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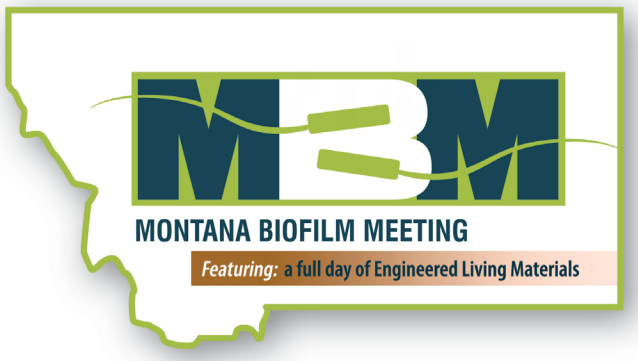

JULY 11-13, 2023

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Grishma Prabhukhot, University of Maryland, US Department of Agriculture

SESSION 1: MULTISPECIES BIOFILMS

War and peace: Polymicrobial interactions during cystic fibrosis airway disease

Presenter: **Dominique Limoli**, Assistant Professor
Co-authors: Andrea Sanchéz-Peña, Kaitlin Yarrington, Elizabeth Warren
Affiliation: Microbiology & Immunology, University of Iowa, Iowa City, IA, USA.

Interactions between microbial species have a profound influence on health and disease. Our laboratory seeks to understand the cellular mechanisms driving these interactions to modulate polymicrobial community behavior and improve patient outcomes.

To survive, bacterial cells must sense and integrate signals from their surrounding environment. While the most ubiquitous extracellular signals faced by microbes are often from other microbes, how interspecies signals are sensed, the nature of these signals, and how signaling contributes to community behaviors necessary for human infection comprise a critical yet understudied arm of bacterial physiology.

Current studies focus on the two most problematic pathogens commonly coisolated from chronic airway infections, wounds, and biofilms on indwelling devices: *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa). Our recent clinical studies reveal high densities of these organisms coinfect the airways of people with cystic fibrosis (CF) for decades, despite intensive, life-long antimicrobial therapies. Contrary to firmly held beliefs at the time, Pa does not replace Sa, instead coinfection only increases over time. Critically, we and others also report that coisolation of these organisms is associated with decreased lung function, heightened inflammatory response, and shortened lifespans.

Given this clinical insight, my group seeks to address the fundamental question of whether microbial interactions promote bacterial survival and virulence, leading to poor patient outcomes. To accomplish this, we visualize interspecies interactions in real time, using strategies we developed to live-image interspecies behaviors on a single cell-level, revealing behaviors previously obscured in prior population-level work. This project discovered Pa can sense Sa-secreted factors from a distance, subsequently increasing Pa surface-based motility towards Sa, forming multispecies communities with increased antibiotic tolerance. While Pa spends most of its life on a surface, it remains unclear how the direction of movement is controlled or how surface-associated bacteria cope with the presence of other species. Pa movement on surfaces is primarily driven by the type IV pili (TFP), hair-like projections that extend and retract to move the cell body across a surface through a motion referred to as twitching motility. Using a combination of high-throughput genetic screens, high-speed live-imaging, and site-directed mutagenesis, we determined that Pa is attracted to secreted peptides from Sa and we have mapped a suite of putative protein networks necessary for Pa to sense interspecies signals. Notably, we determined that Pa uses a designated chemoreceptor to detect interspecies peptides while traveling on surfaces and we identified several essential features of this protein for chemoreception and signal relay.

Finally, we have also determined that the second messenger signaling molecule, cyclic diguanylate (cdG) is necessary for Pa to sense interspecies signals and modulate TFP chemotaxis. We describe a signaling module composed of two diguanylate cyclases (DGC), two phosphodiesterases (PDE) enzymes, that synthesize and degrade cdG, respectively, and two putative PilZ domain containing effectors. Upon cdG binding, the PilZ domains change conformation and physically interact with TFP-chemosensory proteins to modulate directional movement towards other bacterial species.

Understanding how microbes communicate and survive during infection may also reveal a new window for treatment design – that is, we might therapeutically mimic natural strategies microbes use to compete or interfere with interspecies interactions to weaken the community.

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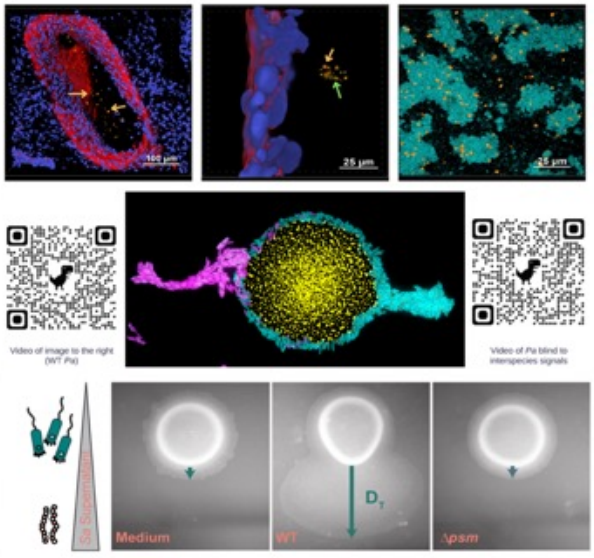

Figure 1. *Overarching research vision to identify social behaviors driving disease. (1) Next-gen. fluorescent in situ hybridization imaging of explanted CF lungs, where mixed species aggregates are observed (top row, left and middle. Pa: green, Sa: orange, airway cell nuclei: blue, mucus: red). R: Representative in vitro airway model recapitulating mixed species aggregate formation (top row, right Pa: cyan, Sa: orange). (2) Micro- and (3) macroscale live-imaging reveals Pa (magenta and cyan) biases the directionality of movement towards Sa (yellow, middle row), or a gradient of secreted Sa peptides (bottom row). QR codes and hyperlinks are available to view the video for the representative middle image and Pa mutant blind to interspecies signals (pilJ ligand binding domain mutant video).*

A biocide study for microbial control in the International Space Station wastewater recovery system

Presenter: **Liz Sandvik**, Research Engineer
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Water management and wastewater recycling is a critical aspect of life support in space exploration. The Water Processor Assembly (WPA) on the International Space Station (ISS) is the longest microgravity wastewater recovery system to date, recovering product water from crew urine distillate and the cabin humidity condensate. While the WPA has been operational for over a decade, intermittent microbial fouling has occluded flow, at times required component replacement to return the system to operation identifying a need for biofilm mitigation strategies. In this study, we screened 23 biocides in minimal biofilm inhibitory concentration (MBIC) assays against four bacterial and one fungal strain isolated from the ISS WPA in a synthetic wastewater based on WPA water chemistry. Of the biocides tested, hydrogen peroxide, silver fluoride, and iodine were selected for testing against a multidomain biofilm in CDC biofilm reactors. Daily dosing of 20ppm silver fluoride or iodine reduced biofilm growth and are promising as candidate biocides. In contrast, daily dosing of 20ppm hydrogen peroxide was subinhibitory resulting in more growth than the untreated control. Protection of hydrogen-peroxide sensitive organisms in a multi-species biofilm will be discussed.

Using a fluorescent probe staining method and COMSAT to assess cell viability in *Aspergillus niger* biofilms treated with antimicrobial agents

Presenter: **Aswathy Shailaja**[1,4], Postdoctoral Associate
Co-authors: Patrick Gerard[2], Terri F. Bruce[2], Charles A. Pettigrew[3], Julia L. Kerrigan[1]
Affiliation: [1]Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, USA.
[2]School of Mathematical and Statistical Sciences, Clemson University, Clemson, SC, USA
[3]Clemson Light Imaging Facility, College of Sciences, Clemson University, Clemson, SC, USA.
[4]Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA.

