupons, dispersed and analyzed by general and selective plating. Selected coupons were also analyzed by confocal scanning laser microscopy. Initial experiments, conducted using a three-channel prototype reactor, showed high variability both between channels within runs and between separate runs. The use of a redesigned four-channel reactor resulted in thicker biofilms and greatly reduced variability between channels within a run. Selective plating indicated the seven-day-old biofilms contained significant populations of Streptococci, *A. naeslundii*, *T. forsythensis*, *F. nucleatum*, and *P. gingivallis*. We were not able to recover *A. actinomycetemcomitans* or *P. intermedians* by selective plating. The model was further evaluated using chlorhexidine as an anti-plaque agent. Treatment of the seven-day-old biofilms

with 0.2% chlorhexidine for 10 minutes resulted in 1.2 to 1.5 log reductions in biofilm populations. Overall, the results suggest this is a promising model for evaluating novel anti-plaque treatments. Ongoing research to evaluate bacterial composition of the biofilms using molecular biology techniques and further evaluation of chlorhexidine treatments is being performed.

W05-S10
PCR-Based Community Analysis of Environmental Biofilms: Competence and Caveats

***Mark McBroom**, Recent MSU-CBE MS Graduate, Environmental Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Analysis of microbial DNA has opened a door of insight that until recently was shrouded behind the limitations of culturing methods. Though useful, these methods have limited our view of microbial diversity to approximately 1% of environmental species. Over the last decade advanced PCR-based methods have allowed us to identify individual species and their relative densities and activities within microbial communities across an array of habitats. With optimized methods, representative community profiles can be obtained to observe temporal and spatial shifts of both community structure and activity. This ability allows biofilm inhibition and stimulation treatments to be optimized in the field by tracking the effects of specific applications.

Unfortunately, PCR-based methods are as much an art as they are a science, requiring constant optimization specific to individual samples. Too often, specific conditions of published methods are accepted as the standard. However, differences in sample chemistry, physical structure, species diversity, and the methods employed require optimized conditions specific to the given sample, particularly when generating representative community profiles. This presentation covers the great potential of PCR-based community analysis, as well as the possible limitations and biases introduced with each of the methods involved. A thorough understanding of the potential solutions to these limitations can result in methods specific to given samples and a truly representative community profile.

W05-S11
What is the "Detection Limit" When Using Dilution Series Methods for Counting Bacteria?

***Julie Sharp,** MSU PhD Candidate in Statistics and **Marty Hamilton,** MSU-CBE Professor Emeritus, Statistics, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

The extent of biofilm growth is conventionally measured by the density of viable bacteria, recorded in units of cfu/cm^2. To determine the density, a sample of biofilm is removed from a specified area of the substratum, placed in a beaker, and disaggregated to form a suspension of individual bacteria. Let N denote the (unknown) number of bacteria in that beaker. To estimate N, a dilution series is formed. Then a small volume is sampled from each dilution tube, plated, incubated, and the colonies are counted at the first dilution for which the colonies do not overlap.

If the investigator anticipates a high viable cell density, he may decide that it is unnecessary to plate samples from the first few dilution tubes because the colonies would be too numerous to count on those plates. Sometimes, it turns out that N is smaller than anticipated, and no colonies are observed on any of the plates. If there were no viable cells in the biofilm (N=0), then there would be no colonies. However, one cannot conclude that N=0 just because viable bacteria did not end up on the plates. It is conceivable that there was a population of viable bacteria in the beaker, but that it was diluted to extinction by the diluting and plating process.

It is of interest to know the largest potential value of N that is consistent with the observation of a zero cfu count. That value is known as the 'detection limit,' denoted here by. N_d The detection limit is determined by the dilution series and plating strategy that the investigator employed. If, for example, the investigator plated a sample from the original beaker, then N_d is relatively small. On the other hand, if no plates were formed prior to the third decimal dilution, then N_d will be larger. Note that N_d is not an estimate of N; it is an upper bound on N. The density result when zero cfu's are counted is typically recorded with a < sign; e.g, if N_d = 105, the investigator would report a density of '< 105.'

Among the many methods that have been suggested for defining and calculating N_d, there are two that seem particularly appropriate in this context: the probability definition and the expected value

definition. The probability method that is used in the physical sciences is defined and compared to the expected value approach that predominates in microbiology. The presentation concludes with numerical examples that illustrate the differences between the two definitions of the detection limit.

SESSION 3: Biological Fuel Cells

W05-S13
Sustainable Power in Microbial Fuel Cells

***Joseph Menicucci**, MSU-CBE PhD Candidate, Chemical & Biological Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

In microbial fuel cell research, power and current measurements are often reported as a peak value (reported values are the maximum of each individual measurement). These measurements tell nothing about the long-term power generation of the cell. We have developed a method that can simply identify the sustainable load in a microbial fuel cell. The definition of sustainable load implies that the power generated does not change with respect to time and the cell potential remains constant after applying the load. Our technique does not require expensive equipment and can be completed in less than thirty minutes.

