Montana State University

Center for Biofilm Engineering

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Center for Biofilm Engineering posters

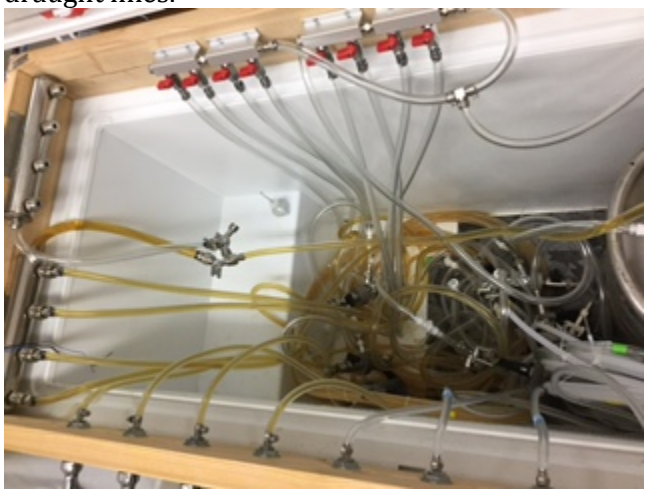
Presentation Abstracts

SESSION 1: Food Biofilms

Biofilm formation in draught beer dispense lines

Presenter: **Kelli Buckingham-Meyer**, Research Assistant III & **Lindsey Lorenz**, Research Assistant III
Co-Authors: Darla Goeres
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilm formation in beer draught lines may contribute to undesirable flavors and odors in beer. While consumption of beer with off flavors and odors does not pose a health risk to customers, it could prove detrimental to the sale of beers carefully crafted for a particular flavor profile. In an effort to provide their customers with a quality product, the Brewers Associated funded the CBE Standardized Biofilm Methods Laboratory (SBML) and NSF International to develop and validate a relevant laboratory model for growing repeatable biofilm in draught beer dispense lines. A draught beer system was set up in the laboratory to replicate refrigeration conditions, system components such as tubing material types, lengths, diameters and connectors, flow velocity and volume of beer. The laboratory system is inoculated with *Acetobacter aceti*, *Pediococcus damnosus* and *Saccharomyces cerevisiae* microbes common to beer spoilage. The model was designed so that once a mature biofilm is formed, replicate draught lines can be isolated and independently treated with the intent of optimizing the clean-in-place guidelines recommended in the Draught Beer Quality Manual. In this presentation, we will provide an overview of the laboratory draught beer system, protocol for operating the model and preliminary results on the biofilm growth in beer draught lines.



Biofilms in the dairy industry

Presenter: **Carolina Mateus**, PhD, R&D Director
Affiliation: Milk Quality & Animal Health, DeLaval Inc., Kansas City, MO, USA.

Biofilms on the surfaces of milk harvesting and processing equipment can risk the quality and safety of dairy products, as well as the safety of the herd. The presence of biofilm forming organisms in milk may originate from contaminated sources in the dairy, excretion from the udder of infected animals, the transportation of milk from the farm to the processing plant, poor cleaning routines in transport and processing lines, and the limited effectiveness of pathogen intervention steps.

Biofilms on dairy processing lines can develop fairly quickly. The type and species forming the biofilm varies depending on the location of the biofilm. Biofilms formed in extraction lines can contain both gram negative and gram positive organisms associated with the environment or the animal, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biofilms present in the processing plant are normally the result of cross contamination or inherent resistance to intervention steps such as pasteurization. They are normally composed of species such as Acinetobacter and Bacillus species.

Biofilm control both at the farm and the plant level currently relies on equipment design and the effectiveness of the clean-in-place (CIP) system. Milk harvesting and processing equipment are designed to facilitate cleaning by eliminating dead ends and providing smooth, non-porous food-contact surfaces. In general, surfaces that come into contact with milk comply with these design characteristics, but there exist some porous or awkward-surface components in the system that present a cleaning challenge, and provide a potential niche for biofilm formation. Since the introduction of the CIP concept, very few modifications have been made to the cleaning routine, where an alkaline cycle is targeted for the removal of proteins and an acid cycle for the removal of minerals. Changes to the CIP routine are focused on increasing the efficiency of the cleaning cycle by reducing cleaning temperature, cleaning time, or by eliminating detergents with a potentially negative environmental impact. However, limited work has been done to confirm which cleaning or sanitizing chemicals and routines are most effective to remove biofilms. Data will be presented comparing the biofilm removal efficacy of common hard surface sanitizers, as well as current attempts to systematically address the effectiveness of those sanitizers in simulated use conditions.

Creating a test system for the removal of biofilm using microfiber fabrics

Presenter: **Fei San Lee**[1], Undergraduate Student, Chemical & Biological Engineering
Co-Authors: Diane K. Walker[1] and Mike Hardegree[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Tietex International, Ltd., Spartanburg, SC, USA.

A laboratory system was created that would apply a constant contact pressure and simulate a wiping motion in order to test the removal of biofilm from a hard, non-porous surface by three specialty microfiber fabrics; two hand wipes and one floor mop. For this study, each fabric was weighted atop a coupon with a *Pseudomonas aeruginosa* biofilm grown according to ASTM Standard Test Method E2562. With the weighted fabric immobilized, the biofilm-coated coupon circled on a rotary shaker, which simulated the wiping motion. After a set number of wipes, the coupon was swabbed to recover any cells remaining on the coupon post-wipe. Results indicate that each fabric removed more than 99.999% *P. aeruginosa* biofilm from the surface of the coupon. This project was sponsored by Tietex International.

SESSION 2: Biofilm Imaging

Live/dead staining challenges

Presenter: **Elinor deLancey Pulcini**, Assistant Research Professor, Chemical & Biological Engineering
Co-Authors: Garth James
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The LIVE/DEAD® BacLight™ Viability Kit (Molecular Probes, Inc.) has become a popular fluorescence based-method for staining biofilms. The Kit consists of two nucleic acid stains, SYTO-9 and propidium iodide. SYTO-9 stains all cells green and propidium iodide, a red fluorescent stain, penetrates cells with damaged membranes. Live/Dead can stain eubacteria, archaea and eukaryotic cells. While the use of this staining method can result in beautiful fluorescent images of biofilms, it is not always a reliable indicator of cell viability.

The kit relies on the penetration of propidium iodide into cells with damaged membranes to establish which cells are dead or non-viable. Unfortunately, there are numerous factors which can affect propidium iodide uptake including bacterial species, Gram positive versus Gram negative membranes, the type of treatment used, and the ability of some species to repair propidium iodide permeable membranes. Cells with intact membranes might not be alive and membrane compromised cells might not be dead. In addition, since the emission spectrum of SYTO-9 overlaps that of propidium iodide, there is a potential for the occurrence of fluorescence resonance energy transfer (FRET) or the transfer of energy from one fluorescent molecule to another, resulting in propidium iodide signal enhancement.

This presentation will review the limitations of the LIVE/DEAD® BacLight™ Viability Kit and describe possible methods to address stain limitations.

BiyoTrap: A rapid, efficient, and cost-effective bacteria concentrator

Presenter: **Recep Avci**[1,2], Research Professor, Director
Co-Authors: G. Elif Ugur[1,2], Elizabeth Vinson[1,2], Merve Evcil[1,2], Garth James[3]
Affiliation: [1]Department of Physics,
[2]Image & Chemical Analysis Laboratory (ICAL),
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

This talk addresses handling a critical safety issue through the development of a low-cost, reliable and easy-to-use method of capturing and concentrating *trace amounts* of the pathogens that might be present in environments such as drinking and washing water supplies, contaminated food sources, and community health care facilities and hospitals. Typically, the concentrations of infectious bacteria required for infection to occur are far below the detection limits of conventional techniques. The technology we discuss here is expected to bridge this gap. It it absolutely *necessary* to *detect* and *identify* drug-resistant or other bacteria in the environment *rapidly, efficiently* and *reliably* before they pose a serious health threat.

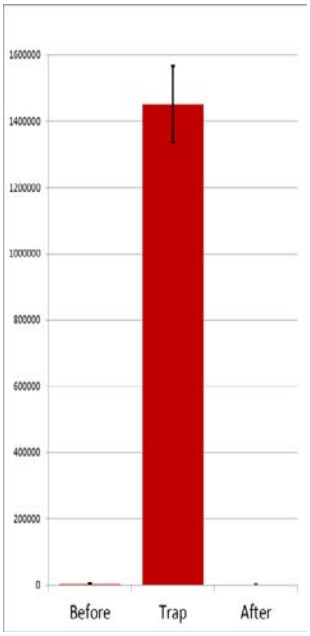


Fig.1: Histogram showing the concentrations of bacterial cells prior to the trap, inside the trap, and after the trap using ATP assay. The sample was 500 mL of seawater containing trace *Marinobacter*. Preliminary work indicated that the BiyoTrap concentrates with equal efficiency all Gram-negative and Gram-positive organisms in a variety of liquids including water, PBS, seawater and fuel.

We achieve this using an efficient, rapid bacteria concentration technology named BiyoTrap (*patent pending*). A prototype device has been constructed based on activated 3D glass microfiber networks that are chemically modified to *trap*, *capture* and *concentrate* nonspecific bacteria with 100% efficiency, using coulumbic charge as a means of capturing charged particles and/or charged macromolecules such as bacteria and DNA without the limitations of physical entrapment; i.e the activated fibers act as a *trap* and *not* as a *filter* for bacteria and/or DNA. A flow-through system is employed that can process large volumes (i.e. liters) of liquid and concentrate trace bacterial contaminations, down to <1 cell/mL, into a volume of ~200 µL of the trap medium. In this way one can easily achieve 2 to 4 orders of bacteria concentration in less than 20 min (better than 500-mL per 3.0±0.5 min flow rate). An example of its application is shown in Fig. 1. Currently, the detection and identification limit of conventional technologies is ~100-1000 cells/mL. BiyoTrap improves on this by trapping and concentrating trace pathogens efficiently, rapidly and cost-effectively to a level at which current detection technologies can be utilized. We have tested the limit of detection and identification of MRSA-700698 strains at a trace concentration level of a single bacterium in 20 mL of liquid. This is achieved by combining BiyoTrap with emerging rapid, reliable and highly sensitive biodetection technologies. The combined detection system has the potential to identify a targeted pathogen within, conservatively speaking, less than 30 min from start to finish. This is a considerable improvement over the 1-2 days of culturing and growing bacteria needed to achieve concentrations at which current technologies can be used.

Structuring microbial communities with 3D printing

Presenter: **James N. Wilking**, Assistant Professor, Chemical & Biological Engineering
Co-authors: Reha Abbasi, Aaron Benjamin, and Madison Owens
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Many biofilms contain multiple microbial species that interact with one another in beneficial and detrimental ways. The spatial locations of these microbes determine nutrient, waste, and signaling molecule concentrations, which in turn govern gene expression, metabolic rates and cell function. Understanding the impact of spatial location on cell function and biofilm physiology is critical for developing biofilm removal strategies as well as optimizing biofilm properties for engineering applications. However, methods for structuring biofilms are lacking. To address this need, we have developed stereolithography-based 3D printing techniques for structuring microbe-loaded hydrogels into "biofilms" with well-defined structures and properties. Our hope is that these methods will enable biofilm researchers to explore the complex structure-function relationships that exist in microbial biofilms.

Imaging mass spectrometry–A technology to advance understanding and diagnosis of bacterial infections

Presenter: **Timothy Hamerly**[1,2]
Co-Authors: Jake A. Everett[3], Margaret H. Butler[4], Jonathan K. Hilmer[1], Nina Paris[1], Steve T. Fisher[5], Arivarasan Karunamurthy[6], Garth A. James[5], Kendra P. Rumbaugh[3], Daniel D. Rhoads[7,8], Brian Bothner[2]
Affiliation: [1]Department of Infectious Disease and Immunology, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA.
[2]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA.
[3]Department of Surgery and TTUHSC Surgery Burn Center of Research Excellence, Texas Tech University Health Sciences Center, Lubbock, TX, USA.
[4]BioScience Laboratories Inc., Bozeman MT, USA.
[5] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[6]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.
[7]Department of Pathology, Case Western Reserve University, Cleveland, OH, USA.
[8]Department of Pathology, University Hospitals Cleveland Medical Center, Cleveland, OH, USA.

A major challenge for clinicians is the inability to detect, identify, treat, and monitor treatment of bacterial biofilms in a timely manner during infection. I have led a project with the long-term goal of rapid identification of bacteria directly from wounds using Imaging Mass Spectrometry (IMS). This technique generates a molecular-based image of tissue, providing information on the metabolic status of the wound and bacteria present. The ability to generate two-dimensional images of a wound, for example, with information about the distribution of bacteria overlaid with the distribution of drugs and metabolites could enhance our understanding of bacterial infection and the wound healing processes. In addition, the rapid determination of bacterial load and species present in a wound within minutes of biopsy would be a significant benefit to clinicians for treatment. In this interdisciplinary work, we demonstrate the use of IMS to improve our understanding of bacterial and antimicrobial distribution in model wounds and the potential application of this technology in a clinical setting to detect and identify bacterial pathogens. We have successfully applied IMS to map the distribution and persistence of chlorhexidine in a human tissue wound model. Subsequent work demonstrated the ability to detect and localize *Staphylococcus aureus* in this tissue model. Molecular heat map style images with sub-millimeter resolution show the promise of using IMS to investigate the presence and status of bacteria on skin. Expanding upon this work, we are using a murine model of thermal injury to investigate the potential of IMS for detecting bacterial infection in situ. In this work, thermally injured mice were inoculated with a low concentration of *Pseodomonas*

aeruginosa and tissue was excised and analyzed by imaging mass spectrometry. We demonstrate for the first time the use of IMS to detect *P. aeruginosa* on thermally injured tissue. A set of molecular features unique to the bacteria were used to generate molecular heat maps. This project represents a new line of investigation taking advantage of leading edge analytical tools to advance basic and clinical research of infection and biofilms.