Aspergillus niger is a filamentous fungus that adheres to different substrate surfaces and forms biofilms consisting of dense hyphal networks embedded in a self-produced gelatinous matrix composed of extracellular polymeric substance (EPS). These characteristics contribute to its prevalence and resistance to antimicrobial agents. The aim of this study was to visualize and assess the efficacy of various antimicrobial agents on *A. niger* biofilms using a fluorescent probe staining method and the COMSTAT program, which we had previously developed and tested. This method allows for the visualization of live and dead cells with confocal laser scanning microscopy (CLSM). *A. niger* biofilms, modeled to reflect those that form under drip flow, were created in a controlled reactor under low-shearing force on glass coupons. The samples were treated with different concentrations of standard bleach with sodium hypochlorite, a proprietary chlorinated-alkaline disinfecting cleaner with sodium hypochlorite, clove bud essential oil containing phenyl terpenoid

and control group that was not treated. Specimens were stained with a combination of SYTO9 and propidium iodide (PI) fluorescent dyes. The cell viability of biofilms was calculated from their biomass (µm3/µm2) using COMSTAT program. The least square student's t-test was performed, and significant differences in efficacy were observed between the different treatment types and different concentrations. The results showed that proprietary chlorinated-alkaline disinfecting cleaner showed the highest antimicrobial efficiency, while clove bud essential oil negatively impacted the development of *A. niger* biofilms instead of completely killing the biofilm cells. These findings contribute to our understanding of *A. niger* biofilm resistance and provide insights into potential antimicrobial strategies.

Food fights and conflict avoidance: a systems biology analysis of contrarian *Pseudomonas aeruginosa* substrate preferences

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

Microorganisms must acquire nutrients and energy to grow. Microbial growth is a primary pathogenicity factor; therefore, understanding microbial responses to nutrient environments is central to treating problematic microorganisms. When presented with a milieu of energy sources, microorganisms prioritize the catabolism of certain substrates over others in a metabolic strategy known as carbon catabolite repression (CCR) or diauxie. Competitive prioritizing of substrates is central to microbial fitness. Opportunistic pathogen *Pseudomonas aeruginosa* uses a substrate preference strategy nearly opposite to many model organisms. This contrarian strategy is termed "reverse CCR" or reverse diauxie. An integrated systems biology study identified a potential ecological basis for the metabolic strategy based on nutrient investment into the proteome. The metabolic strategy is likely an adaptation to life in multispecies consortia where direct competition for substrates can lead to exclusion. Understanding *P. aeruginosa* substrate preference opens the possibility of modulating pathogen phenotype through niche engineering and a phenomenon known as metabolic potentiation.

PANEL DISCUSSION

Challenges & rewards of using a multispecies biofilm in the lab

Moderator: **Chris Jones**
Panelists: **Ross Carlson** (MSU, CBE), **Dominique Limoli** (University of Iowa), **Liz Sandvik**, (CBE) **Aswathy Shailaja** (Duke University Medical Center)

Biofilms are known to thrive as complex ecological communities in the environment, clinic, engineered systems, and the built environment. Yet most biofilm research is still conducted using a single, surrogate species which leads to extrapolating the interpretation of the laboratory data and perhaps provides sound justification as to why laboratory data is not always translatable. In this panel we will discuss the practical challenges associated with using multispecies in a single experiment and how researchers overcome them. We explore the insights that only become apparent when a multispecies biofilm is used, and perhaps when it is acceptable to use a single species in research.

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SESSION 2: MEASURING BIOFILM

Micro-sensor technology

Presenter: **Stephan Warnat**, Assistant Professor
Co-authors: Matthew McGlennen[1,2], Haley Ketteler[1,2], Markus Dieser[1,3], Thomas Palen[1,2], Ruby Jackson[1,2], Christine Foreman[1,3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Microbial biofilms are organized communities of surface-attached microorganisms encased in a self-produced extracellular matrix that pose significant challenges in medicine, the environment, and industry. Biofilms can cause chronic infections, biofouling, and equipment failure while existing methods for biofilm detection are slow, costly, and labor-intensive. Recently, microfabricated electrochemical biosensors have emerged as a promising technique for evaluating biofilm growth in real-time, with the advantages of small size, adaptability, low cost, and high sensitivity.

In this presentation, we first review current biosensor technologies that allow in-situ biofilm characterization and discuss the advantages and disadvantages of given technologies in industrial settings. In the second part of the presentation, we will show our recent progress in electrochemical impedance spectroscopy (EIS), a powerful technique for characterizing bulk and interfacial properties in aqueous, solid, and gaseous systems. Emphasis will be placed on implementing a microfabricated sensor platform in a continuous flow system that allows biofilm growth and mitigation studies. Case studies of sensor technology in the food and manufacturing industries will summarize the talk.

Imaging for biofilm characterization

Presenter: **Heidi J. Smith**, Manager
Affiliation: Bioimaging Facility, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Imaging offers unique insight on biofilm structure, spatial organization, and function that other bulk/destructive techniques can't provide. The ability to visualize biofilms has enhanced the understanding of both biofilm structure and function and the CBE has used imaging to study gene expression localization, structural analysis of extracellular material, community structure, fluid flow patterns, temporal diffusivity of solutes, temporal distribution of intracellular signaling molecules, and spatio-temporal patterns of biocide action. To accomplish these tasks, the CBE Bioimaging Facility utilizes its numerous imaging technologies specially configured to accommodate diverse sample types and sample thicknesses with the ability to span different spatial scales. CBE instrumentation is specifically configured to enable real-time imaging of intact biofilm samples, and to achieve this we have recently updated the facility with ~$2.5M of new state-of-the-art instrumentation. Given that the thickness of biofilms ranges from monolayers of cells to hundreds and even thousands of microns, different imaging approaches and techniques are necessary to successfully image over the entire biofilm from the surface to substratum attachment. Therefore, imaging biofilms requires a combination of distinct imaging and sample preparation approaches. This talk will provide methodological insight for imaging both thick and thin biofilms.

Investigating microbial biofilms as indicators of heavy metals in the Clark Fork Basin, Montana

Presenter: **Elliott Barnhart**[1], Research Hydrologist, Microbiologist
Co-authors: Chiachi Hwang[2,4], Nicholas J. Bouskill[3], Michelle I. Hornberger[1] and Matthew W. Fields[2,4]
Affiliations: [1]U.S. Geological Survey, Helena, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Earth Sciences Division, Lawrence Berkeley National Laboratory, USA.
[4]Department of Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA.

Hyporheic biofilms are an important component of riverine systems, whose diverse populations may offer specific measures of stream health and restoration success. The hyporheic biofilm community structure (e.g. microbial population composition and diversity) was analyzed along with trace metal concentrations in bed sediment and biota (benthic insects) across 11 sites in the Clark Fork Basin. Statistical analysis of the microbial eDNA allowed the identification of several bacteria (*Lysobacter* and *Rhodocyclaceae*) that could act as biomarkers of heavy metal contamination. The identification of these biomarkers improves our ability to indicate how changes to the microbial community in the hyporheic zone may be translated to observed perturbations at higher trophic levels.

16S Ratios Method for analysis of active microbial communities

Presenter: **Hannah Goemann**, PhD Student
Affiliation: Microbiology & Cell Biology, Montana State University, Center for Biofilm Engineering, Bozeman, MT, USA.

Determining microbial community composition and diversity is often the major goal of high-throughput next-generation sequencing projects. This technology typically involves sequencing of marker genes of DNA including the 16S rRNA gene used universally for bacteria and archaea, the large subunit (LSU) or internal transcriber region (ITS) for fungi, or various functional genes. DNA metabarcoding can be extremely informative as to the presence, absence, and relative abundance of microbial community members across samples and over time and is sensitive enough to detect rare community members that are difficult or impossible to detect via culture-based or shotgun metagenomic approaches. However, DNA-based analysis does not provide reliable information regarding the activity of microbial community members. Community dynamics are potentially obscured by the unknown contribution of dormant community members and "relic" DNA from microbial necromass. While metatranscriptomics and metabolomics can provide extremely detailed information regarding community activity, these methods are often cost-prohibitive and also require metagenomes to map particular functions to community members which can become extremely computationally intensive. Another method of determining activity is to analyze the ratios of 16S rRNA/rRNA gene sequences of community members. This method utilizes similar workflows to traditional 16S metabarcoding analyses and allows for differentiation between active and dormant community members. In this presentation we will provide a short overview of this method and its potential applications for analysis of microbial communities relevant to biofilm research.

SESSION 3: MEDICAL BIOFILMS & THE HOSPITAL ENVIRONMENT

Hydrogen peroxide vs biofilms

Presenter: **Phil Stewart**, Regents Professor[1,2]
Co-author: Mark Owkes[3]
Affiliation: [1]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Bozeman, MT, USA.
[3]Department of Mechanical Engineering, Montana State University, Bozeman, MT, USA.