W05-S14
Power Management in Microbial Fuel Cells

***Avinash Shantaram**, MS Candidate, Chemical & Biological Engineering, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Monitoring parameters characterizing water quality, such as temperature, pH and concentrations of heavy metals in natural waters, is often followed by transmitting the data to remote receivers using telemetry systems. Such systems are commonly powered by batteries, which can be inconvenient at times because batteries have a limited lifetime and have to be recharged or replaced periodically to ensure that sufficient energy is available to power the electronics. To avoid these inconveniences, we have designed and tested a self-renewable power source—a microbial fuel cell—which has the potential to eliminate the need for batteries to power electrochemical sensors used to monitor water quality and small telemetry systems used to transmit the data acquired by these sensors. To demonstrate the utility of the microbial fuel cell, we have combined it with low-power, high-efficiency electronic circuitry providing a stable power source for wireless data transmission. To generate enough power for the telemetry system, energy produced by the microbial fuel cell was stored in an ultracapacitor and used in short bursts when needed. Since powering commercial components of electronic circuits requires 5 Volts, and our cell was able to deliver a maximum of 2.1 V, we used a DC-DC converter to increase the potential. The DC-DC converter powered the transmitter, which gathered the data from the sensor and transmitted them to a receiver. To demonstrate the utility of the system, we initially measured temporal variations in temperature followed by the implementation of a chemical sensor to measure copper and lead concentrations in water, this data was then wirelessly transmitted to a remote receiver.

Special Presentations

W05-S15
Biofilms: Clinical Communities are Asking Us to Support Clinical Management: Are We Ready for Reduction to Practice?

***Bill Costerton**, Director, Dental Biofilm Center, University of Southern California*

In December of 2004 the American Society of Bone and Joint Surgeons convened a workshop whose expressed purpose was to take the research that has been done in the etiology of osteomyelitis and of device-related orthopedic infections, and to reduce it to a code of practice that the ABJS would recommend to its members. This process was initiated and followed with relentless pressure to make very definite recommendations to clinicians, with some provisos for the suspension of legal exposure for giving medical advice without the requisite medical licenses. The exercise was extremely salutary, and it is the second time that the CBE biofilm team has been pressed to give definite recommendations, because the FDA has asked for similar guidance in the setting of test criteria for claims of anti-biofilm efficacy with respect to sterilants.

The purpose of this talk will be to summarize the scientific data that was presented to the ABJS workshop, and to report which scientific conclusions the ABJS personnel demanded be clarified and "reduced to practice" in their advice to their members. The discussions started with our contention that orthopedic devices must be free of organic accretions and biofilm residues acquired during manufacture, so

that the "fouling rates" associated with contaminated surfaces would not compromise implant sterility. The second area of interest was diagnostics, and it centered on our demonstration that prostheses may be very heavily colonized by bacterial biofilms without yielding positive cultures of aspirates from synovial fluid or from the surfaces of prostheses that had been removed because of "aseptic loosening." Cultures were seen to be a very insensitive measure of the presence of biofilms, while serological methods that measure specific anti-biofilm antibodies were valued much more highly. The clinicians helped us to conclude that symptoms of aseptic loosening of orthopedic devices should be treated as biofilm infections in all cases, and appropriate antibiotic therapy should be included in all revisions of such cases until an accurate serological method can be introduced to exclude the presence of a bacterial infection. Biofilm theory was applied to the use of dissolving antibiotic-containing beads in osteomyelitis, and in revisions of prosthesis-centered infections, and we concluded that these beads serve both to deliver high concentrations of antibiotics to the local area, and to provide an alternate surface for biofilm formation. The clinical success of antibiotic-containing beads was noted, and we concluded that the UWEB technology of controlled release by ultrasonic energy would improve the performance of these beads, and that the bioelectric effect and the direct ultrasonic effect might be used to further improve their performance. The proceedings of this ABJS workshop will be sent to all members as recommendations for the treatment of patients. The biofilm community should ready itself for requests from more clinical entities for definite direction in the reduction of biofilm research to clinical practice.

W05-S16
Diffusion in Biofilms: Now in Hypertext

***Al Cunningham**, MSU-CBE Professor, Civil Engineering, **Rocky Ross**, MSU-CBE Professor, Computer Science, **Phil Stewart**, MSU-CBE Interim Director, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Al Cunningham will give an update on the CBE's latest educational initiative: the development of an electronic biofilms "hypertextbook" built with specialized Java applets as well as widely available web technologies. Rocky Ross will present the features and advantages of the hypertextbook format, and Phil Stewart will demonstrate these features and advantages with a tour of the hypertextbook's prototype module on Diffusion in Biofilms.