SESSION 3: Biofilm Methods

Drip flow reactor training video: Second video in the SBML methods technology transfer initiative

Presenter: **Darla Goeres**, Associate Research Professor
Co-Authors: Kelli Buckingham-Meyer, Lindsey Lorenz, Diane Walker
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The mission of the National Science Foundation Center for Biofilm Engineering is to advance the basic knowledge, technology, and education required to understand, control and exploit biofilm processes. With this mission in mind, the Standardized Biofilm Methods Laboratory in collaboration with Bryan Warwood and Stephen Pedersen (BioSurface Technologies), Abbey Nelson and Kelly Gorham (MSU Visual Media Department) and Kristen Griffin and Joey Parchen (CBE Communications) are pleased to present the second SBML method training video on how to grow a biofilm in the drip flow biofilm reactor.

Biofilm reactors engineered to include a continuous flow of nutrients under various fluid shear conditions are complex. The purpose of the training videos is to demonstrate how to clean, assemble, and grow a repeatable biofilm using the standardized biofilm reactors. The second video focuses on growing biofilm in the drip flow biofilm reactor (DFR), Figure 1. The DFR grows a biofilm under low fluid shear close to the air/liquid interface. As the name implies, nutrients are dripped on the top of glass microscope slides housed in individual chambers. The short residence time allows the researcher to alter the nutrient source to study biofilm response or to expose a mature biofilm to biocide or antibiotics. The DFR is often used for medically relevant applications and the method may easily be modified to study biofilm growth on a variety of surfaces. The DRF video focuses on the standard operating procedure described in ASTM Method E2647 and ends with a trouble-shooting section that addresses some of the more common challenges encountered when using this reactor. The training videos are a compliment to the Knowledge Sharing Articles which focus on the statistical analysis of surface disinfectant efficacy data.

Figure 1. Assembled DRF reactor.

Understanding the development of mixed fungal-bacterial biofilms

Presenter: **Erika J. Espinosa-Ortiz**, Postdoctoral Research Associate
Co-Authors: Robin Gerlach
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Associations between bacteria and fungi, mostly in the form of biofilms, exist in many natural and industrial environments. Development of fungal-bacterial biofilms is influenced by interspecies microbial interactions, which can affect the attachment and surface colonization, as well as the structure and physiology of the biofilm. Our work aims to investigate the surface colonization and development of fungal-bacterial biofilms. Different co-culture species (*Phanerochaete chrysosporium – Pseudomonas putida; Aeurobasidium pullulans – Pseudomonas putida*) were used for the development of biofilms in various bioreactors (drip flow reactor; environmental chamber). The effect of culture conditions on the colonization and development of biofilms were studied, including: pH, inoculation method (colonization sequence) and type of inoculum. Biofilm growth, substrate consumption and morphology of the biofilms were determined. Circumneutral initial pH values (6.5) seem to promote fungal-bacterial biofilm formation, whereas slightly acidic pH values (4.5) seem to limit bacterial growth and favor fungal dominated biofilm development. At a given pH value, inoculation order also appears to have an effect on the development of fungal-bacterial biofilms. The results of this study may allow for an improved understanding of fungal-bacterial interactions and how culture conditions can influence biofilm development, which is of practical relevance for a diverse range of fields, including agriculture, forestry, environmental protection, coatings, food processing, biotechnology, medicine and dentistry.

ISO Method 846 Part C: Evaluation of plastic surfaces for the ability to support bacterial growth

Presenter: **Natalya Polukoff**, Undergraduate Student, Microbiology & Immunology
Co-Authors: Darla Goeres, Al Parker
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Plastics and the products made from them are a staple of modern society. Unfortunately, plastics are susceptible to biological deterioration, a process which at best results in an aesthetically displeasing surface and at worse shortens the expected useful life of the product or results in a biofilm that may serve as a reservoir for pathogenic microbes. Direct deterioration of the plastic results when the microbes utilize components of the plastic as a nutrient source. Indirect deterioration occurs when the microbe's metabolic products weaken or discolor the surface. ISO Method 846 was developed to test the susceptibility of plastics to both fungal and bacterial deterioration.

The Standardized Biofilm Methods Laboratory participates in the International Biodeterioration Research Group (IBRG). IBRG is comprised of five working groups (paints, functional fluids, wet-state preservation, textiles and plastics) that develop test methods focused on biodeterioration and hygiene. The plastics working group is participating in the revision of ISO Method 846. This presentation will focus on proposed revisions to Part C of ISO Method 846 which focuses on the bacterial deterioration of plastics. The intended goal of the revisions is to make the test quantitative.

Four plastics (polyurethane (PUR), polypropylene (PP), two different polyvinyl chlorides (PVC)) and stainless-steel control coupons were gently dipped into a 10^7 CFU/mL broth culture of *Pseudomonas stutzeri* ATCC 17588 then rinsed in a sterile salt solution. One coupon of each type was sampled for viable cells and the remaining coupons were placed into a humidified chamber kept at 30°C for 72 ± 2 hours. At the end of the incubation period, the remaining coupons were sampled for viable cells. Figure 1 shows the change in cell density over time. All four plastics showed an increase in bacterial numbers over the 72 hours. The bacterial counts decreased on the stainless-steel control coupons. Further development of this test method will include the influence of edge effects (where the inner section of the plastic is exposed due to the machining process), the effect aging the plastic has on bacterial numbers and additional repeats of the existing method. See figure on next page.

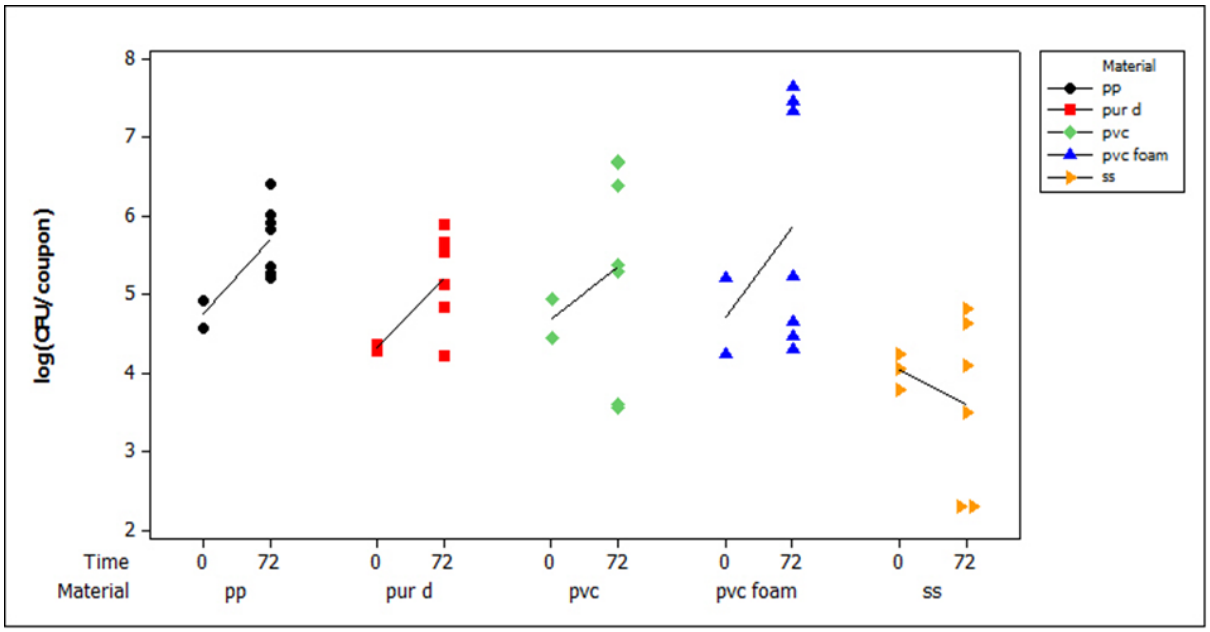


Figure 1. Change in bacterial numbers from time 0 to time equal to 72 hours by coupon material type.

Production and analysis of a *Bacillus subtilis* biofilm with spores using a modified colony biofilm model

Presenter: **Laura Wahlen**[1], Research Associate III
Co-authors: Jason Mantei[2], Research Scientist
Affiliation: [1]Sterility Assurance and [2]Analytical Center of Excellence, Baxter Healthcare Corporation, Round Lake, Illinois, USA

Bacillus subtilis is a Gram positive, spore-forming soil bacterium that is frequently isolated from processing equipment in the food and pharmaceutical industries. *B. subtilis* forms biofilms on the surface of agar plates (colonies) as well as floating biofilms at the air-liquid interface of liquid (pellicles). It is known that sporulation can occur within biofilms, including those formed by *B. subtilis*. It is possible that the spores embedded in the protective EPS matrix could exhibit higher resistance to disinfecting agents than a vegetative biofilm or spores alone, reducing the efficacy of the treatment.

We describe and analyze a method to grow and quantify a reproducible *B. subtilis* biofilm comprised of vegetative cells and spores using a modified colony biofilm model (CBM). The CBM uses an inoculated semipermeable membrane on an agar plate as the biofilm growth surface and nutrient source. In this method, membranes were inoculated and incubated for a total of 8 days to promote sporulation within the biofilm. The biofilm was evaluated over the course of the incubation period using enumerative, microscopic, and spectroscopic methods. At various time points, the total numbers of cells were quantified. Additionally, a spore count was determined by heat shocking the cells (Figure 1). We utilized the Congo red agar (CRA) method to detect the TasA matrix protein, a primary component of the *B. subtilis* biofilm matrix. The presence of TasA was also confirmed using mass spectroscopy. The biofilm morphologies were correlated to the enumeration data with a variety of imaging techniques: confocal microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) (Figure 2).

At the end of the incubation period, the biofilm contained greater than 7 logs total colony forming units with spores comprising approximately 10% of the biofilm. The biofilm generated using this method is suitable for efficacy testing of antimicrobials such as antibiotics, disinfectants, or heat.

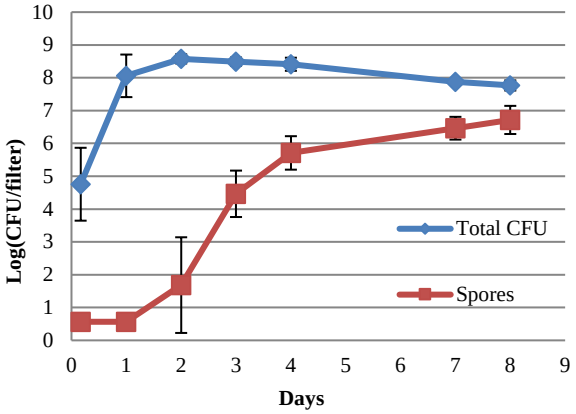


Figure 1: Average log total CFU and spores in a *B. subtilis* colony biofilm over a period of 8 days. Error bars indicate the standard deviation of the mean.

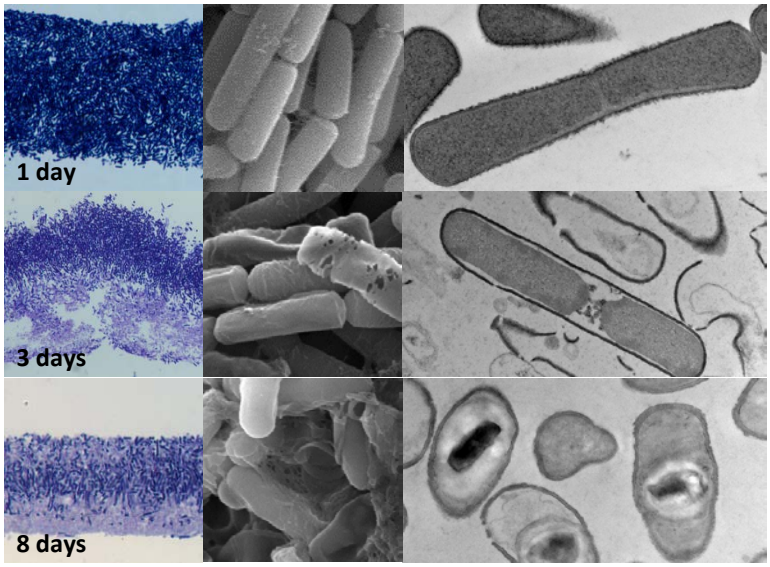


Figure 2: TEM thick section, SEM, and TEM thin section of biofilm at 1, 3, and 8 days.