Hydrogen peroxide (HP) is a common disinfectant and antiseptic. As with other antimicrobial agents, microorganisms in biofilms exhibit tolerance to killing by HP. This is known to be due in part to degradation of HP by bacterial catalases, enzymes that completely neutralize the biocidal activity of HP. It might be anticipated that in prolonged exposure of a biofilm to HP, the top layer of the biofilm would be killed allowing the HP to progressively penetrate and eventually eradicate the entire biofilm. However, using the Biofilm.jl computer model, a mechanism was demonstrated by which the

biofilm can persist during indefinite treatment with a flowing HP solution at concentrations that are lethal to planktonic microorganisms. Indeed, the model predicts that biofilms can become thicker in response to HP treatment. This surprising result is found to be dependent on the neutralization of HP by catalase-containing dead biomass, which provides protection for live biomass deeper within the biofilm (Figure 1). A practical concept for biofilm control following from this study is to treat with an HP dose exceeding a distinct threshold concentration.

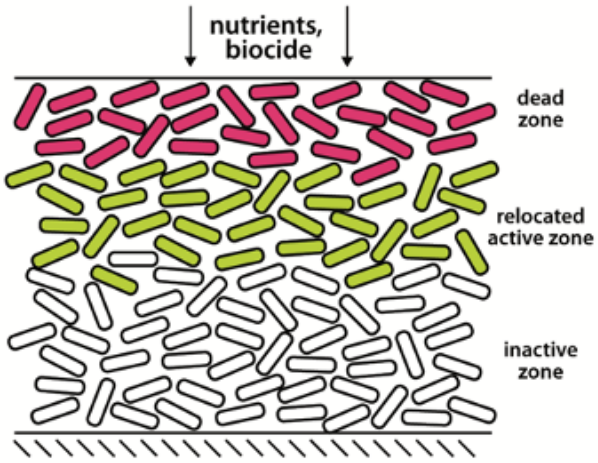


Figure 1. *Diagram of hydrogen peroxide treated biofilm showing stratification of activity. The biofilm is cartooned in cross-section, with the bulk fluid containing nutrients and biocide at the top and the attachment substratum at the bottom. The biocide kills bacterial cells at the top layer of the biofilm (red). A reaction-diffusion interaction prevents hydrogen peroxide from penetration beyond this layer. Nutrients diffuse across the dead zone to feed actively growing cells in an interior stratum (green). Growth in this layer pushes catalase-containing biomass upwards, continuously renewing the protective capacity of the biomass in the dead zone.*

Bacterial ingress through valves used for venous access

Presenter: **Garth James**, PI[1], Associate Research Professor[2]
Co-authors: Marcia Ryder[3], Elinor deLancey-Pulcini[1,2], Albert E Parker[2]
Affiliation: [1]Medical Biofilms Laboratory [2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[4]Ryder Science, Brentwood, TN, USA.

Needleless connectors (NC) were introduced in the 1990s to minimize the risk of needlestick injury and bloodborne pathogen exposure to healthcare workers. Although this addressed the occupational hazards, an unexpected increase in central-line associated bloodstream infections occurred. A wide variety of NC are currently available. Regardless of the NC type, the most important precaution is to disinfect the NC before each access. In some settings compliance can be an issue, so the use of engineering controls is warranted. Ultimately, an NC that is contaminated but allows few microorganisms into the bloodstream (ingress) is preferred.

The Medical Biofilms Laboratory at the CBE has been evaluating NC since 2006. A recent publication `presented the results of testing 20 different NC in a clinically simulated in vitro study. The connectors were attached to catheters and the septa were inoculated with *Staphylococcus aureus*. The connector-catheters sets were then subjected to a series of saline flushes, interspersed with 1%-strength brain-heart infusion broth incubations (locks), and additional daily inoculations over a 5-day period. The number of viable bacteria in the flush (colony-forming units, CFU) was monitored, and on the 4th and 5th days destructive sampling was performed to determine the number of viable biofilm bacteria attached to the intraluminal surfaces of the NC, catheter hub, and catheter tubing.

For bacteria that transferred through the NC into the flush, the NC clustered into 10 statistical groups, with overlap between groups. The least squared mean log CFU/flush ranged from 3.14 to 3.50 for the group with the least bacterial transfer and 5.05 to 5.37 for the group with the most bacterial transfer. Among the NC, hub, and catheter lumen, NC biofilm was the only statistically significant predictor of bacteria in the flush.

The NC were evaluated based on 8 design factors; mechanism of access (luer-activated or blunt cannula), access portal (split septum or surface septum), flow path (mechanical valve, internal cannula, or open), fluid displacement (neutral, negative, positive, or anti-reflux), hydrodynamics (simple or complex), seal length (including septum split length and/or

circumference of surface septum), flow path surface area, and flow path volume. The single most important design factor was flow path. When the flow path was through an internal cannula, there were fewer bacteria in the flush and biofilm bacteria in the NC compared to mechanical valve or open path designs, even after accounting for all other design factors.

Overall, the results of this study indicated that bacterial transfer and biofilm formation in the connector-catheter system varied statistically significantly between the 20 NC, suggesting that NC design can help lower the risk of developing catheter-related blood stream infections.

Electrochemical bandage

Presenter: **Haluk Beyenal**, Professor
Affiliation: The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, WA, USA.

Electrochemical biofilm control is an emerging technology where surface properties of inert electrically conductive materials are controlled by applying an electric potential or electrical current to delay or prevent cell attachment or remove existing biofilms. By precisely controlling potential in an electrochemical system, it is possible to generate biocides on the electrode surface. Such an electrochemical system can be made very small, a wearable device that can generate hydrogen peroxide or hydrochloric acid at low concentrations and can be used to control biofilms growing on surfaces. We developed an electrochemical bandage (e-bandage) that generates hydrogen peroxide or hydrochloric acid to eliminate biofilms on surfaces. e-Bandages are composed of flexible, biologically-compatible conductive carbon fabric tuned by controlling voltage to treat biofilm infections. We have constructed prototype e-bandages and demonstrated that they are active against pathogenic biofilms. Our research demonstrated that e-bandage could be a novel antibiotic-free approach to treating biofilm infections.

Metabolic niche composition affects bacteriophage replication in adherent-invasive *Escherichia coli* biofilms

Presenter: **Robert Brzozowski**, Postdoctoral Researcher
Co-authors: Patrick R Secor
Affiliation: Division of Biological Sciences, University of Montana, Missoula, MT, USA.

The gut microbiota of healthy individuals is composed of a diverse array of bacteria, viruses (phages), and other microbes. Reduced bacterial diversity and an expansion of the gut phage community is associated with a wide range of human diseases including obesity, cancer, and inflammatory bowel disease (IBD). A major factor governing gut microbiota composition is the host environment. In the healthy gut the terminal electron acceptors O_2 and NO_3 are delivered to the lumen of the small intestine by the host, allowing facultative anaerobes to respire simple sugars such as glucose to produce energy. In contrast, O2 and NO3 levels in the colon are strictly limited, which allows obligate anaerobes to flourish, promoting dietary fiber fermentation. Recent work demonstrates that individuals with disease have elevated levels of O2 and NO3 in the colon that “imbalances” the gut microbiota, allowing pathogenic facultative anaerobes such as *Escherichia coli* to colonize the colon. However, how terminal electron acceptor availability affects phage replication in enteric bacteria is poorly understood.

Here, we explore how fluctuations in terminal electron acceptor availability affects phage replication in adherent-invasive *E. coli* (AIEC) that forms biofilms in the colon that contribute to intestinal cancer and IBD. We find that mature 48h-old AIEC biofilms grown under anoxic or oxic conditions were completely resistant to phage infection. However, when AIEC biofilms were transferred from an anoxic environment to environments containing O2 or NO3, robust phage replication and lysis of the pre-formed biofilm was observed. The phage expansion phenotype was not observed under any conditions in planktonic culture, indicating a biofilm-associated phage expansion phenotype. In addition, supplementation of biofilms with acetate (a major fermentation product) exacerbated the terminal electron acceptor-induced phage expansion phenotype while supplementation with glucose prevented phage expansion, suggesting that in addition to terminal electron availability, carbon source also influences phage expansion. Taken together, these data provide valuable insights into how metabolic niches in the gut promote the expansion of phage populations.

SESSION 4: BIOFILM & SURFACES INTERACTIONS

KEYNOTE PRESENTATION

What's a surface?: Surface sensing in *Pseudomonas aeruginosa*

Presenter: **Matthew Parsek**, Professor[1]
Co-authors: Xuhui Zheng[1], Emma Gomez-Rivas[1]
Affiliation: Microbiology, University of Washington, Seattle, WA, USA.