A hypertextbook is a teaching and learning resource designed to be delivered for use in standard web browsers such as Firefox, Internet Explorer, Netscape, Mozilla, Opera, and others. As such, a hypertextbook can be and do many things that a traditional textbook cannot. In addition to the usual textual presentations and static illustrations of material found in a traditional textbook, a hypertextbook can also include:

- multiple paths through material for different learning levels and needs
- any number of high-resolution images with zoom-in, zoom-out scalability
- slide shows with an audio track
- video clips with an audio track
- various learning accessories, such as pop-up calculators
- quizzes and checkpoints with feedback
- elaborate active-learning models, possibly with feedback, of important concepts with which students interact directly on their computers

Additionally, the fact that a hypertextbook is web accessible means that it can be made available to virtually anyone, virtually anywhere at a low cost.

This prototype module has been prepared to address the topic of "Diffusion in Biofilms" at the Intermediate Level, to be suitable for intermediate undergraduate coursework. Future development of the material will include material suitable for the Beginner and Advanced Levels. Content for this module was prepared by Philip Stewart, Professor of Chemical and Biological Engineering at the Montana State University Center for Biofilm Engineering, and is a direct offshoot of his classroom experience teaching this subject.

SESSION 4: Water Treatment

W05-S18
Control of Biofilms and Microbiological Water Quality in Water Distribution Systems

***Raymond Hozalski**, CBE Visiting Researcher, Associate Professor of Civil Engineering, University of Minnesota*

Historically, the water distribution system was a poorly understood "black box." It was generally presumed that if good quality water was pumped in, then good quality water would exit at the tap. Research over the last few decades has shown than significant and possibly detrimental water quality changes can occur as water travels tens of miles for up

to several weeks through a network of water mains, standpipes, and other structures before entering the pipes of the consumer. These mains, service connections, and pipes are comprised of a wide variety of materials including cast iron, ductile iron, concrete-lined iron, plastic (e.g., PVC), lead, copper and even wood. Many of these mains have been in service for decades and possibly for hundreds of years in older eastern cities such as Boston. Two of the main public health concerns arising from water transport through distribution systems are microbiological water quality and lead exposure. Microbiological water quality can be degraded as biofilms growing on the pipe walls release bacteria into the flowing water. The primary approach for controlling this microbial "regrowth" is to add chlorine as free chlorine or combined chlorine (i.e. chloramines). Another approach, championed by some European researchers and practiced in some European cities (e.g., Amsterdam) is to remove the easily assimilable organic carbon (AOC) or food supply for the bacteria. Lead is primarily released from lead service connections, lead pipes in the home, and from lead containing solder. The most common approach for mitigating lead corrosion problems is to add phosphate, an important microbial nutrient. This research involved bench-scale and pilot-scale experiments and full-scale monitoring to investigate the factors affecting bacterial growth in distribution systems and the effects of lead corrosion control chemicals on microbiological water quality. Unfortunately, given the complexity of meeting various and sometimes conflicting water quality regulations, decisions regarding chemical selection and dose are not always straightforward.

W05-S19
Microbial Biofilms as Indicators of Estuarine Ecosystem Condition

***Andreas Nocker**, MSU-CBE Assistant Research Professor, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

As part of a larger project examining the suitability of estuarine biofilms as indicators of estuarine ecosystem status, this work investigated correlations between biofilm community structures and various environmental conditions. Biofilms were grown over defined periods on glass slides in the Pensacola Bay area and were subjected to community analyses using cultivation-independent molecular techniques. A biofilm-based indicator system would have several advantages compared to traditional water quality testing. Most importantly biofilms grown over a certain period can be considered to integrate temporal and spatial changes as well as the full scale of water quality parameters. We also hypothesized that, since microbes are the fastest responders, microbial communities would change due to environmental impacts before the entire habitat exhibits change.

In order to qualify as indicators, a couple of prerequisites have to be fulfilled. This presentation addresses two key questions: 1) Do microbial biofilm communities exhibit habitat specificity? and 2) Do microbial biofilms respond to changes in water parameters within short time periods?

The first question is addressed by comparing the community composition of biofilms grown in an oyster reef habitat with one of a biofilm grown under the influence of a sandy-mud bottom habitat in close proximity. Total biomass and optical densities of dried biofilms showed dramatic differences for oyster reef vs. non oyster-reef biofilms. The observed variation was reflected in the heterotrophic prokaryotic species composition. Moreover, the biofilm community structure from the oyster reef setting showed greater evenness and species richness compared to the one from the muddy-sand bottom.