SESSION 4: Biofilms in the Built Environment

Building microbiome control

Presenter: **Jordan Peccia**, Professor
Affiliation: Department of Chemical and Environmental Engineering, Yale University, New Haven, CT, USA.

Bacteria and fungi in buildings exert both beneficial and hazardous influences on the human microbiome through inhalation, aerosol deposition, ingestion, surface contact, and human and animal interactions. As the identities and functions of health-relevant microbes continue to emerge, a clear, mechanistic approach is needed to understand how building design and operation can control the microbial health of occupants (Figure 1).

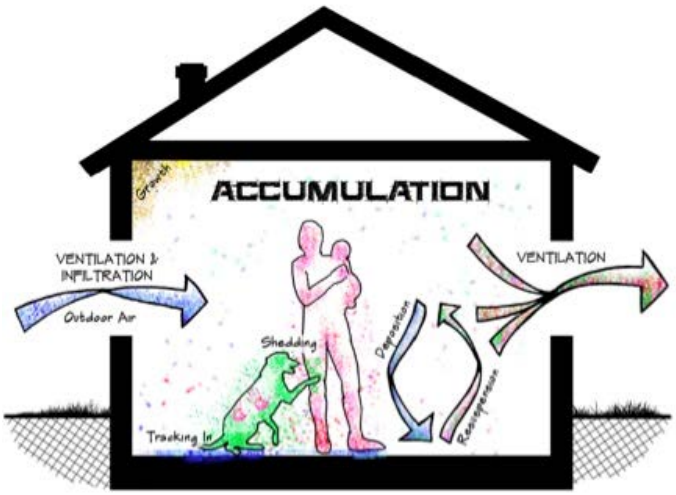


Figure 1. Physical processes affecting building microbiome concentration and ecology.

Objectives of this presentation include the following: (1) previewing indoor microbiome ecology and function in a diversity of buildings, (2) presenting evidence for how bacteria and fungi in buildings may exert beneficial health impacts, and (3) detailing approaches to understand how to control microbiomes through building design and operation.

Mold contamination of indoor materials for the built environment: Shipping, storage and preservation aspects

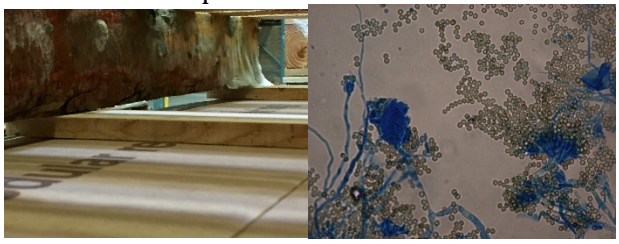
Presenter: **Daniel L. Price**[1], Director of Microbiology
Co-authors: Donald G. Ahearn[2], Brandi M. Prestridge[2]
Affiliation: [1]Interface Inc., and
[2]Georgia State University, Atlanta, GA, USA.

Common construction and finishing materials, wood, wallboards and natural fabrics, have long been recognized as subject to biodegradation by environmental fungi such as *Aspergillus, Chaetomium*, *Paecilomyces* and *Trichoderma*. Protective coatings and/or incorporation of antimicrobial preservatives are necessary for practical applications of these and other materials exposed to wet and humid conditions that may accelerate the decay. It is less recognized that indoor materials may carry dormant fungal propagules or cryptic fungal colonization when obtained by the builder. During the past several decades, product spoilage and recalls resulting from fungal growth or metabolites have been associated in some cases with wood pallet-involved storage and shipping. Such recalls have been most prominent with food and pharmaceutical products. Costs of major regulatory-motivated recalls, which involved millions of items and some adverse consumer events were in million dollar ranges. Indoor construction materials apparently associated with fungi in our experience involve item replacement of several pallet packs (loads) between supplier and builder with notification to keep the product dry.

Recently, we examined mold-contaminated pallets holding luxury vinyl flooring (LVT) tiles. Discovery of rampant mold growth on pallet runner boards resulted in the product being deemed unacceptable for distribution and sale. The affected pallets with LVT tiles were shipped-stored on a container ship from an Asian overseas affiliate. In an attempt to meet a large order commitment, the manufacturer used green (non kiln dried) wood for product shipment pallets. Uncured wood (28-40% moisture

content) combined with favorable growth conditions created in closed shipping containers resulted in over 1597 affected pallets in the US and over 2500 square feet of molded LVT product inside the boxes. Packing configuration and packing materials further complexed the investigation due to the presence of a spacer board at the bottom of the product boxes. Shipping and storage of indoor building materials, particularly on pallets or in packaging that is susceptible to fungal growth raises concerns about pre-installation fungal contamination and the need for appropriate product preservation and ongoing sanitization.

Mold colonized pallet runner Heat tolerant *Talaromyces* sp (400X)



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Living lab studies on biofilms in Finland

Presenter: **Minna M. Keinänen-Toivola**, Research Manager
Co-Authors: Jenni Inkinen, Ahonen Merja, Mäkinen Riika
Affiliation: Satakunta University of Applied Sciences, Pori, Finland

In the Satakunta region of Finland, biofilms studies have been concentrated on real life studies as living labs. Biofilms have been and will be studied on drinking water systems as well as touch surfaces. One specific theme has been use on copper as antimicrobial coating on surfaces. The research work also is in relation to European network of antimicrobial coatings in health care to prevent infectious diseases (http://www.amici-consortium.eu/).

The themes of research work have been:

- Evaluation of the microbiological and chemical quality of the water in a real office building during the first 12 months of operation, especially the amounts of metals leaching from brass joints upon occupation of a new building.
- Characterization of microbial communities in drinking water and biofilms in full and pilot scale studies.
- Exploring the factors affecting water systems' microbial communities: in full-scale system temperature and location, and in pilot system extra chlorine treatment and magnetic water treatment.
- Evaluating the effect of piping material (copper, PEX) on the microbiological and chemical quality of water and biofilms in real life office building and pilot scale systems, and compare the effect of material to other factors.
- Evaluating the antibacterial efficacy of copper containing products (copper, brass) in real life touch surfaces in different facilities. Identifying touch surface types that possess highest bacterial loads and differences between facilities.

The presentation will summarize the main findings of the research work since 2011 and also presents to SAMK's new campus where biofilm studies on water and surfaces are integrated to full scale operations.

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Characterizing biofilm growth on elastomeric roof coatings subjected to ponded water

Presenter: **Joseph Moore**, Senior Chemist
Co-Authors: Victoria Demarest, Roofing R&D Leader
Affiliation: Dow Microbial Control, Collegeville, PA, USA.

Elastomeric roof coatings (ERCs) are white, monolithic, fluid-applied coatings designed to protect the roofing substrate and improve energy efficiency through enhanced solar reflectivity. Sustainably formulated waterborne acrylic roof coatings are known for their excellent cost/performance balance and have offered exceptional performance to the roofing industry for well over 30 years. A remaining opportunity for improvement of ERC lifespan is related to long-term performance in ponded water situations which often arise on flat roofs. It has been observed that biofilms are often established and proliferate in ponded water, and we hypothesize that these biofilms impact dirt pickup, roof surface darkening and, ultimately, coating performance. This presentation describes approaches taken by The Dow Chemical Company to characterize biofilms on ERCs and the resulting performance of ERCs subjected to ponded water conditions. Characterization includes evaluation of coatings exposed to long-term ponded water on roofs and in simulated exterior ponds, macroscopic and microscopic analysis of coating failures, and accelerated approaches to understanding and mimicking ponded water performance.

A multi-scale modeling framework for biofilm development

Presenter: **Ting Lu**, Assistant Professor
Affiliation: Department of Bioengineering, University of Illinois at Urbana-Champaign, Champaign, IL, USA.

Biofilms are ubiquitous around the world, and have profound impacts on human health, environment and agriculture. To control their formation and to further exploit their potential for applications, a central challenge is to quantitatively describe their behaviors and decipher the basic rules that govern their organization that is heterogeneous in space and time. Recently, we developed a multi-scale modeling platform that enables individual-based, biophysical simulation of microbial communities across multiple scales. The platform involves a comprehensive description of molecular networks within individual cells, physical and chemical interactions between cells, and spatiotemporal ecology of cellular populations, along with the environments that cells inhabit. As a demonstration of the platform, we used it to investigate the roles of cellular social interactions in microbial assemblages. Specifically, we explored how the mode of cellular interactions contributes to community structure and how the spatial scale of interaction shapes pattern characteristics, both of which were subsequently examined using experimental biofilm systems. Using engineered cellular interactions, we also further demonstrated the utility of synthetic ecosystems for metabolic engineering applications. Our computational tool enables quantitative description and analysis of the dynamics and patterns of biofilm development. It also serves as a useful testbed for generating and testing hypotheses relating to microbial community formation and control. Additionally, our studies that are enabled by the platform offer fundamental insights into the ecology of complex microbial ecosystems.

SESSION 5: Industrial Biofilms

Biofilms in metal working fluids

Presenter: **Christine Foreman**[1,2], Associate Professor
Co-Authors: Markus Dieser[1,2], Safiye Selen Ozcan[1,2], and Kevin Cook[1,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Department of Mechanical Engineering, Montana State University, Bozeman, MT, USA.

In many manufacturing processes, metal working fluids are applied to ensure reduced tool wear and workpiece quality. Worldwide, more than half a billion gallons of metal working fluids are estimated to be consumed annually. However, microbial contamination is a significant factor in the degradation of these fluids, causing biofouling and corrosion of equipment, degradation of metal working fluids, imperilment of product quality, and posing occupational safety risks. According to the National Institute for Occupational Safety and Health, more than 1 million US machine workers are exposed to metal working fluids in the workplace environment. Once these fluids are microbially contaminated, removing bacteria is a difficult task. Residual bacteria can quickly repopulate, even after meticulous cleaning and recharge procedures. Biofilms within the inaccessible regions of a working fluid system are likely responsible for the rapid post-cleaning repopulation.

Although deficiencies due to microbial contaminants in metal working fluids are known, biofilms remain poorly characterized, regulations regarding the permissible levels of microbial contamination have not been established, and a consensus on monitoring microbiological contaminants needed for accurate risk assessment and proper operation has not been determined. Working with Mechanical Engineering Technology students we have developed a model metal working fluids circulation system suitable for testing a variety of parameters related to biofilm development and eradication.

We have begun testing a suite of agents for their effectiveness against *Pseudomonas aeruginosa* and *P. stutzeri*, known biofilm formers in metal working fluids. In comparison to a control, two agents successfully inhibited 78 and 85% of biofilm formation after 48 hours. Ultimately, better understanding of the metalworking environment and associated biofilm-forming microbial communities will provide economic benefits and reduce occupational safety risks.

Bulk phase resource ratio alters electron transfer mechanisms in sulfate-reducing biofilms grown on metal

Presenter: **Greg Krantz**[1], PhD candidate
Co-Authors: K. Lucas[1], L. T. Hoang[2], G. Siuzdak[2], M.W. Fields[1]
Affiliation: [1]Department of Microbiology & Immunology, Center for Biofilm Engineering,
Montana State University, Bozeman, MT, USA.
[2]Scripps Center for Metabolomics and Mass Spectrometry, The Scripps Research Institute,
La Jolla, CA, USA.

Microbially Influenced Corrosion (MIC) is a major concern for industrial ferrous metal pipelines and can result in pipeline failure. Sulfate-Reducing Bacteria (SRB) have been implicated in contributing to MIC due to their production of corrosive H2S gas and elemental sulfur along with metal-microbe interactions. This project focuses on the effects of Electron Acceptor Limitation (EAL) and Electron Donor Limitation (EDL) on biofilm physiology and corrosion rate on various surface types, including 1018 carbon steel, 316 stainless steel, and borosilicate glass. *Desulfovibrio alaskensis* G20 was grown under steady-state conditions in anaerobic sulfate-reducing biofilm reactors. Under EAL conditions, biofilms on glass and 1018 steel had elevated biomass levels, both in terms of protein and hexose levels. Under EDL conditions, biofilms on 1018 steel had the highest protein and hexose levels. Differential corrosion rates were observed between EDL and EAL conditions on 1018 carbon steel. The results indicated that different ratios of respiration substrates contributed to altered rates of corrosion,

and the difference in corrosion rates could not be explained solely by sulfide, acetate, or carbohydrate levels. Protecting the 1018 metal coupon from biofilm colonization while maintaining exposure to sulfide was shown to dramatically reduce corrosion. Metabolomic mass spectrometry analyses combined with XCMS data processing show an increase in lumichrome, a precursor to FAD, under the EDL condition, suggesting the bacteria are using FAD for extracellular electron transfer from the metal, and as a means to increase metal corrosion when starved for electron donor.

Organic molecules and biofilm driven processes of metal deterioration: Exploring an uncharted territory

Presenter: **Iwona Beech**, Research Professor
Affiliation: Institute for Energy and the Environment, University of Oklahoma, Norman, OK, USA. CAMEC Consulting, USA.