Background

The formation of bacterial biofilms has been implicated in several different types of chronic infections. The first step for biofilm formation is bacteria sensing a surface. In the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, two main types of surface sensing mechanisms have been proposed. The Wsp system is activated by cell envelope stress and the diguanylate cyclase within the system, WspR, synthesizes cyclic diguanylate monophosphate (c-di-GMP). Meanwhile, the Pil-Chp system senses a surface through type IV pili and activates the production of cyclic adenosine monophosphate (cAMP). While both cAMP and c-di-GMP are critical second messenger molecules that control various cellular activities, how the bacterium coordinates these two signaling pathways is largely unclear. In this work, we aim to study the regulation of c-di-GMP and cAMP during surface sensing in *P. aeruginosa*.

Methods

We developed a tri-color plasmid-based reporter to simultaneously measure the levels of both c-di-GMP and cAMP in a single cell. Using the laboratory model strain PAO1 as well as isogenic mutants, we examined c-di-GMP and cAMP reporter activities in various surface sensing models.

Results

We found that the activation of c-di-GMP or cAMP depends on surface conditions. In a continuous flow cell system, we mainly observed an increase in c-di-GMP reporter activity but not cAMP during the first 4 hours of inoculation. On the contrary, on a 1.5% agarose pad, the levels of cAMP reporter activity increased but not c-di-GMP. Using a simplified model for different surfaces, we showed that the presence of liquid on the surface controlled the selective activation of c-di-GMP or cAMP signaling pathway.

Conclusion

Our findings suggest that *P. aeruginosa* senses different surfaces and responds with distinct surface behaviors. Our ongoing work is investigating the mechanisms involved in the selective activation of c-di-GMP/cAMP pathways on different surfaces.

Effects of surface finish on microbiologically influenced corrosion of copper by *Oleidesulfovibrio alaskensis* G20

Presenters: **Amit Acharjee**, PhD Candidate[1,3]; **Yagmur Keskin**, MS Student[2,3]
Affiliation: [1]Mechanical and Industrial Engineering Department, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering Department, Montana State University, Bozeman, MT, USA.
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Corrosion is a natural process which causes immense economic losses, estimated to be around 2.5 trillion USD globally, to a wide range of industries. Microbiologically influenced corrosion (MIC) is responsible for approximately 20% of the total corrosion damage and 50% of all pipeline failures. Sulfate-reducing bacteria (SRB), which are abundant in a broad range of anaerobic environmental conditions, are related to almost half of all MIC related cases. Despite having widescale applications as infrastructure materials in both marine and freshwater environments, limited information is currently available regarding SRB induced MIC of copper and copper alloys which are commonly used in water distribution systems. This work evaluates the effects of extrinsic properties of copper, such as different surface morphologies achieved through metallographic grinding or polishing, on the MIC severity caused by exposure to *Oleidesulfovibrio alaskensis* G20. 3D optical profilometry, Field Emission Scanning Electron Microscopy (FE-SEM), Energy Dispersive X-Ray

(EDX) and X-ray Diffraction Analysis (XRD) were used to investigate corrosion morphology and product development. Characterization was conducted before and after exposure in both biotic and abiotic conditions. Copper dissolution rates and impacts on planktonic growth were also determined. Corrosion rates and pit depth measurements were carried out to assess the corrosion on the different copper surface finishes. The measured corrosion rate was found to decrease with decreasing surface roughness. Corrosion product investigation after biotic exposure showed the presence of two different crystal structures (monoclinic and hexagonal) of Cu2S development. The monoclinic structure of Cu2S is thermodynamically more favorable to form while the hexagonal structure occurs when the environment becomes copper deficient. This phenomenon may offer important insights into the overall MIC related to the extracellular polymeric substance's ability to bind copper ions. Results from this work represent a novel and inexpensive approach to reduce MIC by taking advantage of material surface properties which can be controlled during the manufacturing process.

Mixed domain biofilm responses to coated surfaces

Presenter: **Madelyn Mettler**, PhD Candidate[1]
Co-authors: Brent Peyton[1], Erika Espinosa-Ortiz[1]
Affiliation: [1]Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Undesired biofilm growth and biomass accumulation has been observed in the International Space Station (ISS) water recycle system (WPA), resulting in necessary part replacement and system redesign. To improve astronaut safety and spaceflight sustainability, undesired biofilm growth in spacecraft water recycle systems must be reduced. Material coatings offer one approach to mitigate biofilms in these systems. Combined with additional mitigation strategies like biocides, coatings could greatly reduce biofilm accumulation, resulting in easier water treatment and improved sustainability. This project aims to investigate the effects of different coatings against multi-domain biofilm formation comprised of five organisms commonly isolated from the WPA: four Gram-negative bacteria including *Ralstonia insidiosa*, *Burkholderia contaminans*, *Methylobacterium organophilum*, and *Cupriavidus metallidurans*, and a filamentous fungus, *Lecythophora mutabilis*.

Biofilms were grown in a CDC bioreactor with ersatz-a medium designed to mimic the chemical composition and nutrient availability within the WPA during a potential biofilm clogging event. While experiments occur at normal gravity, the organisms, medium, and materials used closely matched those found in situ on the ISS. Coatings are evaluated on Teflon and Inconel (a nickel-chromium alloy), two materials found in the ISS water system. The system was run for 8 days at room temperature, and pH of 5.55. Uncoated materials were also tested. After 7 days of incubation in continuous flow, the biofilms on uncoated coupons reached a density of ~8.5 log10CFU/cm^2, largely dominated by *R. insidiosa*. Confocal and scanning electron microscopy reveal that the fungus not only contributes much more biomass than its cell density suggests, but that it also acts as a scaffold for bacterial cell attachment. While the coatings tested resulted in similar total cell density at the end of the experiments compared to the uncoated controls, the species distribution varies between coatings.

This presentation will consider the dynamics of a multi-domain biofilm, the application of coatings for biofilm accumulation reduction, and the potential unintended consequences of biofilm control strategies.

SESSION 5: ENGINEERED LIVING MATERIALS

Programming bacteria to grow into macroscopic, tailored materials

Presenter: **Caroline Ajo-Franklin**, Professor[1,2]
Co-authors: Sara Molinari[2], Robert F. Tesoriero, Jr.[2], Esther Jimenez[2]
Affiliation: [1]Department of Biosciences, [2]Rice University, Houston, TX, USA.

We currently face rising global temperatures, dwindling natural resources, and increased needs for human and ecological health. To help meet these challenges, my research group engineers biomolecules and microorganisms for real-time bioelectronic sensing and for sustainable synthesis of materials. We are inspired by how proteins and microorganisms transfer electrons to materials in their environment and how they assemble advanced materials using low energy

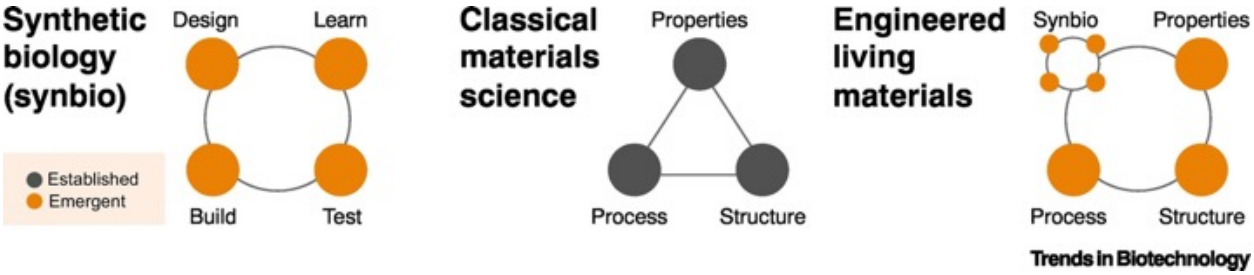
syntheses. We leverage these naturally-occurring pathways to engineer microorganisms with tailored abilities to report on sensing via electron transfer and to assemble macroscopic living materials.

In my talk, I will describe how we have programmed bacteria to grow into macroscopic materials with tunable mechanical properties. We engineered *Caulobacter crescentus* to display and secrete an engineered self-interacting protein. This protein forms a matrix and assembles cells into hierarchically-ordered, centimeter-scale living materials. Changing the composition of this protein changes the mechanical properties of the material. Together, this work provides a new platform for growing macroscopic materials with simultaneous control over the materials and biological properties and a route towards sustainable plastics and rubbers.