The second question is addressed by showing an example of how microbial biofilms in a field-setting change over a temporal oxygen gradient in a nutrient rich system affected by a sewage outfall site. As hypoxia is becoming an increasing problem for estuarine ecosystems, dissolved oxygen (DO) stress serves as an example of an important anthropogenic stressor. The experiment comprised the consecutive deployment of biofilm samplers over a one-month period that coincided with a gradual rise in DO levels for the near benthic water from severe hypoxia to normoxic conditions. We saw a correlation between the DO concentrations on the one hand and overall bacterial diversity and the abundance of sulfate reducing prokaryotes on the other.

In summary, the biofilm communities studied here seemed to show requisite responsiveness and a core stability of the diversity and richness that potentially qualifies them as sentinels of water quality.

SESSION 5: Cell-Cell Signaling

W05-S22
Influence of Quorum Sensing and Hydrodynamics on *Pseudomonas aeruginosa* Biofilm Structure and Behavior

***Laura Purevdorj-Gage**, Recent MSU-CBE PhD Graduate, Microbiology, Center for Biofilm Engineering at Montana State University–Bozeman, 59717*

Biofilm formation by bacterial pathogens is recognized as an important factor in the progression and treatment of many infectious diseases. One of the strongest links between biofilm formation and virulence is established for *Pseudomonas aereuginosa*, a common environmental isolate which is also associated with many nosocomial infections and, most notably, cystic fibrosis infection. Biofilm structural development is currently recognized as a complex dynamic process dependent on many cellular and environmental parameters. Among many other recognized factors, Quorum Sensing (QS) and hydrodynamic conditions of the biofilm growth environment were shown to be important in biofilm structural maturation. Since QS performance is dependent on accumulation of a threshold autoinducer concentration, it was hypothesized that flow dynamics in the bulk fluid surrounding the biofilm, which also determines mass transfer characteristics, would play an important role in QS system, and thus the genes and resulting phenotypes that are under its control. In order to investigate the relative contribution of hydrodynamics and QS on biofilm development, biofilms were grown from wild type *Pseudomonas aeruginosa* PAO1 and the cell signaling lasI mutant PAO1-JP1 under laminar and turbulent flows. Both biofilms in turbulent flow formed streamlined patches, which in some cases developed ripple-like wave structures which flowed downstream along the surface of the flow cell. By visual comparison, the structural morphology and dynamic behavior of the JP1 mutant biofilm did not significantly differ from the WT biofilm but hydrodynamics significantly affected biofilm structure. When morphological features of the biofilms were quantified using Image Structure Analyzer (ISA) software, a multivariate analysis demonstrated that both cell signaling and hydrodynamics significantly ($P<0.000$) influenced biofilm structure, suggesting that QS was not required for biofilm development but affected structural heterogeneity in biofilms. GFP reporter based gene expression analysis of QS regulated lasB (coding for elastase) expression pattern during biofilm development in laminar flow further supported these results.

In addition to biofilm growth, detachment has been recognized as another factor that may define structural morphology of biofilms. Under flow conditions, hollow biofilm clusters were formed as a result of an active detachment process, which we termed "seeding dispersal." On closer inspection, a differentiation of a "seeding" microcolony into an interior motile, swarming, phenotype and a non-motile surrounding, "wall phenotype" formed as a prelude to the actual dispersal process, in which the interior cells swarmed out of the microcolony from local break-out points and spread over the wall of the flow cell. A critical microcolony diameter of approximately 100 µm was required for differentiation, suggesting that regulation was related to cell density and mass transfer conditions. Using the flow cell and the plate assay techniques to screen for this detachment process in mutations known to affect biofilm structure, it was found that while rhamnolipid (rhlA-) biosurfactant was not required, QS system (PAO1-JP2) was shown to be indirectly involved in the detachment process, possibly by sensing nutrient limitation within the biofilm microcolonies.

These results strengthen a current view of multi-cellularity and coordinated behavior in prokaryotes as well as a dynamic network of overlapping pathways and cellular mechanisms that act on biofilm development in a complex, interrelated manner.

W05-S23
Influence of the Quorum Sensing Signal Autoinducer-2 on *Mycobacterium avium* Biofilm Formation

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Background: *Mycobacterium avium* is an opportunistic pathogen often found to colonize water distribution systems by forming highly complex communities called biofilms. Quorum sensing, the cell-density dependent regulation of gene expression, has been shown to be an important factor in the regulation of biofilm formation and maturation many times in different bacterial species. The current study was performed to test the influence of the signaling molecule autoinducer-2 (AI-2) on *M. avium* biofilm formation.

Methods: *M. avium* was grown in Middlebrook 7H9 medium with OADC enrichment at 37°C. After seven days, the culture was centrifuged for 20 minutes at 8000 rpm and the pellet was re-suspended in water. This aqueous culture was then used to inoculate the microtiter plates (150 µl per well) in the presence of different concentrations of chemically synthesized AI-2, resulting in final concentrations of 2.5, 0.25, 0.025 and 0.0025 mM, respectively. The negative control contained water instead of the AI-2 solution. After fourteen days, the biofilms were quantified by crystal violet-staining. The cell density in the supernatant was measured by viable counts on Middlebrook 7H10 agar. The structure of the biofilms was observed using confocal laser scanning microscopy.