Mechanisms by which biofilms on metallic materials influence near-surface chemical processes that can result in deterioration of such materials are subject of extensive studies. Damage of a metal due to the presence and/or activity of biofilms is referred to as microbiologically-influenced corrosion (MIC) or biocorrosion. The necessity of the contact between the cell and the surface has been demonstrated as prerequisite for MIC, however, the issue whether exo/endo metabolites, derived from sessile (biofilm) or planktonic (bulk phase) cells, can exacerbate metal deterioration and if so, by what mechanisms, is still unresolved.

Although profiling biofilm populations, using either traditional microbiological or molecular ecology techniques, is advocated to diagnose biocorrosion and decide on control measures, a tendency still exists to implicate MIC as the cause of materials failures, based solely on characterizing planktonic bacterial populations. Indeed, mitigation strategies are often designed based on the latter data. While planktonic and overall biofilm populations in closed systems are, most likely, not too dissimilar, metal surface-associated population (innermost part of the biofilm) may still have markedly different phylogenetic and/or metabolic profile than the community present in uppermost parts of the biofilm or/and in the bulk phase. It is, however, unclear whether and what specific regions within the biofilm have a most significant impact on corrosion behavior of colonized material, thus, should be a primary target of MIC control.

This presentation discusses the results of laboratory and field investigations carried out employing –omics (metabolomics, metagenomics) laser-based chemical imaging, combined with electrochemistry and surface science methods to elucidate mechanisms of MIC in oilfield and naval systems. Particular emphasis is placed on the role of organic molecules and on demonstrating differences in (i) community structure between biofilm regions, (ii) metabolic activities of these communities and (iii) chemical composition of corrosion products accumulating on biofilm versus not colonized surfaces.

Metabolomic and genomic imaging as novel ways of investigating biofilm-influenced processes in industrial systems and in medical settings

Presenter: **Iwona Beech**

Abstract not available.

Particle size impacts carrying-capacity for biofilm via reduction of free pore space and resources

Presenter: **Sara Altenburg**[1], Research Lab Manager
Co-Authors: H.J. Smith[1], I.S. Miller[1], A.P. Arkin[2], M.W. Fields[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Lawrence Berkeley National Lab, Berkeley, CA, USA.

Sulfate-reducing-bacteria (SRB) occur naturally in a variety of anaerobic environments where sediments are present. *Desulfovibrio* biofilms were grown on silica oxide particles (three different sizes) using modified biofilm reactors in an effort to investigate the impact of physical surface scale on microbial biofilms occurring in anaerobic habitats. Surface area to volume ratios decreased with

increasing bead size, and ranged from 1500 to 58 to 20 cm 1, respectively. Under steady-state conditions, both the biofilm protein and carbohydrate per surface area (mg/cm2) was greater for the intermediate and largest sized particles, compared to the smallest. However, the overall biofilm carbohydrate to protein ratio was similar for the tested particle sizes (0.11, 0.07, 0.09, respectively). The amount of biofilm per unit area decreased with the particle size even as surface area/volume increased. As the biofilm formation proceeds on the beads and in the inter-bead space, the porosity of the packed bed changes thereby affecting biofilm growth. Initial porosity was similar for coupons packed with beads of different sizes (40 to 41%). After biofilm growth, the steady state pore space occupied by biofilm was 24%, 57%, and 87%, respectively. In addition, the ratio of acetate to consumed lactate differed between the particle sizes, and results suggested that pore space limitation impacted metabolism (i.e., more end-product and less biomass). Upon switching the resource ratio (e.g., electron donor and acceptor ratio), the smallest particle size now had 95% of available pore space occupied by the biofilm, and metabolic activity was not sustained (3H-leucine uptake). These results suggest that the consumption rate of resources can be limited by free porosity related to particle size and may contribute significantly to the control of sediment biofilm activity.

SESSION 6: Medical Biofilms

Chemical and biological gradients in biofilm infections

Presenter: **Philip Stewart,** Professor, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Reaction-diffusion models were applied to gain insight into aspects of biofilm infection and persistence by comparing mathematical simulations with experimental data from varied bacterial biofilms. These comparisons, including three in vitro systems and two clinical investigations of specimens examined *ex vivo*, underscored the central importance of concentration gradients of metabolic substrates and the resulting physiological heterogeneity of the microorganisms. Relatively simple 1D and 2D models captured the: 1) incomplete penetration of oxygen into a *Pseudomonas aeruginosa* biofilm under conditions of exposure to ambient air and also pure oxygen; 2) localization of anabolic activity around the periphery of *P. aeruginosa* cell clusters formed in a flow cell and attribution of this pattern to iron limitation; 3) experimentally determined distribution of specific growth rates measured in *P. aeruginosa* cells within sputum from cystic fibrosis patients; and 4) very low specific growth rates, as small as 0.025 h^{-1}, in the interior of cell clusters within a *Klebsiella pneumoniae* biofilm in a complex 2D domain of variable cell density.

Interactions of human neutrophils with nascent *Staphylococcus aureus* biofilm

Presenter: **Niranjan Ghimire**[1,2], Research Fellow, Wake Forest Institute of Regenerative Medicine
Co-authors: Kyler Pallister[3], Jovanka Voyich[3], and Philip S. Stewart[2]
Affiliation: [1]Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA.
[2]Center for Biofilm Engineering,
[3]Department of Microbiology & Immunology, Montana State University, Bozeman, MT, USA.

The ability of human neutrophils to clear newly attached *S. aureus* bacteria from a serum-coated glass surface was examined *in vitro* using time-lapse confocal scanning laser microscopy. The objective of this work was to determine parameters important in the ability of host defenses to eradicate contaminating microorganisms from a biomaterial surface and thereby prevent establishment of a biofilm-based infection. Quantitative image analysis was used to measure the temporal change in bacterial biomass, mean neutrophil speed, and fraction of the surface area policed by neutrophils. In control experiments in which the surface was inoculated with bacteria but no neutrophils were added, prolific bacterial growth was observed (mean specific growth rate 0.67 ± 0.13 h^{-1}). Neutrophils were able to control bacterial growth but only consistently when the neutrophil:bacteria number ratio exceeded approximately one. When pre-attached bacteria were given a head start and allowed to grow for 2.5 h prior to neutrophil addition, neutrophils were unable to maintain control of the nascent biofilm. In the head start experiments, aggregates of bacterial biofilm with areas of 50 μm^2 or larger formed and the growth of such aggregates continued even when multiple neutrophils attacked a cluster. These results show that the ratio of the surface concentrations of neutrophils and bacteria and also the time required to recruit neutrophils to the surface are critical parameters affecting the potential of host defenses to prevent biofilm-based infection. Other factors identified as important in this interaction include the chemistry of the material surface and oxygen tension.

Do biofilms play a role in Lyme disease?

Presenter: **Garth James**, Associate Research Professor, Chemical & Biological Engineering
Co-authors: Brian Parrett, Laura Boegli, Steve Fisher, Kelly Kirker, and Elinor Pulcini
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Lyme disease is caused by bacteria of the genus, *Borrelia*, which are transferred to humans by ticks of the genus *Ixodes* (black-legged tick). In the USA, approximately 30,000 cases of Lyme disease are

reported to the CDC each year, concentrated in the Northeast and upper Midwest. Early symptoms include fever, chills, headache, fatigue, muscle and joint aches, and swollen lymph nodes. In many cases an erythema migrans (EM) rash develops at the site of the tick bite and the rash can have a "bullseye" appearance. Later symptoms of the disease include severe headaches and neck stiffness, additional EM rashes on other areas of the body, arthritis, facial palsy, pain in tendons, muscles, joints, and bones, heart palpitations or an irregular heartbeat, dizziness, shortness of breath, inflammation of the brain and spinal cord, nerve pain, shooting pains, numbness, or tingling in the hands or feet, and short-term memory problems. The various symptoms of Lyme disease are similar to other diseases, complicating diagnosis. Lyme disease can also be complicated by co-infection with other pathogens transmitted by ticks. In the USA, Lyme disease is caused by the species, *Borrelia burgdorferi* and the newly discovered species, *B. mayonii*. These bacteria are spirochetes, but *B. burgdorferi* can assume other morphological forms such as cysts. Small mammals and birds are considered the primary reservoirs of *B. burgdorferi*, which does not naturally live outside of a host. It is one of the few bacteria known to have a linear chromosome, along with numerous linear and circular plasmids. It is fastidious but can be grown in laboratory cultures. Most cases of Lyme disease can be successfully treated with early antibiotic therapy. However, in some cases symptoms may persist or recur following antibiotic treatment, which is termed Post-treatment Lyme Disease Syndrome. There is debate whether this is due to a persistent infection or residual damage from the original infection. *B. burgdorferi* has been shown to form aggregates and biofilms in vitro (1). A recent study, demonstrated the presence of *Borrelia* aggregates and associated alginate in biopsy specimens from borrelial lymphocytomas (2). Atypical forms of Borrelia have also been found in brain tissue samples from subjects with Lyme neuroborreliosis (3). These findings suggest that biofilms may play a role in Lyme disease, although further research will be necessary to confirm and elucidate this role.

1. Sapi E, Bastian SL, Mpoy CM, Scott S, Rattelle A, et al. (2012) Characterization of Biofilm Formation by *Borrelia burgdorferi* In Vitro. *PLoS ONE* 7(10): e48277.

2. Sapi E., Balasubramanian K., Poruri A., Maghsoudlou J. S., Socarras K. M., Timmaraju A. V., et al. . (2016). Evidence of in vivo existence of Borrelia biofilm in *Borrelial lymphocytomas*. *Eur. J. Microbiol. Immunol*. 6, 9–24.

3. Miklossy J, Kasas S, Zurn AD, McCall S, Yu S, McGeer PL. Persisting atypical and cystic forms of *Borrelia burgdorferi* and local inflammation in *Lyme neuroborreliosis*. *J Neuroinflammation*. 2008;5:40.

Detection and treatment of bacterial biofilms in chronic wounds

Presenter: **Gregory Schultz**, Professor
Co-authors: Gojiro Nakagami, Daniel Gibson, Qingping Yang,
Affiliation: Institute for Wound Research, University of Florida, FL, USA.

Bacterial biofilms are known to play key roles in stimulating the chronic inflammation and infection that produces multiple pathological conditions including periodontal disease, chronic sinusitis, osteomyelitis, and catheter associated infections. In 2006, the first report of biofilm structures in chronic wounds was published by James et al, which led to multiple reports of bacterial biofilms in chronic skin wounds from labs and clinics around the world. One obstacle that has limited the ability of clinicians to effectively treat chronic wounds with biofilms is the lack of a rapid, point-of-care (POC) detector that can quickly identify which chronic wounds contain biofilms and where biofilms are located in the bed of chronic wounds. We have developed a rapid, inexpensive, and simple POC device that can detect and localize biofilms on chronic wound beds. This "biofilm wound map" technology uses a positively charged nylon membrane that is typically used for binding DNA, RNA and proteins following polyacrylamide gel electrophoresis (Western or Southern blots) that is pressed onto a wound bed for about a minute to bind components that are typically present in the exopolymeric matrix (EPM) of biofilms like acidic polysaccharides or free bacterial DNA. The membrane is the briefly submerged in a cationic dye solution to 'stain' the molecules bound to the membrane then briefly washed in dilute

alcohol or PBS to remove unbound dye molecules. The retained dye molecules reveal the topographical location of biofilm EPM. This technique was used to detect biofilm EPM in wound beds of 50 chronic wound patients before and after debridement at the University of Tokyo (Nakagami et al., Wound Repair Regen, 2017). Following application of standard wound dressings the patients' wounds were examined the next week and the area of residual biofilm on the wound bed as indicated by the area of staining predicted the area and amount of wound slough covering the wound bed. We also recently reported (Yang et al., International Wound J, 2017) that daily application and wiping for three days with a concentrated pluronic surfactant gel, Plurogel™, was able to totally kill and remove mature Pseudomonas aeruginosa biofilm grown on pig skin explants. Plurogel also enhanced the ability of two topical antibiotics to totally kill mature Acinetobacter baumannii biofilm grown on pig skin explants after three daily applications. Plurogel also was able to prevent formation of biofilms by planktonic bacteria of three major pathogenic species. These results indicate new options for detecting and treating mature, highly tolerant biofilms in chronic skin wounds and preventing the formation of biofilms by key pathogenic bacterial species in skin wounds.

Perspectives, controversies, and research needs regarding perioperative antimicrobial prophylaxis for implantable medical devices

Presenters: **Roger Wixtrom**, President
Affiliation: LSCI, Springfield, VA, USA.

Biofilm Infections have been clearly demonstrated as short- and long-term issues for a multitude of implantable medical devices, resulting in significant morbidity and additional healthcare expenditures. A wide range of perioperative antimicrobial prophylaxis measures have been implemented over the years with varying degrees of effectiveness. Some of these measures were first implemented more than 15 to 20 years ago, in many cases based on low levels of evidence, and are long overdue for a critical reappraisal, particularly in light of recent studies questioning the effectiveness, and perhaps even safety of some approaches under certain circumstances. Breast reconstruction with implants – or with tissue expanders immediately followed by implants – represents the principal method of postmastectomy reconstruction in most countries worldwide. Current breast reconstruction practice, together with examples drawn from other long-term implantable medical devices, will be used to identify current issues and the critical need for updating our current scientific understanding and future approaches.