Engineered living building materials: From concept to commercialization

Presenter: **Wil V. Srubar, III**, Associate Professor
Affiliation: Materials Science and Engineering Program, Department of Civil & Architectural Engineering, University of Colorado, Boulder, CO, USA.

Recent advances in biological engineering have enabled autonomous, high-fidelity biomanufacturing of useful chemical, mineral, and polymer building blocks that can be leveraged in the hierarchical design and fabrication of living architecture at the human scale. This presentation will discuss the integration of synthetic biology, microbiology, materials science, and structural engineering to design, build, and test engineered living building materials (LBMs). LBMs are an emergent class of structural materials that leverage the biomineralization capability of microorganisms within sand-hydrogel scaffolds to produce living, load-bearing structures. In this presentation, LBMs will be discussed in terms of their conception, bench-scale demonstrations, and commercial deployment. In addition, this presentation will propose a taxonomy of engineered living materials (ELM) research and use the categorization to discuss current trends and state-of-the-art advancements, significant opportunities, and imminent challenges for synthetic biologists and materials scientists that must be overcome to create never-before-imagined material solutions for critical societal problems in energy, water, and the built environment.



3D printing of engineered bacteria for the production of biofilms-on-a-chip

Presenter: **Anne S. Meyer**, Associate Professor
Affiliation: Biology, University of Rochester, Rochester, NY, USA.

In order to create crisp, defined patterns of biologically-created materials, new technologies need to be developed and implemented. The Meyer lab is developing first-of-their-kind DIY bacterial 3D printers that can deposit engineered bacteria in specific three-dimensional patterns using simple devices and chemistries. Our affordable, easy-to-build bacterial 3D printers have fully automated, coordinated control of the pumps and printhead, allowing for high spatial resolution (<mm-scale) printing of bacteria onto wet or dry surfaces. Our printers mix an alginate-containing bacterial culture with a calcium chloride slurry upon printing, triggering cross-linking of the alginate molecules to form a stable, biocompatible scaffold to support the bacteria. After printing, the bacteria survive for several weeks and can be induced to produce a variety of biomaterials. We are applying our printer to the fabrication of engineered biofilms, groups of bacteria that live within a spatially structured polymer matrix. Our 3D printer can deposit engineered *E. coli* that are able to produce CsgA fibrils, or curli fibrils, the major protein component of many biofilm polymer matrices. These 3D-printed, engineered bacteria create free-standing, stably patterned biofilms with arbitrary spatial patterning. Our patterned

biofilms develop emergent properties analogous to those seen in natural biofilms, including resistance to anti-bacterial treatment and oxygen depletion within the biofilm interior. These model biofilms will be crucial for future development of therapeutic anti-biofilm strategies, for which no reproducible model biofilm test system is currently available, as well as for the reliable production of beneficial living materials, which could be applied for water and soil purification, mineral extraction, or energy capture.

Polymer platforms for 3D printing engineered living materials

Presenter: **Alshakim Nelson**, Professor
Affiliation: Chemistry, Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA, USA.

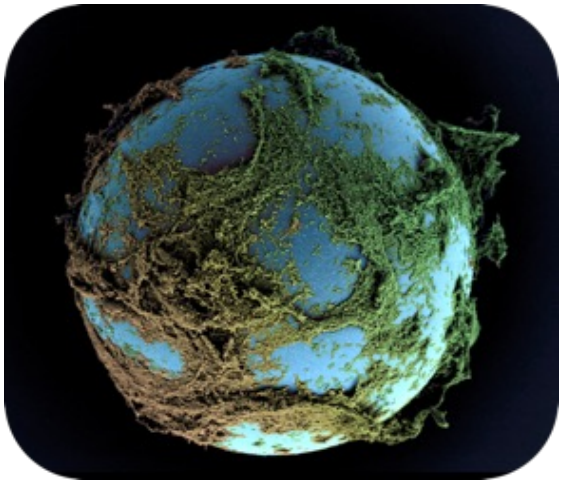
The convergence of synthetic biology with polymer science has led to the rapid emergence of engineered living materials (ELMs), which are composite materials of engineered cells encapsulated within a polymeric matrix. ELMs have the potential to attain a level of precision, control, and sustainability that is not achieved with traditional abiotic materials. While the minimum requirements for functional ELMs are cell viability and metabolic activity, the integration of ELMs in future biomanufacturing also requires processing them into desired form factors and exerting spatial control over multiple engineered cell populations.

Additive manufacturing (or 3D printing) has re-emerged into the spotlight in the last 10 years driven by the rapid progress in hardware and software. While off-the-shelf polymeric materials have been utilized as inks and resins for additive manufacturing, there is still a need to develop functional materials that are specifically designed for these processes. In this seminar, I will present two different polymer platforms that we have developed to 3D print ELMs. In the first part of my seminar, I will discuss the development of stimuli-responsive triblock copolymer hydrogels for fabricating immobilized cell bioreactors. These hydrogels were used to incorporate engineered microbes that served as cellular factories for the on-demand bio-production of chemical products. In the second part of my seminar, I will discuss a two-step additive manufacturing process to fabricate protein-based constructs using a commercially available laser-scanning SLA printer. Methacrylated bovine serum albumin (MA-BSA) was synthesized and formulated into aqueous resins that was used to print complex 3D geometrical constructs with a resolution comparable to a commercially available resin. Compression studies of hydrated and dehydrated constructs demonstrated a broad range of compressive strengths and Young's moduli that could be further modulated by adjusting the type and amount of co-monomer. These protein-based matrices served as biodegradable scaffolds for 3D printing ELMs, and I will present strategies for fabricating bio-augmented and bio-sustained ELMs.

(Abstracts continue on next page.)

Biologically fabricated materials from engineered microbes

Presenter: **Neel Joshi**, Associate Professor
Affiliation: Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA, USA.



The intersection between synthetic biology and materials science is an underexplored area with great potential to positively affect our daily lives, with applications ranging from manufacturing to medicine. My group is interested in harnessing the biosynthetic potential of microbes, not only as factories for the production of raw materials, but as fabrication plants that can orchestrate the assembly of complex functional materials. We call this approach "biologically fabricated materials", a process whose goal is to genetically program microbes to assemble materials from biomolecular building blocks without the need for time consuming and expensive purification protocols or specialized equipment. Accordingly, we have developed Biofilm Integrated Nanofiber Display (BIND), which relies on the biologically directed assembly of biofilm matrix proteins of the curli system in *E. coli*. We demonstrate that bacterial cells can be programmed to synthesize a range of functional materials with straightforward genetic engineering techniques. The resulting materials are highly customizable and easy to fabricate, and we are investigating their utility for practical uses ranging from bioremediation and biodegradable bioplastics to engineered therapeutic probiotics.

INDUSTRY PANEL

What are industry perspectives and considerations regarding the development of ELM technologies?

Moderators: **Nicole Motzer** (MSU), **Nika Stoop** (MSU)
Panelists: **Chuck Pettigrew** (Arxada), **Tony Rook** (Sherwin-Williams), **Marcia Ryder** (Ryder Science), Wil Srubar (University of Colorado-Boulder, Minus Materials, Prometheus)

GOVERNMENT AGENCY PANEL

What are government agency program director and government lab researcher perspectives on the future of ELM research?

Moderators: **Nicole Motzer** (MSU), **Nika Stoop** (MSU)
Panelists: **Giovanna Biscontin** (National Science Foundation), **Michael Carter** (Air Force Research Labs), **Luke Roberson** (NASA)

POSTER ABSTRACTS

CBE Poster #817

Date: 07/23
Title: **Developing high pH/high alkalinity polycultures to accelerate algal biofuel production**

Authors: **Breuklyn Opp**[1], Sandra Rincon-Miranda[1], Huyen Bui[1,2], Robin Gerlach[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]ARUP Laboratories, Salt Lake City, UT, USA

Commercial and laboratory algal growth is, like many other agricultural pursuits, often attempted in monoculture – with much effort spent on keeping the cultures monoalgal or even axenic (pest control). By combining multiple algae and potentially other microbes in co-cultures, a wider range of niches may be occupied by the different community members, thus potentially increasing resource utilization and biomass production efficiency through niche-complementarity. To

successfully grow co-cultures industrially, it is valuable to identify media conditions which optimize the quality or quantity of biomass while preventing domination of a single strain.