Results: Biofilms accumulated more biomass with increasing AI-2 concentration, culminating in the doubling of the biomass in the presence of 2.5 mM AI-2. However, the cell density of the supernatant decreased with increasing AI-2 concentration. Microscopic observation revealed significant differences in biofilm structures depending on the AI-2 concentration, with complexity of the biofilm structure increasing with the AI-2 concentration.

Discussion: AI-2 does not appear to have a metabolic effect on the growth of mycobacterial cells, as has been shown for other selected bacteria. However, this study does suggest that AI-2 plays a role in *M. avium* biofilm formation by regulating the complexity of the biofilm architecture, and by inducing the switch from the planktonic to the biofilm mode of growth.

SESSION 6: Environmental Biofilms

W05-S25
Use of Solid Phase Iron Oxide Minerals as Terminal Electron Acceptors in Anaerobic Respiration: A Selective Pressure for Surface-Associated Bacterial Growth During Early Life on Earth

Gill Geesey, *MSU-CBE Professor, Microbiology, Montana State University–Bozeman, 59717*

During early evolution of life, what prompted bacteria and archaea to associate with surfaces of particulate matter? While electron donors such as hydrogen and methane were likely distributed between the gas and liquid phases, Fe(III) was likely the most abundant electron acceptor for microbial respiration prior to the appearance of molecular oxygen. During anaerobic photosynthesis, bacteria such as *Chromatium*, a large Purple sulfur bacterium common to marine sediments and brackish waters, carry out the following reaction:

$4\ FeCO_3 + 10\ H_2O \Rightarrow$
$4\ Fe(OH)_3 + (CH_2O) + 3\ HCO_3^- + 3\ H^+$

Fe(III) is very insoluble in water unless complexed with organic compounds. Most of the Fe(III) available for microbial respiration in organic carbon-limiting environments resides in amorphous or crystalline minerals such as hematite, goethite, and magnetite. While it has been known for some time that bacteria such as *Geobacter* spp. and *Shewanella* spp. can utilize Fe(III) associated with amorphous Fe-oxides, only recently have we recognized that Fe-reducing bacteria such as *Shewanella oneidensis* MR-1 has the ability to reduce Fe(III) associated with crystalline minerals such as hematite. This was demonstrated by observing the accumulation of cells on hematite surfaces when this crystalline mineral served as the sole electron acceptor in the system. While the mechanism by which these cells extract Fe(III) from the crystal matrix for subsequent reduction to Fe(II) remains a mystery, close association—but not necessarily direct cell contact—with the surface appears essential. Evidence of dissolution of the crystal matrix in the form of pitting has been observed on some but not all areas of the hematite surface colonized by the bacteria. Synchrotron radiation microscopy revealed that some of the reduced iron remains associated with the colonized hematite surface. The colonization behavior of mutants is currently being compared to wild-type strains in order to elucidate the role of different proteins at the cell surface in electron transfer from the cell to the solid phase mineral. This information will advance our understanding of how electrons are transferred from the bacterial cell surface to the surface of solid phase minerals and engineered materials. It will also lead to a better understanding of how microorganisms influence deterioration and corrosion of engineered materials.

W05-S26
Exploring Microbial Capabilities Through Biochemical Network Analysis

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Cells grow by oxidizing nutrients using a complex network of biochemical reactions. During this process new biological material is produced along with energy needed to maintain cellular organization. Because metabolic networks are highly branched, these tasks

can be accomplished using a wide variety of unique reaction sequences. However, evolutionary pressures likely select organisms that utilize highly efficient pathways. Using elementary-mode analysis, we demonstrate that the metabolism of the bacterium *Escherichia coli* contains four unique pathways that most efficiently convert glucose and oxygen into new cells under any level of oxygen limitation. We have applied this information, together with published glucose uptake rate data, to map rates through each intracellular reaction for steady-state growth over a wide range of doubling times and any level of oxygenation. This operating space of rates is governed by four discrete metabolic states that occur when only one of the four elementary mode pathways is operational. Generally, for a given growth rate, any point in this operating space that supports most efficient cell growth can be described as a linear combination of two pathways. Predicted regulatory patterns, oxygen uptake rates and some metabolite secretion rates are in excellent agreement with experimental observations. This supports the conclusion that, in spite of the many possible pathway choices, cell growth of wild-type *E. coli* likely operates on the basis of the identified most efficient pathways. Because the entire rate structure of a cell is known, the cell physiology under a range of operating conditions is completely determined, and specific cell behavior can be analyzed. The concise defining of cellular performance using the network's simplest flux units is a logical starting point for analyzing and engineering other complex systems, such as recombinant hosts used for bioprocesses and interacting populations of cells like those found in biofilms.