Advancing biofilm removal via surgical wound lavage–A collaborative development

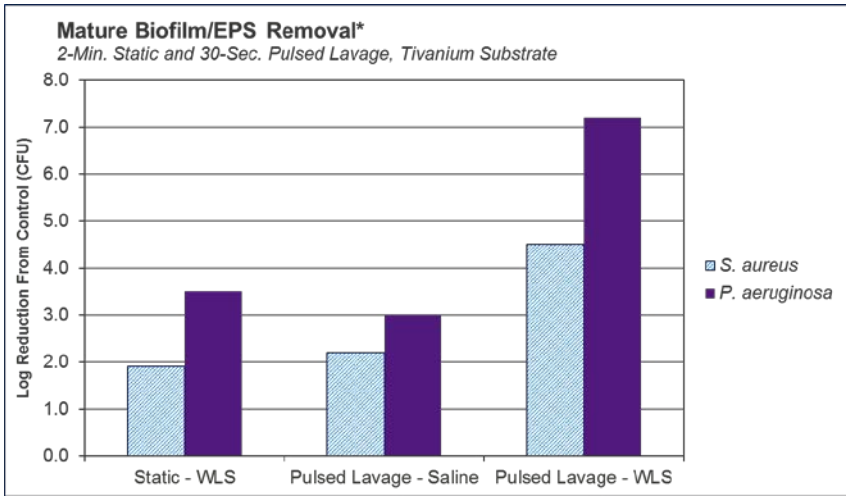
Presenters: **Matthew F. Myntti**, VP of Research and Development, Next Science, LLC
Christopher Hosler, Associate Brand Director
Affiliation: Next Science, LLC, Jacksonville, FL, USA.
Zimmer Biomet Surgical, Dover, OH, USA.

Wound contaminants can lead to surgical site infections. In orthopedics, out of the 2.4 million primary procedures performed in the EU alone, it is estimated that approximately 2% will develop an infection. These infections are particularly devastating in implant procedures, where a biofilm can form on the hardware that is untreatable by current anti-infection technologies. Treating these infections necessitates the removal of colonized hardware and treatment of the infection, often followed by a second surgery to implant new hardware. In a California study, the median cost of an infection in a total knee arthroplasty was found to be over $160,000. As such, there is a definite need for a proactive means of preventing surgical site infections for orthopedic and general surgery use.

In an effort to prevent primary procedure infections and treat revision surgeries, Zimmer Biomet and Next Science collaborated [with help from the Montana State University Center for Biofilm Engineering (CBE)] in the development of a lavage product to remove debris and biofilm within a surgical wound prior to closure. This development occurred in a defined process, whereby Zimmer Biomet and Next Science defined the product requirements and then performed Design of Experiment (DOE) trials to down-select from formulation ingredients and properties that fall within the Next Science intellectual

property. This down-selection started with the evaluation of planktonic bacteria efficacy, followed by Biofilm DOE testing in the CBE Drip Flow Model.

After the optimal formulation was created, additional design requirements were closed-out through functional evaluations, compatibility analysis for implant applications, animal testing for safety and toxicity, and additional biofilm testing at the CBE on specific implant substrates and against other products used in this space. The final formulation created, Bactisure™ Wound Lavage, is a lavage product which has broad-spectrum efficacy yielding over 4-log of biofilm removal in only 30 seconds of lavage treatment. Bactisure Wound Lavage was commercially launched by Zimmer Biomet in February 2017.



Poster Abstracts

Academic Posters (non-CBE)

Title: **Uropathogenic *E. coli* regulators mediate biofilm formation in response to oxygen**
Date: 05/2017
Authors: **Allison R. Eberly**[1], Kyle A. Floyd[1,2], Spencer Colling[1], Ellisa W. Zhang[1], Maria Hadjifrangiskou[1]
Affiliation: [1]Department of Pathology, Microbiology & Immunology,
[2]Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, TN, USA.

Urinary tract infections (UTIs) account for the vast majority of antibiotic prescriptions in the United States. The primary causative agent of UTIs is uropathogenic *Escherichia coli* (UPEC), a Gram-negative facultative anaerobe that can form biofilms on catheter material, urinary tract tissues, and within bladder epithelial cells. Adhesive pili assembled via the chaperone usher pathway, such as type 1 pili (*fim*), mediate UPEC adherence to diverse niches including the human urothelium. Previous studies indicated that *fim*-expressing UPEC subpopulations localize to the most oxygenated regions, suggesting the presence of an oxygen-responsive mechanism that regulates piliation and contributes to biofilm heterogeneity in UPEC. To study this further, we investigated biofilm formation by UPEC in different oxygen concentrations and observed that reduction in oxygen concentration from atmospheric (21% O_2) to anoxic (0% O_2) conditions coincided with gradual reduction in Fim-dependent biofilm formation. Under the conditions tested, addition of alternative terminal electron acceptors typically used by *E. coli* was not sufficient in restoring *fim* production and biofilm formation. To identify the oxygen-responsive regulator(s) of *fim* gene expression, we created a saturated transposon library and screened for mutants that became insensitive to changes in oxygen concentration. Identified mutants were validated using *fim* gene and protein expression analyses. In parallel studies, we identified the fumarate/nitrate-reductase regulator (FNR) as a regulator of pili and flagellar expression under aerobic conditions. We found that deletion of FNR restored *fim* gene transcription and Fim subunit abundance under anoxic conditions, suggesting that FNR represses the *fim* locus. However, similar defects were observed under aerobic conditions, suggesting that FNR exerts a regulatory function in the presence of oxygen.

Title: **Two-step approach for cleaning and disinfection of *Bacillus cereus* biofilm**
Date: 07/2017
Authors: **Amanda Deal,** Dan Klein, Rebecca Hostettler, and Paul Lopolito
Presenters: Amanda Deal & Rebecca Hostettler
Affiliation: STERIS Corporation, Saint Louis, MO, USA.

Methodology has been evolving for the testing of disinfectants against bacterial single-species biofilms, as the difficulty of biofilm remediation continues to gain much needed attention. Bacterial single-species biofilm contamination presents a real risk to GMP regulated industries. However, mixed-species biofilms and biofilms containing bacterial spores remain an even greater challenge for cleaning and disinfection. Among spore-forming microorganisms frequently encountered in pharmaceutical manufacturing areas, the spores of *Bacillus cereus* are often determined to be the hardest to disinfect and eradicate. One of the reasons for the low degree of susceptibility to disinfection is the ability of these spores to be encapsulated within an exopolysaccharide biofilm matrix. In a series of experiments, we evaluated the disinfectant susceptibility of *B. cereus* biofilms relative to disassociated *B. cereus* spores and biofilm from a non-spore-forming species. Further, we assessed the impact that pre-cleaning has on increasing that susceptibility.

Title: **Modelling mono-species biofilms for environmental efficacy testing**
Date: 06/2017
Authors: **Fergus Watson**
Affiliation: Biological Science, University of Southampton, UK.
Sponsored by: Bill Keevil and Sandra Wilks (University of Southampton), and John Chewins (Bioquell UK).

A pilot study was devised to investigate a novel approach to efficacy testing of antimicrobial cleaning agents; focusing primarily of vaporised hydrogen peroxide (HPV). Contaminated surfaces are recognised modes of pathogen transmission within healthcare facilities (HC); and increase risk of pathogen acquisition in newly admitted patients - particularly if the prior room occupant was a known harbourer. Studies have shown these pathogens can survive on surfaces for an extended period of time despite routine cleaning (e.g. >5months for *Acinetorbactor sp.*). This resilience is characteristic of biofilms – a resistant community of microbes. Recent publications have revealed biofilms are present in clinical settings, such as intensive care units. In contrary to this, the efficacy of many cleaning agents is tested using planktonic (less resistant) organisms deposited on a surface. Our study used a drip flow reactor (DFR) to contrive a biofilm model that is more representative of the challenge faced in a HC arena. The DFR's unique liquid-air interface and shear forces – experiences relatable to those in a hospital – generated mono-species biofilms with a population exceeding that of a hospital microbiome. Additionally, epifluorescence (EF) microscopy using Live/Dead staining revealed an abundance of micro colonies in a heterogeneous matrix of extracellular substances. Micrographs highlighted varying structural arrangements indicative of the attachment and dispersal stage of a biofilm life cycle. Upon exposure to HPV (≈10gm^{-3}) complete inactivation was achieved in as little as 80 minutes accompanied by mass disruption of the colony formations and the protective extracellular substances. These pathogenic reservoirs previously demonstrated through environmental samples could be linked to the difficulties seen by clinicians tackling infection rates. Moreover, why these orthodox cleaning agents proven against planktonic organisms fail to achieve the desired results.

Center for Biofilm Engineering Posters

CBE Poster #694

Title: **Effect of coal particle size on microbial methanogenesis in the presence of oxygen**
Date: 07/2017
Authors: **Rita Park,** Margaux Meslé, Adrienne Phillips, and Matthew Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: The Undergraduate Scholars Program at MSU, the Center for Biofilm Engineering, and the US Department of Energy

This project is a sub-experiment within the Department of Energy (DOE)-funded Microbial Enhanced Coal Bed Methane (MECBM) project, which studies shallow coal beds of the Powder River Basin (PRB) located in southwestern Montana. Through this experiment I observed the effects of coal particle size on: methane production yield, microbial diversity, dissolved oxygen (DO) concentration in the coal bed methane (CBM) water, and bioavailability of organics from the coal. Cultures containing coal and CBM formation water from the PRB coal beds were set up (in triplicate) in 100 mL glass bottles sealed with a rubber septum to allow for regular sampling. Coal of two particle sizes was obtained with sieves: small ($0.075 < Ø < 0.85$ mm) and large ($18.3 < Ø < 28.3$ mm). These cultures were inoculated with a methane producing inoculum from a PRB coal bed. Control conditions included cultures with CBM water de-oxygenated through a gas retention membrane (Stephen et al., 2014), as well as non-inoculated bottles. Techniques including gas chromatography (GC), DNA extraction, PCR amplification, cell counts and gas chromatography coupled with mass spectrometry (GC-MS) to identify the intermediary products leading to methane formation, were used. Based on preliminary and unpublished data, we know that

coal acts as an oxygen-scavenger in the CBM water, allowing for a natural degassing of the water over time, and the potential generation of anaerobic conditions that would be favorable to methanogenesis. We hypothesized that more methane would be produced from cultures containing small-size coal due to lower amounts of oxygen dissolved in the CBM water, and potentially higher access to the organic resource of the coal. It is important to discover the conditions that facilitate methane production in subsurface coal as methane could one day be utilized as the primary source of natural gas in many homes.

CBE Poster #695

Title: **Urease transport and distribution to better understand its subsurface behavior**
Date: 04/2017
Authors: **Zach Frieling,** Arda Akyel, Robin Geralch, Adie Phillips
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: The US Department of Energy, the State of Montana, and the Undergraduate Scholars Program

Biomineralization is a very promising technology being developed and researched today. Biomineralization is the formation of a precipitate, namely calcium carbonate, from a sufficiently saturated solution that can be used to remediate toxic metals, plug very small pores, seal fissures, and many more. The application of this technology is as diverse as it is exciting, and its diversity leads to increasingly creative projects each year as new challenges arise. A major use of biomineralization has been the sealing of wellbores to prevent further leakage. There is also promise in its use to store supercritical CO2 and stop the leaking of methane from wells into the atmosphere. The bacterium *Sporosarcina pasteurii* has been studied in great depth as a source of biomineralization as it produces large amounts of the enzyme urease. This enzyme breaks down urea into ammonium and carbonate which increases the pH. If calcium is present at sufficiently high concentrations calcium carbonate starts to precipitate out of solution and biomineralization has occurred. A eukaryotic source of enzyme, jack bean meal, has also been studied, and shows promise in conjunction with the bacterial method. A major drawback of microbially induced calcium carbonate precipitation (MICP) is that *S. pasteurii* does not grow well at temperatures greater than 30°C whereas the urease is functional up to 80°C. Additional research indicates that MICP appears to create a much stronger seal than enzyme induced calcium carbonate precipitation. Based on these findings, a column study at 60°C was conducted using inactivated *S. pasteurii* as a source of urease. Ureolysis and calcium disappearance within the column appeared to occur quickly indicating that urease functioned well at this elevated temperature. These rates appeared to be greater closer to the inlet of the column, which may indicate that there was a urease concentration gradient within the column. Analysis showed that calcium carbonate was induced to form within the column creating a seal that fixed the sand in place. This technology shows promise in using MICP at elevated temperatures to form a calcium carbonate seal for use in many different applications.

CBE Poster #696

Title: **Image analysis via maximum likelihood and posterior estimation: Case study of a photochemical generated chlorine dioxide anti-biofilm agent**
Date: 07/2017
Authors: **Al Parker**[1], Lindsey Lorenz[1], Jacob Adams[2], Chuck Pettigrew[2], Kelli Buckingham-Meyer[1], Jennifer Summers[1], Andres Christen[3], Darla Goeres[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Procter & Gamble, Global Microbiology, Mason, OH, USA.
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We present a novel image analysis approach for confocal laser microscopy (CLSM). Because it is well known that quantitative outcomes from image analysis (e.g., volumes) can be biased when the image is thresholded by the user, our approach does not require the user to choose a threshold. Instead, an edge detector is applied to the light intensity data in the 3D image to find the maximum likelihood estimate (or the Bayesian maximum posterior estimate) of the surface of the biofilm. "Error bars" (e.g., a confidence or probability interval) are also provided that quantify the uncertainty in the location of the surface and of summary statistics, such as a bio-volume.