The research to be presented involves the growth of three different photosynthetic strains – a cyanobacterium, a green alga, and a diatom – with the goal of maximizing productivity through a co-culture of these species. Most recently, we characterized the effect of different environmental factors on the growth of these strains, such as pH, alkalinity, silicate concentration, and light intensity. These screenings have been performed in 24-well plates with growth being monitored through absorbance measurements at 600 and 750 nm as well as chlorophyll fluorescence. The work to be presented focuses on identifying optimal growth conditions along with an in-depth characterization of growth parameters such as growth rate (optical density, cell count), dry weight, ash-content, pH, bulk inorganic nitrogen and phosphorus concentrations, and chlorophyll fluorescence. Experimentation over a range of silicate concentrations has shown both critically low and high silicate concentrations for the diatom. Neither the very low nor the very high concentrations of silicate have prevented growth of the green alga or the cyanobacterium, although reduced growth with high silicate concentrations has been observed. Observations of the impact of pH, alkalinity, and light intensity on the growth of these cultures are currently being addressed.

CBE Poster #818

Date: 06/23

Title: **Rapid MRI profiling of two phase drainage and imbibition dynamics in porous media**

Authors: **Quirine Krol**[1,3], Sarah Codd[1,2], Alex Hansen[3], Joseph Seymour[1],

Affiliations: [1]Magnetic Resonance Laboratory, Montana State University, Bozeman, MT, USA.
[2]Mechanical Engineering, Montana State University, Bozeman, MT, USA.
[3]Physics Department, NTNU, Trondheim, Trondelag, Norway.

Flow in porous media have an abundance of applications in physics, bio-chemistry and engineering. Experimental methods to capture this phenomena are often limited to two dimensional setups, refractive index matching and/or are too slow to capture the dynamics at a resolution relevant to the physics at the pore scale. We utilize nuclear magnetic resonance methods to retrieve 1D spin-echo intensity and phase-angle profiles with a temporal and spatial resolution of 50 ms and 70 microns, respectively. We compare first and secondary imbibition, drainage and steady state two-phase flow of a wide variety of three-dimensional microstructures ranging from bead-packs to sticky hard spheres. We extend analysis of standard 1D spatial profiles used to determine water saturation, to measure velocity and correlate signal attenuation to local velocity fluctuations. With this study we demonstrate that size and duration of local surges can be related to the heterogeneity of the porous media and has future potential to measure effective dispersion coefficients in steady state two-phase phase flow and biofilm populated porous media.

CBE Poster #819

Date: 06/23

Title: **NMR relaxometry characterization of water adsorption in corn stover anatomical fractions**

Authors: **Matthew C. Young**[1], Sarah L. Codd[2], Dylan S. Cousins[1], William G. Otto[1], David B. Hodge[1,3], Joseph D. Seymour[1]

Affiliation: [1]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Division of Sustainable Process Engineering, Luleå University of Technology, Luleå, Sweden.

Despite being major contributors to global CO_2 emissions, fossil feedstocks are finite natural resources frequently used to produce high value goods including fuels and plastics. One alternative is to replace fossil feedstocks with renewable

agricultural feedstocks due to their ability to sequester carbon during growth. While a promising alternative, the use of food crops as feedstocks brings its own set of challenges. Recent emphasis has been placed on deconstruction of agricultural residues, such as corn stover, into fuels and chemicals. Polysaccharides from lignocellulosic plant cell walls can be converted to glucose, but biomass recalcitrance to enzymatic hydrolysis presents a practical challenge to this pathway. Pretreatment steps help improve enzymatic access to plant cell walls and once optimized, allow for these processes to be scaled. Nuclear magnetic resonance (NMR) relaxometry is applied to corn stover to gain a better understanding of these systems and the impacts of pretreatment. These measurements directly measure water adsorption in anatomical fractions of corn stover. NMR transverse T_2 relaxation time distribution measurements indicate multiple water populations, which vary with anatomical fraction and water adsorption. Measured T_2 data are used to calculate thermodynamic properties of Brunauer-Emmet-Teller (BET) adsorption theory using a model to estimate mono and bilayer relaxation. T_2 data are used directly to determine rotational diffusion correlation times indicating adsorption interaction strength. T_1-T_2 longitudinal-transverse relaxation time correlation measurements quantify differences in the molecular level structural order of the adsorbate surface water as a function of water activity, i.e. relative humidity or water vapor partial pressure. The T_1/T_2 ratio provides a measure of the surface energy related to the adsorption strength and surface diffusive mobility of the water adsorbate, and differentiates the anatomical fractions. The results indicate that direct measurement of NMR relaxation times can be used to characterize corn stover biomass water adsorption, which are data relevant to biomass processing and handling. These procedures may be extended to monitor biofilm growth at air-liquid interfaces in plants, providing pertinent information for wetland water treatment.

CBE Poster #820

Date: 07/23

Title: **Developing a novel tool to image organic matter in soil and ice samples**

Authors: **Nicole Krysiak**, Madelyne Willis, Evan Eshelman, Christine Foreman

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Organic matter (OM) is a mixture of natural compounds found in freshwater and agricultural environments. OM can affect reactivity and microbial communities within an environment. Through the use of fluorescence spectroscopy, the dynamics and biogeochemical composition of OM can be determined. To measure OM quality, Excitation Emission Matrix Spectroscopy (EEMS) can be used to scan a sample across a range of excitation/emission wavelengths, and measure fluorescence intensity. EEMS identifies different fluorophores and positions of excitation/emission maxima within the OM. In the past, to analyze soil samples the OM must first be extracted; this process is time consuming and results in destruction of the sample. We have been developing an organic matter bioimager known as SEEMS: Spatial Excitation-Emission Matrix Spectroscopy. SEEMS takes false color images of a solid sample to spatially resolve OM fluorescence on the surface. These images display the fluorescence of the OM present in the sample and does not require an OM extraction. To validate SEEMS, the data collected was compared to the EEMS data collected using a Horiba F4 spectrofluorometer. We will present data from soils and icy environments showing analyses on both instruments and the increased spatial information that can be obtained using SEEMS.

CBE Poster #821

Date: 07/23

Title: **Engineering synthetic biofilm with novel laser lithography-based 3D bioprinting methods**

Authors: Isaak Thornton[1,4], **Kathryn Zimlich**[2,3], Jim Wilking[3,4], and Matthew W. Fields[2,4*]

Affiliation: [1]Mechanical & Industrial Engineering, [2]Microbiology & Cell Biology, [3]Biological and Chemical Engineering, Mayo Clinic, [4]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: Army Research Office

Attached microbial growth (biofilm) more closely resembles in situ conditions for microorganisms in diverse environments than free-living growth. Biofilms play vital roles in ecosystem functions, industrial processes, and human

health that have profound implications for the grand societal challenges of water, food, energy, and health. Biofilm growth is a ubiquitous phenotype with unique physiology, ecology, and evolution that requires deeper investigation to improve our understanding of the microbial world and to drive biotechnological innovation by building and utilizing synthetic biofilm . The spatial structure of biofilms often gives rise to emergent properties likely forged early in microbial evolution. However, biofilms are typically defined and studied as self-assembled systems and the underlying mechanisms that drive biofilm formation and behavior are often difficult to systematically manipulate. Methods of controlling and manipulating biofilm structure and composition are needed. We have developed novel 3D bioprinting methods using laser lithography that provide control over the structure and composition of living materials at the scale of tens to hundreds of micrometers. Using our methodologies, we 3D printed hydrogels with an encapsulated bacterium (*Pseudomonas fluorescens*) and developed methods to spatiotemporally quantify growth in the hydrogel matrix. We anticipate this technology will advance into a powerful method for identifying key physical and chemical constraints that likely drive structure-function relationships within biofilms and will bolster capabilities to engineer complex, spatially structured microbial communities.

CBE Poster #822

Date: 07/23

Title: **Characterization of a green algal strain in a high alkalinity environment**

Authors: **Charles Holcomb**[1,2], Sandra Milena Rincon[1], Huyen Bui[1], Robin Gerlach[1,2]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: National Science Foundation (NSF) Grant No. 2125083 – MIM: Deciphering and Optimizing Cross-Domain Interactions to Increase Productivity in High pH-High Alkalinity Microalgae Communities.
Montana Nanotechnology Facility (MONT) part of National Nanotechnology Coordinated Infrastructure (NNCI)
NSF (Grant No. ECCS-1542210), HIDAC – Transforming High pH/High Alkalinity Cultivation through Beneficial Microbiomes and Improved Pond Design Award Number: DE-EE0009273

Using algae to produce high-value products and biofuel is a way to support energy and raw material demands. Due to space and nutrient demands, along with the high costs associated with algal pond operation, the economic feasibility of industrial-scale algal production is a consideration. Strains producing high-value products at a high rate, such as lipids, along with the optimization of media components, are keys to making algal pond operation profitable.