W05-S27

Biofilms on Plant Surfaces: Impacts on Plant Production, Biology and Ecology: Part One—Above Ground

***Cindy Morris**, CBE Visiting Research Scientist, Institut National de la Recherche Agronomique, Plant Pathology Research Unit, Avignon, France and Affiliate Professor, MSU Dept. Plant Sciences and Plant Pathology*

The surfaces of aerial parts of healthy plants harbor ca. 104 to 108 bacteria/cm^2 as well as a multitude of other microorganisms. These microorganisms play well-known roles in plant production and food technology, such as the generation of field and post-harvest diseases and decay, and fermentation leading to the production of alcoholic beverages and fermented vegetable products. Other less-known but important effects that these microorganisms can have on plants include the induction of frost damage, the biological control of plant pathogens, and enhanced growth via non-symbiotic nitrogen fixation or the production of hormones. Plant surfaces can also harbor certain human pathogens implicated in food poisonings. Currently, additional impacts of these epiphytic microorganisms on plants and their environments are being explored: their role as ice nuclei in atmospheric processes leading to precipitation and in modifying plant volatile compounds that affect air quality.

Bacteria, the dominant microbe on aerial plant parts, are known to establish biofilms on these surfaces. Nevertheless, a large proportion of the epiphytic (plant surface) bacterial population is outside of biofilms, and there is likely a dynamic shuffling of cells between these two states. Many questions remain unanswered about the exact role of biofilms per se in the impact that epiphytic bacteria have on plants and the environment. These questions are analogous to those posed for biofilms in medical, industrial and water distribution systems—where biofilms have roles in protection from stress, in enhancing genetic exchange and chemical communication, etc. Management of the biological activity of plant-associated microbes, to enhance their beneficial effects and limit deleterious effects, will depend on targeting the key players and understanding *in situ* behavior. This poses a special technical challenge, as leaf surfaces are living tissues with marked topography and are not readily represented by current tools based on colonization of inert surfaces.

Biofilms on Plant Surfaces: Impacts on Plant Production, Biology and Ecology: Part Two—Below Ground

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Plant root-microbe interactions in soil are mostly unknown, although it is suspected that there must be a complex communication network that is largely chemically-mediated. SEM imaging clearly shows the presence of microbial biofilms attached to root surfaces (the rhizoplane), and large numbers of microbes in the soil immediately adjacent to plant roots (the rhizosphere). In addition, it has been shown that many different types of organic compounds are

released by plant roots, both in the form of liquid exudates and contained within "root border cells" released from defined regions of root surfaces. These compounds can perform a positive function as microbial chemoattractants or can have negative biocidal effects. Thus, there is no doubt that complex symbiotic, commensal, and/or antagonistic relationships exist between plants and microbes in the soil environment. Understanding the intricacies of plant root-microbe interactions holds great potential for improving plant production, controlling plant disease, developing bioremediation strategies, and for discovering new pharmaceutical products.

W05-P326

Ultrasonically Controlled Antibiotic Release from Hydrogel Coatings for Biofilm Prevention

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Medical devices are routinely employed in healthcare settings since they provide clinicians with a useful means of administering nutrients, drawing blood samples and drug delivery. In spite of these advantages, local and systemic infections are frequently associated with their use. In fact, implanted devices often provide a highly suitable surface for bacterial adhesion and colonization resulting in the formation of complex, differentiated and highly structured communities known as biofilms. Once a biofilm infection is established, conventional treatments frequently fail because bacteria growing in biofilms are much less susceptible to antibiotics than their planktonic counterparts. As a result, a variety of implantable drug-delivery systems have been developed. However, drug release tends to decay over time, and these systems are prone to uncontrollable leaching. To overcome this problem the University of Washington Engineered Biomaterials (UWEB) group has developed a novel drug-delivery polymer matrix consisting of a poly 2-hydroxyethyl methacrylate hydrogel coated with ordered methylene chains forming an ultrasound-responsive coating. The polymer hydrogel was loaded with ciprofloxacin, an antibiotic well known for its action against gram-negative bacteria. This system was able to retain the drug inside the polymer in the absence of ultrasound, but showed a significant drug release when low intensity ultrasound was applied. When ultrasound application was complete, the hydrogel returned to its original configuration and again exhibited minimal drug leaching. With the combination of this polymer system and ultrasonic energy, drug delivery can effectively be turned on or off as needed for treatment. We have incorporated these hydrogel coatings into a flow cell reactor in order to observe biofilm formation and growth over time using confocal microscopy. *Pseudomonas aeruginosa* biofilms were grown on hydrogel surfaces in flow cells with a bulk fluid flow of 1 ml/min. Ultrasound was applied for twenty minutes every twenty four hours for three days using a 43 kHz ultrasonic bath with a power density of 2–3 W/cm^2. Confocal images were taken both before and after ultrasound application. The confocal data was then analysed quantitatively using the biofilm analysis software package COMSTAT. It was shown that, over the course of three days, the average maximum thickness of the biofilm colonies decreased. Control experiments with the same hydrogel configuration without ultrasound application showed that the average maximum thickness of the biofilm colonies increased over time. Control experiments with hydrogels that were not loaded with ciprofloxacin also showed increased average maximum thickness, both with and without ultrasound application. The results of our studies may ultimately facilitate future development of medical devices sensitive to external ultrasonic impulses, capable of treating or preventing biofilm growth via "on demand" drug release.