This technique was applied to efficacy data generated by a novel approach invented by Procter & Gamble for the photochemical generation of chlorine dioxide. Our lab tested the efficacy of photochemically generated ClO^2 using a modified version of the Single Tube Method (ASTM E2871) against a *Pseudomonas aeruginosa* biofilm grown in the CDC reactor (ASTM Method E2542).

In two independent experiments, biofilm bacterial abundances (CFU/cm^2) and image data (surfaces (um) and bio-volumes (um3)) were collected side-by-side at 4 time points (10, 20, 60 and 120 minutes).

Based on these data, we investigated: the repeatability of the quantitative imaging outcomes over multiple fields of view, coupons and experiments; the responsiveness of the quantitative imaging outcomes to the contact time of the antimicrobial treatment; and the relevance of the imaging outcomes with plate count data collected from the same experiments.

CBE Poster #697

Title: **Temperature-dependent kinetics of urea hydrolysis catalyzed by jack bean meal**
Date: 06/2017
Authors: **Vincent Morasko**[1,2], Marnie Feder[1], Adrienne Phillips[1], Robin Gerlach[1,2]
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Sponsored by: The US Department of Energy

Ureolysis driven mineralization has become an extensively researched field in recent years to better understand how biocement is created using various sources of urease. Microbially induced calcite precipitation from the enzyme urease has the potential to seal subsurface hydraulic fractures, manipulate subsurface flow paths to improve oil recovery, treat fractures in well bore cements, and minimize dust dispersal from various sources. For certain prospective applications higher temperatures, pressures, and extreme pH conditions may be encountered, potentially influencing the effectiveness of different urease sources. This research presented focuses on determining the urea hydrolysis and enzyme inactivation kinetics of *Canavalia ensiformis* (jack bean) in ground meal form for the temperature range 20-80°C. Different kinetic models were applied with the intention of deriving the most computationally simple but efficient model. A first order enzyme inactivation mechanism

provided the best fit among the models tested; series, series-parallel, and first order inactivation. The findings will aid in the implementation of urea-hydrolysis induced calcium carbonate precipitation technologies in higher temperature environments. Work is currently being conducted on higher pressures and a wider pH range.

CBE Poster #698

Title: **Stoichiometric network analysis of cyanobacterial acclimation to photosynthesis-associated stresses identifies heterotrophic niches**
Date: 07/2017
Authors: **Ashley E. Beck**[1,2], Hans C. Bernstein[3], Ross P. Carlson[1,4]
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Sponsored by: Pacific Northwest National Laboratory, National Science Foundation

Metabolic acclimation to photosynthesis-associated stresses was examined in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 using integrated computational and photobioreactor analyses. A genome-enabled metabolic model, complete with measured biomass composition, was analyzed using ecological resource allocation theory to predict and interpret metabolic acclimation to irradiance, O_2, and nutrient stresses. Reduced growth efficiency, shifts in photosystem utilization, changes in photorespiration strategies, and differing byproduct secretion patterns were predicted to occur along culturing stress gradients. These predictions were compared with photobioreactor physiological data and previously published transcriptomic data and found to be highly consistent with observations, providing a systems-based rationale for the culture phenotypes. The analysis also indicated that cyanobacterial stress acclimation strategies created niches for heterotrophic organisms and that heterotrophic activity could enhance cyanobacterial stress tolerance by removing inhibitory metabolic byproducts. This study provides mechanistic insight into stress acclimation strategies in photoautotrophs and establishes a framework for predicting, designing, and engineering both axenic and photoautotrophic-heterotrophic systems as a function of controllable parameters.

CBE Poster #699

Title: **Monitoring single bacterial cell growth using drop-based microfluidics**
Date: 07/2017
Authors: **Shawna Pratt**[1,2], Geoffrey Zath[1,2], Robert Schaefer[1,2], Tatsuya Akiyama[1,3], Kerry Williamson[1,3], Michael Franklin[1,3], Connie Chang[1,2]
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Sponsored by: Montana INBRE

Drop-based microfluidics is a technology by which monodisperse water-in-oil emulsions are created through the manipulation of fluids in a microfluidic device. The resulting drops act as individual, contained environments that can carry biological cargo; in the case of this study, the cargo is single *Pseudomonas aeruginosa* bacterial cells. Here, the growth of two strains of *P. aeruginosa*, wild type and a mutant hibernation promotion factor knockout strain, Δhpf, was monitored using specially developed microfluidic drop incubation technology. The Δhpf gene helps cells to successfully enter dormancy

when undergoing starvation. Here we have developed an incubation technique that utilizes a uniquely engineered microfluidic device to hold drops in a set position and prevent drop evaporation for the duration of a 24 hr growth period. This technique allows for the growth of individual cells to be monitored, meaning that insights such as the heterogeneity of cell growth are lost to bulk data. During the growth period, drops are continually imaged through confocal technology to determine changes in fluorescence output, which reflects cell growth. This technique demonstrates the ability to monitor the growth of single cells to produce growth curves for each individual cell. In this study the developed microfluidic incubation technology facilitates a deeper investigation of the demographics of growth between the two strains explored, and allows for probing the heterogeneity of bacterial populations at a single cell level.

CBE Poster #700

Title: **Minimizing the potential for environmental impacts of coal combustion residuals**

Date: 07/2017

Authors: **Abby Thane**[1], Ben Gallagher[2], Lee Spangler[3], Adrienne Phillips[1]

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Sponsored by: Montana Research and Economic Development Initiative (Montana REDI)

Due to its potential impacts on environmental and human health, waste generated by coal-fired power plants (coal combustions residuals or CCRs) has become a growing concern. In the wake of the 2008 Kingston Fossil Plant spill, the Environmental Protection Agency published a final rule placing standards on CCR storage sites. These were aimed at minimizing potential atmospheric and groundwater contamination by controlling fugitive dust emissions and infiltration. However, traditional corrective actions such as in-situ stabilization (ISS) may be impossible to implement in large CCR facilities. Microbially-induced calcite precipitation (MICP) is a potential alternative to ISS. In this process, a common soil organism *Sporosarcina pasteurii* produces the urease enzyme to promote the hydrolysis of urea. In the presence of calcium (Ca^{2+}), this reaction results in the formation of solid calcite (limestone). By utilizing low viscosity fluids that can infiltrate into the pore spaces of particulate materials, the produced calcite can form around loose particles and bind them together to create biocement. Recent laboratory work has shown that applying MICP to CCRs effectively binds the material together resulting in increased strength and decreased permeability. This demonstrates the potential to control fugitive dust emissions and infiltration in operating storage sites as well as an alternative method for site closure.

CBE Poster #701

Title: **Drop-based microfluidic applications at the single cell level**
Date: 06/2017
Authors: **Geoffrey Zath**[1], Shawna Pratt[1,2], Tatsuya Akiyama[1,3], Nick Reichart[1,4], Kerry Williamson[1,3], Michael Franklin[1,3], Roland Hatzenpichler[1,4], Connie Chang[1,2]
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Sponsored by: Montana State University Office of the Provost, DARPA

The power of drop-based microfluidics promises reduced biological assaying times and greater sample throughput than standard laboratory procedures. Here we present three microfluidic methods where microdroplets are used for the encapsulation and manipulation of singe bacterial cells. First, in collaboration with the Hatzenpichler Lab at MSU we present the sorting of single *E. coli* cells fluorescently labeled with a biorthogonal non-canonical amino acid tagging (BONCAT) method. At a rate of 100 drops per second, we were able perform a binary sort of positive and negatively labeled cells based upon a fluorescence threshold. While this is two orders of magnitude slower than a standard fluorescently activated cell sorter (FACS), the advantage of drop-based microfluidics is in the preservation of the drop and isolated environment the cell is contained in for further downstream processing. Second, in collaboration with the Franklin Lab at MSU, we used our Drop-Spot device to image single *P. aeruginosa* cells in drops over time on a confocal microscope. Our Drop-Spot device captures drops in wells and keeps them stationary for up to 24 hours. By using fluorescently tagged cells, we observed the change in fluorescence intensity over time to determine a growth curve for each drop. This allowed for finding a maximum growth rate (μ_{max}) and lag phase time for each individual drop, and therefore each initial cell, rather than a population as would be the case for standard optical density measurement methods. And finally, we present our drop merging technique which is aimed at investigating virus therapies that co-evolve with the virus itself. The Evolution Chip can split a fraction of one drop and combine it with a second drop downstream. This allows us to introduce a virus population at varying generations in a drop to another drop containing mammalian cells at high speeds (>100 Hz) and observe the resulting interactions downstream. The three methods presented here are a sample of techniques and technologies available in our microfluidics lab in the Center for Biofilm Engineering.

CBE Poster #702

Title: **Structure and diffusion of aerobic granular sludge using magnetic resonance**
Date: 07/2017
Authors: **Catherine Kirkland** [1,2], J.R. Krug [3,4], F. J. Vergeldt[4], S. L. Codd[1,5], H. Van As[4], M. K. de Kreuk[6], and J. D. Seymour[1,2]
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Sponsored by: National Science Foundation, Organization for Scientific Research (NL)

Magnetic resonance imaging (MRI) and nuclear magnetic resonance (NMR) allow non-invasive measurements describing both internal structures and transport properties of opaque, complex materials like biofilms. High-field MRI was used to image aerobic biofilm granules collected from full-scale wastewater treatment plants in the Netherlands. T1 and T2 relaxation-weighted images reveal heterogeneous internal structures that include high- and low-density regions and solid inclusions. Additionally, pulsed field gradient (PFG) NMR methods and multi-dimensional correlation and exchange experiments were used to measure diffusion and transport properties within undisturbed granules. Our results show differences in rates of water diffusion within the heterogeneous granule structure and suggest that models employing a single diffusion coefficient may be insufficient to capture the complexity of transport behavior within the granules.

CBE Poster #703

Title: **Rheological and atomic force microscopy investigation of carotenoid pigmented Antarctic heterotrophic bacteria**
Date: 06/2017
Authors: **Hanna Showers**[1,2], Christine Foreman[1,2], Recep Avci[3,4], Jennifer Brown[1]
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The Earth's biosphere contains a wide range of cold temperature environments (snow, ice, permafrost, deep oceans), yet the intricacies of the biology of organisms from these environments are not well understood. Organisms that survive these harsh conditions are actively involved in biogeochemical cycling, and important contributors to global cellular and carbon reservoirs. Psychrophiles, or cold-loving extremophilic organisms, are of particular interest because they possess adaptations to protect themselves from extremes of temperature, multiple freeze-thawing events, and desiccation, which cause severe damage to non-adapted organisms. Polar carotenoid pigments in cell membranes cause a phenotypic trait exhibited by Antarctic isolates, which is their bright colors. These pigments have been hypothesized to contribute to their survival. This project investigates the mechanical properties of the membranes of pigmented and non-pigmented organisms through rheological and atomic force microscopy, as membrane fluidity is integral to structural integrity and cellular function. A suite of laboratory techniques probed the microstructural and hydraulic properties of the membranes of these microorganisms as a function of pH, extreme temperature exposure, and applied shear stress. Bacteria isolated from glacial environments were imaged with an atomic force microscope (VEECO Multimode

V), motility techniques were determined to create deflection curves of membrane elasticity in tapping mode. Rheology was used to investigate the viscosity of bacteria after exposure to pH changes and multiple freeze thaw cycles to observe changes to the membrane's mechanical structure. Understanding the mechanism behind enhanced survivability of pigmented organisms has broad implications for our understanding of the evolution of life, and presents opportunities for biotechnological exploration.

CBE Poster #705

Title: **Growth and applications of Antarctic biosurfactants**
Date: 07/2017
Authors: **MiKalley Williams**[1,2], Markus Dieser[1], Christine Foreman[2]
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Sponsored by: Montana Space Grant Consortium

Biosurfactants are amphiphilic compounds produced by microbes that disperse media, biofilms and hydrocarbons by reducing interfacial tension. These compounds can be grown from a multitude of different organisms, and so can be easily optimized to maximize functionality in different environments. Bacteria isolated from polar regions that are capable of producing biosurfactants therefore have potential as bioremediation tools or biofilm growth inhibitors in extreme environments. The focus of this project was the growth and interrogation of microbes previously isolated by the Foreman Research Group from a supraglacial stream on the Cotton Glacier, Antarctica. Two *Janthinobacteria sp*. isolates were selected for their ability to produce biosurfactants in the presence of canola oil, as was revealed by the production of high E24 emulsion stability values and confirmed by haemolytic activity tests. The produced biosurfactants were chemically extracted from the cell culture and dissolved in various solvents. The biofilm inhibiting capabilities and hydrocarbon degradation capacity of these microbial dissolved extracts were then quantified to determine their efficacy in bioremediation applications.