A green algal strain, designated as ALgE, was isolated along with its microbiome from Alkali Lake, Washington (USA). Alkali Lake is a soda lake with an alkalinity of 3.4 meq and a pH of roughly 9. When ALgE is grown in Bolds Basal Medium (BBM) at an initial pH of 10.3 with an addition of 100 mM sodium bicarbonate, a doubling time of 0.90 days along with a lipid content of 0.7 grams of fatty acid methyl esters (FAMEs) per liter of culture was obtained. These results indicate that ALgE can be grown at much higher alkalinity and pH than in its natural environment. Also, it shows that under these media conditions, the alga produces a large quantity of lipids. Growing algae at higher pH and alkalinity can prevent limitations in inorganic carbon and increase the production of storage compounds. Furthermore, the high pH increases CO_2 mass transfer from the atmosphere, often eliminating the need for a CO_2 sparge. Using productive strains adapted to high pH and alkaline conditions allows for direct air capture strategies for producing high-value bioproducts, which works toward decreasing the costs of algal pond operation.

CBE Poster #823

Date: 07/23

Title: **Pharmaceutical fate and effects on active microbial communities during treatment with aerobic granular sludge**

Authors: **Kylie Bodle**[1,2], Rebecca Mueller[1,3], Madeline Pernat[1,2], Catherine Kirkland[1,2]

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Research Service, Albany, CA, USA.
Sponsored by: Montana IDeA Network of Biomedical Research Excellence (INBRE), National Institutes of Health

Aerobic granular sludge (AGS) is a novel wastewater treatment biotechnology in which numerous bacterial species coexist in a spherical biofilm. Oxygen and nutrient gradients throughout each granule allow complete wastewater treatment in a single reactor, and extracellular polymeric substances (EPS) in granules provide a diffusive barrier that protects bacteria from toxic shocks and improves granule settleability. For these reasons, AGS may be capable of enhancing removal of emerging contaminants, such as pharmaceuticals, from wastewater using less energy and a lower footprint than conventional systems.

This research evaluated the effects of three model pharmaceuticals on AGS morphology, microbial communities, and wastewater treatment capacity. Pharmaceutical fate (adsorption versus biodegradation) was also monitored. Pharmaceutical exposure caused a significant reduction of the lipid barrier in exposed granules, and key active wastewater-treating communities declined in relative abundance. Phosphate and total nitrogen removal decreased and did not recover over the 80-day dosing period. Pharmaceutical removal was temporary and appeared to occur via both adsorption and biodegradation.

CBE Poster #824

Date: 07/23

Title: **Fate and transport of environmentally relevant bacterial isolate through a simulated groundwater environment**

Authors: **James Marquis**[1,4], Kaelee Massey[2,4], Heidi J. Smith[1,4], Alfred B. Cunningham[3,4], Matthew W. Fields[1,4]

Affiliation: [1]Department of Microbiology & Cell Biology, Montana State University, Bozeman, MT USA.
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Sponsored by: US Department of Energy

Understanding the fate and transport of environmentally relevant bacteria in groundwater is critical to the prediction of fate and transport of microbial biomass and activity as well as the enhancement of potential bioremediation in subsurface porous environments. In this study, we examined the transport and adsorptive capacity of *Stenotrophomonas* EB106-03-01-XG87 isolated from the contaminated Bear Creek Aquifer in Oak Ridge, Tennessee that contains high levels of nitrate and heavy metals. A limited understanding of the transport processes of relevant bacteria at this site remains a large hurdle in estimating the distribution and dispersal of microbial biomass, and *Stenotrophomonas* ASVs observed from field samples are represented equally distributed as groundwater or sediment-associated populations. Therefore, *Stenotrophomonas* EB106-03-01-XG87 represents a base case bacterial population for insight into fate and transport in a simulated porous media. In this study, *Stenotrophomonas* EB106-03-01-XG87 was inoculated into packed bed reactors (PBR), which mimicked key environmental conditions from the aquifer. Namely, the PBR was composed of sand particles that represented particle size distributions observed at the field site and two flow rates (low and high) that simulated the upper and lower bounds of groundwater flow in situ (triplicate reactors). Samples for cell counts, nitrate, acetate, and qPCR were collected daily from the effluent and from the reactor sediment at the final timepoint. Results indicate that relatively small changes in flow rate may have a substantial impact on binding affinity of *Stenotrophomonas* to sand particles. To determine the intrinsic binding affinity of *Stenotrophomonas*, static (no flow) binding affinity experiments with either varying concentrations of viable or non-viable *Stenotrophomonas* cells were completed. Results indicate that under static conditions, independent of cell concentration, the binding affinity of *Stenotrophomonas* is unaffected. However, there was a ~20% greater binding affinity for live versus dead cells. Indicating that *Stenotrophomonas* will not selectively partition in either the planktonic or attached phase. Combined, these results suggest that *Stenotrophomonas* may be treated as an impartial microbial tracer, which may have utility in future efforts to understand bacterial transport in porous media environments.

CBE Poster #825

Date: 07/23

Title: **Environment constrains fitness advantages of division of labor in microbial consortia engineered for metabolite push or pull interactions**

Authors: Ashley E. Beck[1,4], Kathryn Pintar[1], Diana Schepens[2], Ashley Schrammeck[1], Timothy Johnson[1], Alissa Bleem[1], **Martina Du**[1], William R. Harcombe[3], Hans C. Bernstein[5], Jeffrey J. Heys[1], Tomas Gedeon[2], Ross P. Carlson[1]

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[4]Department of Biological and Environmental Sciences, Carroll College, Helena, MT, USA.
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Fitness benefits from division of labor are well documented in microbial consortia, but the dependency of the benefits on environmental context is poorly understood. Two synthetic *Escherichia coli* consortia were built to test the relationships between exchanged organic acid, local environment, and opportunity costs of different metabolic strategies. Opportunity costs quantify benefits not realized due to selecting one phenotype over another. The consortia catabolized glucose and exchanged either acetic or lactic acid to create producer-consumer food webs. The organic acids had different inhibitory properties and different opportunity costs associated with their positions in central metabolism. The exchanged metabolites modulated different consortial dynamics. The acetic acid-exchanging (AAE) consortium had a 'push' interaction motif where acetic acid was secreted faster by the producer than the consumer imported it, while the lactic acid-exchanging (LAE) consortium had a 'pull' interaction motif where the consumer imported lactic acid at a comparable rate to its production. The LAE consortium outperformed wild type (WT) batch cultures under the environmental context of weakly buffered conditions, achieving a 55% increase in biomass titer, a 51% increase in biomass per proton yield, an 86% increase in substrate conversion, and the complete elimination of byproduct accumulation all relative to the WT. However, the LAE consortium had the tradeoff of a 42% lower specific growth rate. The AAE consortium did not outperform the WT in any considered performance metric. Performance advantages of the LAE consortium were sensitive to environment; increasing the medium buffering capacity negated the performance advantages compared to WT.

IMPORTANCE: Most naturally occurring microorganisms persist in consortia where metabolic interactions are common and often essential to ecosystem function. This study uses synthetic ecology to test how different cellular interaction motifs influence performance properties of consortia. Environmental context ultimately controlled the division of labor performance as shifts from weakly buffered to highly buffered conditions negated the benefits of the strategy. Understanding the limits of division of labor advances our understanding of natural community functioning which is central to nutrient cycling and provides design rules for assembling consortia used in applied bioprocessing.

CBE Poster #826

Date: 06/23

Title: **Synthesis and biological evaluation of novel 2-aminoimidazole antimicrobial agents**

Authors: **Amethyst R. Demeritte**[1,2], Heidi N. Koenig[1,2], Tom Livinghouse[2], Phil Stewart[1]

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To combat antimicrobial-resistance (AMR), a pertinent approach is the identification of adjuvants that augment the activity of current antibiotics. Recent literature has shown poly-substituted 2-aminoimidazoles (2-AI) possess the ability to inhibit biofilm formation, disperse preformed biofilms and re-sensitize multidrug-resistant bacterial strains to conventional

antibiotics. Structural formation of these novel 2-AI focuses on incorporation of sulfide linkers for autoinducers activity enhancement via inhibition of quorum sensing.