W05-P335

Computer Model of Persister Cell Protection Mechanism of Biofilms Against Antimicrobial Agents

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Hypothetical mechanisms based on the formation of persister cells protecting microorganisms in biofilms from killing by antimicrobial agents were incorporated into a three-dimensional model of biofilm growth. The results of two simulations, a base case with a non-resuscitating persister cell type and a second case with a resuscitating persister cell type, show that the persister cells offered the population protection from antibiotics and a means for rapid recovery. The high numbers of persister cells that accumulated in the biofilm began to resuscitate and regrow once the antibiotics had been relaxed. Near the end of the simulation, the biofilm with resuscitating persisters had recovered to a more viable state than the biofilm with the non-resuscitating persister cell type.

W05-P342
Biofilm Growth Induced Transformation of Porous Media Dynamics

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Magnetic resonance microscopy (MRM) has been applied to study hydrodynamic dispersion in porous media impacted by biofilm growth. MRM measures the averaged propagator of motion which provides the probability of displacements to occur over experimentally controlled times. The transition from pre-asymptotic to asymptotic hydrodynamic dispersion in a homogeneous porous medium constructed from monodisperse spheres is clearly visualized by the time evolution of the propagator to a Gaussian distribution. The growth of biofilms in the porous media induces a transition in the hydrodynamic dispersion from normal to anomalous transport which is visualized by the propagator transition from Gaussian to that modeled by a subdiffusive fractal kinetics model based on continuous time random walks (CTRW's). This transition is consistent with the porous media structure changing from homogeneous to non-homogeneous; connections to fractal dimensions are discussed. The MRM data can be analyzed in the q-space domain, i.e., the wavelength space reciprocal to displacement. They provide information on system dynamics for scales above and below a single pore. Fractional kinetics models for subdiffusive processes predict stretched exponential Gaussian behavior; the q-space data, when fit to stretched exponentials, exhibit a transition from Gaussian to subdiffusion distribution due to biofilm growth.

W05-P344
Artifact in Viability Staining of Biofilm Bacteria with *Bac*Light

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The diffusion of fluorescent stains from the LIVE/DEAD *Bac*Light bacterial viability kit into *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* biofilms was visualized by fluorescent light and confocal scanning laser microscopy. Propidium iodide diffused through biofilms up to 200 microns thick in a few minutes. SYTO 9 failed to diffuse entirely through the same sized biofilms within several hours. This study provides direct visual confirmation that propidium iodide does penetrate biofilm during traditionally accepted staining time periods, whereas SYTO 9 does not. The differential penetration of the two stains may confound interpretation of viability patterns in some cases.

W05-P345
Parameter Estimation of Initial Bacterial Attachment Using a Dynamic Model

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Attachment of drinking water bacteria to sintered glass was investigated in batch systems and packed columns. A dynamic model was developed to predict the removal of such bacteria in filter columns. Parameters obtained from the batch experiments were used in the model. Simulations were in good correspondence with measured results after adjustment of initial surface-attached bacteria concentration. Simulations provided insight into mechanisms involved in bacterial attachment and showed important aspects for improving the experimental design of future experiments.

W05-P346
Escherichia coli Capture from the Bulk Fluid by an Established *Pseudomonas aeruginosa* Biofilm in a Capillary Flow Cell

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Much research has been performed examining the ability of different organisms to form biofilms. Very little, however, has been done to examine the ability of an established biofilm to capture planktonic cells of another species out of the bulk fluid. This project examines and characterizes *Escherischia coli* capture and the events following *E. coli* capture by an established *Pseudomonas aeruginosa* biofilm in a continuous flow chamber using confocal microscopy, flow cytometry, and standard plate count methods. The *P. aeruginosa* are tagged with the green fluorescent protein, and the *E. coli* are tagged with DsRed, both carried on a plasmid. The Pseudomonas base biofilm averages about 75 microns in depth prior to *E. coli* inoculation, with a spatially heterogeneous structure. *E. coli* cells are then added in the bulk fluid, and their subsequent attachment and colonization are imaged using confocal microscopy. Visual observations are then correlated to a quantitative analysis of the effluent, both using plate counts and flow cytometry.