CBE Poster #706

Title: **Spatial control of biofilms using 3D SLA hydrogel printing technology**
Date: 07/2017
Authors: **Reha Abbasi**, James Wilking, Aaron Benjamin, Madison Owens
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Biofilms typically contain multiple microbial species that interact with one another in beneficial and detrimental ways. The spatial locations of these microbes determine nutrient, waste and signaling molecule concentrations, which in turn govern gene expression, metabolic rates and cell function. Understanding the impact of spatial location on cell function and biofilm physiology is critical for developing biofilm removal strategies as well as optimizing biofilm properties for engineering applications. However, methods for structuring biofilms are lacking. To address this need, we have developed stereolithography-based 3D printing techniques for structuring microbe-loaded hydrogels into "biofilms" with well-defined structures and properties. Our hope is that these methods will enable biofilm researchers to explore the complex structure-function relationships that exist in microbial biofilms.

CBE Poster #707

Title: **Oxygen profile characterization in biofilm systems using 19F nuclear magnetic resonance oximetry**
Date: 07/2017
Authors: **Jeffrey W. Simkins**[1,2], Joseph D. Seymour[1,2], Kayla E. Keepseagle[1,2], Philip S. Stewart[1,2]
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Sponsored by: National Institutes of Health Award Number R01GM109452

^{19}F magnetic resonance has become a popular method in the medical field for quantifying oxygenation in blood, tissues, and tumors. The technique, called ^{19}F NMR oximetry, exploits the strong affinity of molecular oxygen for liquid fluorocarbon phases, and the resulting strong linear dependence of ^{19}F spin-lattice relaxation rate R_1 on local oxygen concentration. The success of ^{19}F NMR oximetry in clinical contexts naturally introduces the possibility of repurposing this method to measure oxygen in different systems. Bacterial biofilms, aggregates of bacteria encased in a self-secreted matrix of metabolic products, are ubiquitous in industrial and clinical settings and, in both cases, oxygen gradients are a critical parameter in biofilm behavior. However, measurement of oxygen distributions in biofilms is often cumbersome and in some cases intractable due to limitations of traditional methods (e.g. microelectrode). In the present work, we develop methodology for quantifying oxygen distributions using ^{19}F NMR oximetry in different biofilm systems, focusing primarily on planar *Staphylococcus aureus* biofilms grown on agarose gel, a model of a chronic wound infection, and biofilms grown on packed bed columns, of which the porous geometry models, for instance, wastewater and soil biofilms. We explore the applicability of different fluorocarbons for different biofilm systems, based on properties such as signal density, fractional sensitivity to oxygen, emulsion stability, and volatility.

CBE Poster #708

Title: **Scale-up of microbially enhanced coalbed methane stimulation techniques using a column flow reactor**
Date: 09/2016
Authors: **Katherine Davis**[1,2], R. Hiebert[1,6], R. Hyatt[6], A.B. Cunningham[1,3], E.P. Barnhart[1,4,5], R. Gerlach[1,2], and M.W. Fields [1,4]
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Sponsored by: Department of Energy, Montana Board of Research & Commercialization Technology

It is difficult to access the subsurface and expensive to run experiments there, making subsurface environments inherently difficult to study *in situ.* Therefore, it is necessary to mimic these environments in laboratory microcosm studies to increase understanding of the abiotic and biological processes, pathways, and kinetics. Benchtop batch reactor studies are good surrogates for investigating specific processes, pathways, and engineering strategies under controlled conditions. Though batch systems can provide a lot of insights into the subsurface conditions, comparability to the *in situ* condition can be restricted due to limited substrate or accumulation of byproducts that may influence reactions or metabolisms. Using flow reactors to mimic subsurface conditions can overcome some of

the batch limitations and allow for greater understanding of *in situ* conditions, especially when developing techniques for subsurface engineering.

We have developed an upflow column reactor to mimic subsurface *in situ* flow conditions. This system is designed to maintain an anaerobic environment for applications in upscaling microbially enhanced coalbed methane (CBM) strategies. The coal packed column creates a surrogate for the subsurface coal seam where CBM producing microbial processes take place. A gas trap is in place to capture all gases produced for quantification and analysis. Each iteration of the system has been tested with a monoculture of *Methanosarcina acetivorans* to ensure anaerobicity and to collect and quantify produced gases, primarily methane. The system is currently being used to apply CBM enhancement strategies that have been developed in batch systems to increase the understanding of the stimulation process and improve techniques for application in a field based *in situ* trial.

These upflow reactors can be run under different flow conditions and can be easily modified for investigations of other subsurface environments, especially where gases or volatile compounds are of interest.

CBE Poster #709

Title: **Resource ratio impacts *Desulfovibrio vulgaris* reduction and response to Cr(VI)**

Date: 07/2017

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Desulfovibrio spp. are capable of heavy metal reduction and are well-studied systems for understanding metal fate and transport in anaerobic environments. *Desulfovibrio vulgaris* Hildenborough was grown under environmentally-relevant conditions (i.e., temperature and nutrient limitation) to elucidate the impacts on Cr(VI) reduction and cellular physiology. Growth at 20°C was slower than 30°C and the presence of 50 μM Cr(VI) caused extended lag times for all conditions, but once growth resumed the growth rate was similar to that without Cr(VI). Cr(VI) reduction rates were greatly diminished at 20°C for both 50 μM and 100 μM Cr(VI), particularly for the electron acceptor limited condition in which Cr(VI) reduction was much slower and the growth lag much longer (200 h). An increase in sulfate levels in the presence of Cr(VI) improved the cellular response via a shorter lag time to growth, and a sulfate permease triple mutant was more sensitive to Cr(VI) exposure under a balanced electron donor to electron acceptor condition. The results suggest that temperature and resource ratios greatly impacted the extent of Cr(VI) toxicity and subsequent cellular health via Cr(VI) influx and overall metabolic rate.

CBE Poster #710

Title: **Nutrient and temperature stress for lipid accumulation in a novel environmental green microalgae**

Date: 06/2016

Authors: **Luisa Corredor Arias**[1,2], J. Nagy[1,2], R. Gerlach[1,2], A. B. Cunningham[1,2], E. Barnhart [3], M. W. Fields[1,2]

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U.S. Department of Energy-Advancements in Sustainable Algal Production (ASAP) contract DE-EE0005993

Microalgae as sustainable feedstocks for biofuel production offer great advantages over higher plants such as efficient CO_2 sequestration/photosynthesis, fast growth rates, and wide tolerance to extreme environmental conditions. In addition, some photoautotrophs can be cultivated in low-quality water and tolerate more extreme conditions. Natural gas development in the Rocky Mountain northwest is associated with large amounts of production water that is not useful for agriculture or municipalities but could be used for the production of algal biomass and/or biofuel. Our goal was to isolate and characterize an algal population native to natural gas production water. In addition, numerous studies have been published about model microalgae under different stress conditions, but a body of knowledge is lacking regarding environmental isolates with potential for industrial applications. Nitrogen limitation has been the most commonly reported factor triggering lipid accumulation in microalgae. We have combined nitrate and temperature stresses to evaluate lipid production in an environmental isolate, from the *Chlamydomonadaceae* family, collected from high alkalinity and salinity natural gas production water (CBM-W). The microalga isolate, CBMW, was grown in buffered and unbuffered Bolds Medium with modified nitrate concentrations (0.5 mM, 2 mM and 3 mM) at different temperatures (20°C, 25°C, 30°C, 35°C).

Growth performance was evaluated by cell counts and total chlorophyll extraction. Media filtrates were used to track nitrate concentration changes and lipid production was measured using Nile Red (NR) fluorescence. Fatty acid methyl esters (FAMEs) speciation was quantified by Gas Chromatography-Mass Spectrometry (GC-MS) after biomass lyophilization and transesterification. The optimum conditions for growth and lipid accumulation were 30°C at 0.5 mM of nitrate (40% w/w of FAME per dry cell weight). Lower and higher temperatures in combination with higher nitrate concentrations had much lower biomass and lipid production. Buffered media appeared to inhibit lipid production. The results indicate that CBMW is a fast growing microalga with high tolerance to nitrogen starvation, temperature stress and potential to produce lipids for biofuel production in actual natural gas production water.

CBE Poster #711

Title: **Culturing novel thermoalkaliphilic aigarchaeota**

Date: 06/2017

Authors: **Andrew Gutknecht**[1,2], Dana Skorupa[1,2], Noelani Boise[1,2], Brent Peyton[1,2]

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Sponsored by: W.M. Keck Foundation

Yellowstone National Park holds the greatest concentration of geothermal features in the world, with a broad range of temperature and pH represented. Many studies have looked into microbial communities in acidic and circum-neutral systems, with a relatively small amount having been carried out in alkaline systems. This work focuses on the culturing and isolation of Aigarchaeota, a novel phylum of Archaea present in geothermal systems worldwide, but with no cultured representative. Two alkaline hot

springs in the Lower Geyser Basin and Heart Lake Geyser Basin of Yellowstone National Park were utilized for this study, which is a follow-up on previous work that has confirmed the presence of Aigarchaeota at these locations. Utilizing geochemical data along with metagenome and transcriptome findings, a synthetic media was developed to mimic the hot spring environments and promote archaea cultivation. Growth media was enriched with several different carbon sources, including amino acids, cellobiose, sterilized cyanobacterial mat, fatty acids, sucrose, yeast extract, and peptone. Headspace compositions ranged from anaerobic, to 1% and 10% oxygen, to fully aerobic. Several enrichment studies were run with transfers occurring on 3 to 10 day intervals with transfer inoculum volumes ranging from 2.5% to 5%. Treatment with vancomycin was carried out to inhibit growth of bacteria that may be outcompeting the oligotrophic aigarchaeota. Overall enrichment effectiveness was tracked via DNA sequencing along with the potential to use fluorescent in situ hybridization as a more efficient form of tracking cell growth.

CBE Poster #712

Title: **Transcriptional and post-transcriptional regulation of *Pseudomonas aeruginosa* Hpf, encoding the ribosome-binding hibernation promoting factor**
Date: 06/2017
Authors: **Tatsuya Akiyama**, Michael J. Franklin
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The dormant subpopulations of bacteria residing in biofilms are highly resistant to antibiotics, and can cause chronic biofilm-associated infections. Transcriptome analysis of the dormant subpopulations of *P. aeruginosa* biofilms revealed the presence of mRNA encoding the ribosomal accessory protein, hibernation promoting factor (HPF). Previously, we demonstrated that HPF is essential for ribosome maintenance, and for resuscitation of *P. aeruginosa* from starvation-induced dormancy. Therefore, HPF is likely expressed as cells enter a dormant state. To test this hypothesis, we characterized the regulatory mechanisms of *hpf* expression, using single-copy transcriptional and translational fusions to the yellow fluorescent protein. The *hpf* gene is downstream of *rpoN*, and is predicted to be part of the *rpoN* operon. The results here demonstrate that *hpf* is expressed as part of the *rpoN* operon. However, *hpf* is also expressed from its own promoter that has a putative *rpoD* or *rpoS* consensus sequence and UP element, and is located downstream of the *rpoN* gene. Transcription from the *hpf* promoter was modulated by stringent response factors. However, *hpf* expression was not eliminated in *rpoS*, dksA2, or *relA/spoT* mutants, suggesting that *hpf* expression is not completely controlled by the stringent response. To identify potential post-transcriptional regulatory mechanisms, the role of the 5' untranslated region (5'UTR) was assayed by constructing *hpf-yfp* reporters containing different 5'UTR regions, placed behind an arabinose inducible promoter. The results show that disruption of the putative hairpin loop (HPL) reduced *hpf* expression. In addition, a translational fusion including part of the *hpf* 5' coding sequence had increased expression compared to the promoter alone. The results suggested that the 5' coding region is important for *hpf* expression. Disruption of the putative HPL containing both the 5'UTR and 5' coding region increased *hpf* expression, while stabilizing this HPL reduced expression, indicating that *hpf* expression is controlled in part by a riboswitch. Finally, mutants that had reduced *hpf* expression compared to wild-type were impaired in resuscitation from dormancy. These results indicate that *hpf* expression is regulated from its own promoter at both the transcriptional and post-transcriptional levels, and that the cellular amount of HPF is important for *P. aeruginosa* survival during dormancy.

CBE Poster #713

Title: **Functional characterization of the *Pseudomonas aeruginosa* hibernation promoting factor (HPF) by site-directed mutagenesis**
Date: 06/2017
Authors: **Elizabeth L. Sandvik**[1,2], M.J. Franklin[1,2]
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Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic biofilm infections. During biofilm infections, subpopulations of the bacteria enter a dormant state, and are able to survive long-term nutrient-limitation conditions and antibiotic treatments. In previous work, we identified hibernation promoting factor (HPF) to be critical for ribosome preservation during starvation, and for resuscitation of *P. aeruginosa* from starvation. Here, we investigated the functional relationship between conserved amino acids in HPF, and the ability of *P. aeruginosa* to recover from dormancy. For these studies, we developed a microtiter plate screening assay to quantify the rate of recovery of the *P. aeruginosa* Δ*hpf* mutant following starvation. Recovery rate was then compared to the Δ*hpf* strain complemented with *hpf* containing point mutations. HPF mutations were generated by site-directed mutagenesis of highly conserved amino acids, and by random mutagenesis using error-prone PCR. The results showed that *P. aeruginosa* Δ*hpf* had a greatly increased lag time compared to the wild-type strain, following starvation in phosphate buffered saline (PBS). Complementation of the Δ*hpf* mutant with wild-type *hpf*, restored the wild-type recovery time. Surprisingly, mutations of highly-conserved residues throughout HPF had little effect on HPF function, and strains containing these mutations had recovery times that were similar to the wild-type strain. Therefore, to characterize HPF further, we used random mutagenesis and screening following starvation, to identify amino acid changes in HPF that had intermediate to complete loss of function. Sequence analysis of the random mutations with the highest loss of function found these mutations were in the two alpha-helices of the protein and likely affected protein folding. When these amino acids were replaced with alanines, the wild-type function was restored. Finally, we complemented the *P. aeruginosa* Δ*hpf* strain with HPF homologs from distantly related organisms, including *E. coli*, *S. aureus*, and *C. difficile*. All of these *hpf* homologs were able to complement the survival phenotype in *P. aeruginosa*. The results indicated that HPF is a robust protein that tolerates many amino acid changes with the exception of amino acids that affect protein folding.

CBE Poster #714

Title: **Biofuel production by microbial consortia**
Date: 07/2017
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The purpose of this project was to explore the interactions of microbial consortia. The three microorganisms studied were *Clostridia phytofermentans*, *Escherichia coli*, and *Methanosarcina barkeri. C. phytofermentans* is an anaerobic cellulose degrader which liberates glucose, *E. coli*, is a biofuel producing oxygen scavenger, and *M. barkeri* is an acetate utilizing methanogen that detoxifies byproducts. These microorganisms were first grown as monocultures before being grown as consortia planktonically. Monoculture and consortia growth were compared to determine how much the other microorganisms were able to feed on the byproducts from *C. phytofermentans*. The primary challenge for this community growth was ensuring that the anaerobic *C. phytofermentans* and *M. barkeri* had sufficient growth to complete their respective purposes in the community, but were not exposed to oxygen. Once community growth was achieved, HPLC was used to quantify the concentrations of various media components and microorganism byproducts that were present. In addition, plate counts

were performed on selective media for each organism to determine the concentration of colony forming units present. This information will then be used to try and replicate the consortia as a biofilm.

CBE Poster #715

Title: **Application of laser etching and 3D printed polymers for modeling ice vein habitats**
Date: 06/2017
Authors: **Taylor Oeschger** [1, 2], Markus Dieser [1,2], Christine Foreman[1,2]
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Sponsored by: MSU Undergraduate Scholar Program, Montana Space Grant Consortium

If microorganisms exist on Mars or other extraterrestrial bodies, it is probable that they are contained within ice or permafrost. With approximately 10 percent of the Earth's land mass covered by glacial ice, the Earth's cryosphere provides an analogue environment crucial to assessing suitable extraterrestrial niches for astrobiological signatures. Although it is well documented that microbes are metabolically active within ice and may persist there for hundreds of thousands of years, surprisingly little is known about their habitat and biogeochemical contributions. Studies have shown that bacterial cells present in ice are physical located within the veins at grain boundaries and triple junctions between ice crystals; aqueous micro-niches that may stretch tens of micrometers across. This project aims to reconstruct and mimic this ice vein system to better observe how extremophiles can survive in sub-zero conditions. Laser etching of Plexiglas using a X660 laser system was used to create a two-dimensional model of an ice vein system into which GFP labeled psychrophilic microbial isolates could be injected. The system was examined for changes in activity using Confocal Laser Scanning microscopy and a cryostage that mimics freeze thaw cycles of Antarctica. Another large scale three dimensional model, seen in Figure 1, was designed using Fusion 360 and printed using a Ultimaker 3D printer in order to assist in outreach demonstrations to explain how microorganism are able to survive in seemingly solid blocks of ice.

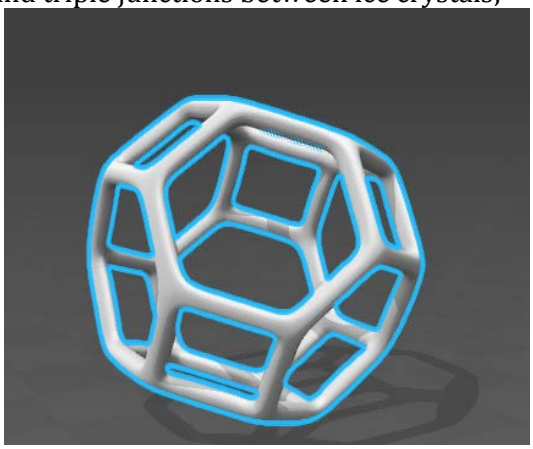

Figure 1. Computer-Aided 3D Design of Large Scale Outreach Model

CBE Poster #716

Title: **Microscopic analysis of biofilms in human tissue**
Date: 06/2017
Authors: **Garth James**[1,2], Steve Fisher[2], Laura Boegli[2], Erika Avera[2], Elinor Pulcini[1,2]
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Microscopic analysis of tissue samples, diabetic foot ulcers and intervertebral disc samples, obtained from human patients was conducted by the Medical Biofilms Laboratory. Debrided wound samples were obtained using standard practices for debridement, under IRB approval, from patients with moderate to severe diabetic foot infections. Specimens were shipped on wet ice overnight to the CBE. Upon arrival at CBE the samples were fixed in 4% paraformaldehyde and dehydrated in a solution of 30% sucrose in phosphate-buffered saline. The samples were then cryo-embedded in OCT. Frozen samples were cut into 5 mm-thick sections using a cryostat, placed on plus slides and stored at -70°C.

Sections of each specimen were stained using Sytox® green and Texas Red® labeled Wheat Germ Agglutinin. Samples were screened for biofilm using epifluorescence microscopy or confocal scanning laser microscopy (CSLM). Each specimen was semi-quantitatively characterized for the presence of biofilm with a rank of 0 equaling no observed microorganisms up to a rank of 5 equaling thick (> 10 um) continuous film of microorganisms. A ranking of 2 or higher was considered positive for biofilm. Bacterial biofilms were visualized in a majority of tissue debridement specimens.
Frozen intervertebral disc tissue samples were sent under CDC PHS PERMIT NO. 2016-05-119 to the Center for Biofilm Engineering at Montana State University on dry ice. Upon receipt at CBE, the disc tissue was defrosted and fixed using 4% paraformaldehyde, cryo-embedded in OCT, and cryo-sectioned into 7-10 µm tissue sections. The sections were placed on plus glass slides, stained with SYTO9 and imaged using CSLM. In order to determine the prevalence of *Propionibacterium acnes*, the samples were then analyzed using fluorescent in-situ hybridization (FISH). Two probes were used: a general eubacterial probe EUB338 (GCT GCC TCC CGT AGG AGT) and a *P. acnes* specific probe PAC16s598 (GCC CCA AGA TTA CAC TTC CG). Bacterial biofilms were visualized in the majority of tissue specimens. FISH probes indicated that many of the samples contained aggregates of *P. acnes.*

CBE Poster #717

Title: **Investigation into the coal carbon sources for microbial methane production from coal**
Date: 04/2017
Authors: **George Platt**[1,2], Katie Davis[1,2], Heidi Smith[1], Robin Gerlach[1,2]
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As the world begins to transition away from high-emissions fossil fuels, natural gas has become increasingly relevant. One natural gas reserve is found in subsurface coal seams, known as coalbed methane (CBM). Biogenic methane production occurs in shallow, anoxic environments where microorganisms catalyze the conversion of coal to methane through fermentation and methanogenesis. This study focuses on the upstream biogeochemical processes that promote the degradation of the coal matrix into bioavailable organic intermediates. A series of solid-liquid extractions were performed with coal from the Powder River Basin (PRB) using methanol, dichloromethane, and water in various treatments and sequences. The residual coal from the extractions was used in anaerobic bioreactors inoculated with a microbial consortium from the PRB to assess and quantify the variation in methane production via Gas Chromatography (GC). Additionally, the liquid fraction of the extracts was analyzed using Gas Chromatography – Mass Spectrometry (GC-MS) and the bioreactor contents were analyzed using Fluorescence Excitation-Emission Matrix Spectroscopy (EEMS) to assess their dissolved organic carbon signatures, respectively. While the bioreactors produced limited amounts of methane compared to previous CBM studies, the EEMS analysis showed that the bioreactor contents experienced a shift in fluorescent signature, indicating potential microbial activity and biogeochemical conversion of the coal. Dissolved inorganic and organic carbon measurements showed significant differences between treatments, indicating that the coal pre-treatments affected the abiotic and biotic processes associated with coal conversion.

CBE Poster #718

Title: **Assessment of ureolysis induced mineral precipitation material properties compared to oil and gas well cements**
Date: 06/2017
Authors: **Dicle Beser**[1,2], West C.[1,2], Cunningham, A.[1,2], Fick, D.[2], Daily, R.[1,3], Gerlach, R.[1,3], Spangler, L.[4] and Phillips, A.J.[1,2]
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Sponsored by: US Department of Energy, Schlumberger, State of Montana

Novel methods are needed to prevent or mitigate subsurface fluid leakage (stored carbon dioxide or fuels, during unconventional oil & gas resource development or nuclear waste disposal); therefore, ureolysis-induced calcium carbonate precipitation (UICP) has been investigated as a method to plug leakage pathways and fractures in the wellbore environment. The urease enzyme catalyzes the hydrolysis of urea to react with calcium and form solid calcium carbonate (similar to limestone). UICP test specimens were prepared in triplicate by filling 2.5 cm diameter x 5 cm long molds with sand and injecting both microbial and plant based enzymes into the molds. The microbial and plant based enzymes were then allowed an overnight attachment period to ensure bondage with the sand particles. Urea and calcium solutions were injected into the specimens to promote microbial growth (Urea) and precipitation (Calcium). For comparison, Class H well-, fine-, and Portland Type I cement specimens were prepared by mixing the cement paste (API 10B) then mixing with sand (ASTM C305) to produce a mortar. Compression strengths of the plant-based enzyme specimens reached 77% and 66% of the compressive strength of the 28-day well-cement and Type 1 cement mortars respectively, while reaching 83% of the 14-day fine cement strength. The results of this study indicate that the UICP produced specimens have adequate strength for various field applications.

CBE Poster #719

Title: **Engineering human gut tissues in the lab**
Date: 07/2017
Authors: **Barkan Sidar**[1,2], Andy Sebrell[3], Diane Bimczok[3], James N. Wilking[1,2]
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[2]Center for Biofilm Engineering,
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Human organoids are three-dimensional, millimeter-scale human tissues that replicate much of the structure and function of naturally formed organs. They are grown in the lab through directed differentiation of stem cells by carefully timed chemical cues and resemble organ tissues in the embryonic stage of human development. A variety of human organoids have been developed, including gastrointestinal, brain, kidney, liver, pancreas, prostate, lung, heart, and breast organoids. These tissues have a variety of potential applications in biotechnology, including drug formulation testing, regenerative medicine and microbiome research. Despite their potential applications, knowledge of how growth, material transport and mechanical properties influence organoid structure is lacking. The main goal of this project is to understand and optimize the structure of gastrointestinal organoids to improve their viability and reliability as model systems. To achieve this, we use a combination of time-lapse microscopy, image analysis and modeling to develop an understanding of organoid growth and development. Knowledge gained from this work will improve the manipulation and scaffolding techniques that are used today by organoid researchers and may provide insight into water transport mechanisms across the epithelial tissue, which are poorly understood.

CBE Poster #720

Title: **Characterizing algae growth and biomass composition under autotrophic, mixotrophic, and heterotrophic conditions**

Date: 07/2017

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Sponsored by: US Department of Energy, MSU Undergraduate Scholars Program

A great deal of interest exists in using low quality waste streams with high nutrient concentrations for the cultivation of algae. This is largely due to the potential use of waste streams to offset the high cost and energy requirements of producing traditional fertilizers, as well as the limited availability of clean water in many parts of the world. The majority of waste streams that have been considered for algae cultivation contain high concentrations of organic carbon, which has the potential to increase algal productivity if the strains selected are capable of utilizing it. Simultaneously, the ability to utilize organic carbon has the potential to offset the inorganic carbon used during algae growth. The algal research group in the Center for Biofilm Engineering at Montana State University has a library of over 100 algal cultures. During the current study the cultures in the collection were screened for their ability to grow under phototrophic (presence of light and inorganic carbon), mixotrophic (absence of inorganic carbon; presence of light and organic carbon), and heterotrophic (absence of light, presence of inorganic and organic carbon) conditions. Initially, the strains in the culture collection were evaluated for each of the conditions in 5mL volumes using screw top test tubes. From this initial screening each organism's ability to utilize organic carbon in the presence or absence of light was determined. In addition, we were able to compare growth for each strain under the different conditions. Upon completion of the initial screening, an organism was selected that showed promising growth under all three conditions for a larger scale experiment using 1.25L tube reactors. During the larger scale study, we were able to gain information about each of the organism's growth under the different conditions and changes to the chemistry of the growth medium resulting from cultivation. We determined growth rates and characterized the produced biomass with respect to lipids (GC-MS), carbohydrates (spectrophotometric assay), proteins, and elemental composition (elemental analysis).