W05-P347
Feasibility of Using Hyperspectral Imaging Incorporated into a Mobile Biofilm Unit to Detect Perturbations in Water Quality

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A Mobile Biofilm Unit (MBU) has been designed and constructed that enables characterization, both microscopically and spectroscopically, of biofilms that develop under natural conditions. An integral component of the MBU is a portable flow cell system coupled to a microscope and an image processing unit. A microscope-coupled imaging spectrometer allows collection of reflectance spectra in the visible range (0.5 to 0.9 μm) for all pixels of the image. Using this system, development of biofilms inoculated and fed from water channeled into the flow cell from acid mine waste drainage was followed. Data from the contaminated site was compared to data collected at a relatively uncontaminated site further downstream.

W05-P348
Individual-Based Computer Models to Evaluate Biofilm Removal Strategies

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Extracellular polymeric substances (EPS) constitute a matrix that embeds bacterial cells in biofilms and may play an important role in the cohesiveness of these attached microbial communities. Based on this hypothesis, methods for removal of biofilms using chemical agents that attack the EPS in a biofilm have been investigated, so far with mixed results. Such chemical agents may be enzymes of bacterial origin, such as dispersin B produced by *Actinobacillus actinomycetemcomitans* that has been shown to enhance the detachment of *Staphylococcus epidermus* biofilms by degrading an N-acetylglucosaminidase containing extracellular polysaccharides.
In the current study, quantitative 2D and 3D mathematical biofilm modeling is used to investigate the feasibility of potential strategies for removing unwanted biofilms that use chemical agents to degrade the EPS matrix and thus induce biofilm detachment. The model used is based on a mathematical description of the process involved in biofilm formation using first-principles, and the spreading of the biofilm matrix is described using individual based modeling (IbM).

POSTER ABSTRACTS

W05-P349
Microbial Copper Corrosion

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Copper is the most widely used metal for plumbing systems, and copper corrosion in potable water is a critical problem. Some of the worst health effects of exposure to copper include gastrointestinal distress, vomiting, nausea and abdominal pain. Lead and Copper Rule (LCR) 1991 sets the action level of copper in the distribution system as 1.3 mg/L. Copper corrosion can also lead to distribution system damage. Copper corrosion in potable water is a complex chemical and microbial process, and very few studies focus on the microbial aspect. Microbially influenced corrosion (MIC) can produce excessive copper corrosion in water. In the presence of disinfectants the copper level decreases as MIC reduces the metal. Disinfectants also improve the color and turbidity of the water.

W05-P350
Drip Flow Biofilm Reactor: Commercializing a Research Tool

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Biofilm consists of bacteria attached to a surface by means of secreted plastic-like substances called extracellular polysaccharides, which form the protective slime layer around the cells and bind cells together. An extremely important difference between individual, suspended bacteria and the organized community of biofilm bacteria is that biofilm bacteria can tolerate very high concentrations of disinfectants and antibiotics. New products are needed to control biofilm bacteria. Applied biofilm research directed at developing these new products requires laboratory methods and apparatus that incorporate the defining parameters for each environment where biofilm exists.

The Center for Biofilm Engineering (CBE) at Montana State University and BioSurface Technologies Inc. (BST), a Montana company that manufactures and markets laboratory supplies, have a history of successful collaborative efforts in producing reliable, repeatable, and marketable laboratory reactors. This collaborative project will provide BST with a new product to manufacture and sell: the Drip Flow Biofilm Reactor (DFR). In biofilm research there are four environments of particular interest: high fluid shear (high velocity fluid flow over the surface), moderate shear, low shear, and no shear. Currently, BST manufactures reactors that represent the high and moderate shear environments. The no-shear environment does not require a sophisticated reactor system. That leaves a void for a reactor that grows biofilm under low shear conditions. The DFR will fill that void. Funding has been provided by the Montana Board of Research and Commercialization Technologies to develop a laboratory apparatus (DFR) that simulates an environment different from the reactors in the current BST product line, design the DFR to meet the BST manufacturing and marketing criteria, and develop a standard operating procedure (SOP) for the DFR that is suitable for use as an official standard method.

W05-P351
Imaging Biofilms on Tissue Using Scanning Electron Microscopy

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A Scanning Electron Microscope generates an electron beam that is used to excite secondary electrons on the surface of an object. These electron emission patterns are collected and used to create topographical, images of the surface at the microscopic level. This technology is being applied to human and animal tissue samples at the CBE's Biofilm Behavior Laboratory to identify biofilm on biological surfaces. One of these projects involves the study of human skin wounds to determine whether biofilm plays a role in the development of chronic infectious wounds. Biofilm has been detected on eight of twelve persistent wounds already with the use of SEM and this technology will continue to aid in the elucidation of bacteria and biofilm on the surfaces of tissue.