A library of novel 2-AI has been synthesized and the preliminary screening for anti-biofilm activity against Methicillin-resistant *Staphylococcus aureus* (MRSA USA300 LAC) and highly virulent *Pseudomonas aeruginosa* (PA14) biofilms and structural activity relationship (SAR) analysis will be disclosed. To evaluate these novel adjuvants as possible therapeutic agents for pathogenic biofilms, Kirby-Bauer Disk Diffusion assays, Minimum Inhibitory Concentration (MIC) assays and Minimum Biofilm Eradication Concentration (MBEC) assays were conducted and the lead compounds from this study will also be presented.

CBE Poster #827

Date: 06/23

Title: **Fungal mycelium as a scaffold for living building materials**

Authors: **Ethan Viles**[1,2], Erika Espinosa-Ortiz[1,3], Adrienne Phillips[1,4], Robin Gerach[1,3], Chelsea Heveran[1,2]

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Sponsored by: NSF

Engineered living materials (ELMs) have garnered considerable excitement as a frontier of materials science. ELMs are characterized by the functionalities provided by living cells, such as sensing or self-healing. Most ELMs to date have been soft materials. Engineering stiff ELMs would be a major accomplishment, since these materials may provide more sustainable alternatives to conventional building materials. The few attempts to engineer load bearing 'living building materials' (LBMs) have utilized microbial biomineralization to bridge aggregate or stiffen hydrogels. However, microbial lifespan is still low in these structures, spanning days to a few weeks. A new idea in LBM design is to use fungal mycelium as a living scaffold that can be biomineralized to increase its stiffness. A living fungal scaffold has the potential to confer exciting functionalities to a material, including self-biomineralization and potentially self-repair. Alternatively, fungal mycelium could serve as a scaffold for biomineralization by another microorganism, such as ureolytic bacteria.

In this work, we demonstrate success in self-biomineralizing living *Neurospora crassa* scaffolds as well as biomineralization of nonviable fungal scaffolds using the ureolytic bacteria, *Sporosarcina pasteurii*. The resulting composites have different morphological, mineralogical, and biological characteristics. Bacterial biomineralization was more efficient in mineralizing the scaffold compared with fungal self-mineralization and achieved large calcium carbonate crystals that bridge together hyphae. Fungal self-biomineralization achieved smaller calcium carbonate crystals that have a markedly different distribution around fungal hyphae compared with bacteria-produced specimens. The self-biomineralized fungal scaffolds remain viable for at least 2 weeks after mineralization and drying, demonstrating their potential for self-healing or other functionalities. The bacterially biomineralized scaffolds show early evidence of bacterial viability. Ongoing work is investigating the viability of *S. pasteurii* 4 weeks after biomineralization and drying.

CBE Poster #828

Date: 06/23

Title: **Tissue disruption during device implantation masks contaminating *S. aureus* aggregates**

Authors: **Timothy Borgogna**[1,2], Brian Pettygrove[3], Cassandra Robinson[2], Owen Burroughs[2], Jovanka Voyich[2], Phil Stewart[1]

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Implanted device related infections account for 50-70% of all nosocomial infections. These infections are frequently caused by commensal skin organisms and are complicated by their ability to form and grow as biofilms. As biofilms mature, they become increasing recalcitrant to antimicrobial therapies and immune defenses. Previous studies, using confocal microscopy, have established *Staphylococcus aureus (S. aureus)* aggregates >50 μm^2 are resistant to human neutrophil killing, thus highlighting the importance for early therapeutic intervention. Despite this consensus, the immediate host-pathogen interactions occurring both within the peri-implant tissue and at the implant interface remain poorly described. Using an in vivo mouse model of subcutaneous implant infection, we explore the hypothesis that low doses of contaminating *S. aureus* on implanted devices remain undetected by the host immune system. Flow cytometry analysis demonstrated that the presence of attached low doses ($5.5x10^5$ CFU/cm^2) of contaminating *S. aureus* does not significantly impact the percent of recruited leukocyte populations compared to sham or sterile controls; however, attached high doses ($1x10^8$ CFU/cm^2) of *S. aureus* promote increased neutrophil recruitment. Histological analysis of tissue directly adjacent to sham, sterile, low, and high contaminated implants revealed increased numbers of leukocytes at the implant interface only in implants contaminated with high doses of *S. aureus*. On-going efforts aim to identify factors to enhance neutrophil detection of *S. aureus* immediately following implantation. Preliminary in vitro experiments have revealed exogenous adsorbed fibrinogen drastically enhances neutrophil detection of *S. aureus* and may facilitate neutrophil killing of *S. aureus* aggregates >50 μm^2. Collectively, these data imply the dominant immune stimuli following implantation surgery originates from mechanical disruption of the underlying and peri-implant tissue (versus bacterial contamination of the implant site). Furthermore, the extensive tissue damage occurring during implantation surgeries likely prioritizes polarization of recruited leukocytes to tissue repair phenotypes over bacterial killing. In turn, this may enable low doses of contaminating *S. aureus* to remain undiscovered and form nascent biofilms.

CBE Poster #829

Date: 06/23

Title: **Fabrication of 2D micromodels for studies of multiphase flow and bacterial activities in porous media**

Authors: **Md Ahsan Habib**[1], Diego Armstrong[1], Bo Guo[2], and Yaofa Li[1]

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Sponsored by: NSF Career (2144802)

Drying and multiphase flow in porous media is central to a broad range of natural and engineering applications, including oil recovery, CO_2 storage, and critical zone science. In many scenarios, these porous solid matrices display multi-scale variability in pore structure and physical properties such as porosity and permeability. For instance, in critical zone, soil is often viewed as a hierarchical organization: primary particles of a few micrometers in size form microaggregates of hundreds of micrometers in size, which in turn form macroaggregates, effectively leading to dual porosity in the porous media. The resultant multi-scale flow dynamics and inter-/intra-aggregate interaction in this system are recognized to control numerous processes, such as water and gaseous transport. However, the underlying physics including fluid mechanics and thermodynamics, is not well understood. In this study, we fabricated surrogate porous media called micromodels employing microfabrication techniques. The fabrication process includes HMDS oven exposure, photolithography, plasma etching in an Oxford ICP machine, and anodic bonding. The new micromodels are unique in that they are in a glass-silicon-glass bond. Previous micromodels use one glass wafer per silicon wafer and are analyzed from the glass side of the micromodel. These new double glass micromodels allow for an innovative approach for flow measurements as two microscopes are used. The new micromodels provide solutions to more efficient analysis of multiphase flow. Analysis includes velocimetry, drying process of water in porous media, and water-air interface behavior through porous media, which will provide valuable insight into the underlying physics during drying of porous media. This new method also opens the door to in-situ analysis of bacterial activities in porous media.

CBE Poster #830

Date: 07/23

Title: **Log density of biofilm growth in the CDC reactor relative to coupon position and orientation: *P. aeruginosa* and *S. aureus***

Authors: **Elizabeth Buckner**[1], Kelli Buckingham-Meyer[1], Lindsey Miller[1], Al Parker[1], Chris Jones[1], Darla Goeres[1]

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Sponsored by: CBE Industrial Associates

The Single Tube Method was developed to determine the efficacy of biocides against biofilm grown in the CDC Biofilm Reactor. These data may be used to support "kills biofilm" product claims if the biocide achieves a greater than six log kill against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. When conducting the test, the laboratory technician randomly selects three coupons that are used as the control coupons and five random coupons that are used as the test coupons. The assumption underlying the use of randomly chosen coupons is that biofilm growth is not influenced by the placement of the coupon within the reactor.

In the biofilm reactor, three coupons are held vertically in a coupon holder with one side of the coupon facing the spinning baffle in the center of the reactor, and one side facing the glass container on the outside. The goal of this study was to determine whether CDC biofilm reactor coupon orientation (glass v. baffle) and rod placement (top, middle, bottom) result in different cell growth densities for *P. aeruginosa* and *S. aureus*. *P. aeruginosa* and *S. aureus* biofilms were grown in the CDC biofilm reactor according to ASTM E3161 then scraped, homogenized, diluted and plated according to ASTM E2562. Three runs with each species were completed.

Despite morphological differences between the glass and baffle orientated surfaces revealed through microscopy, the density of *P. aeruginosa* cell growth found by plate counts was shown to be statistically equivalent amongst all coupon orientations and positions. The density of *S. aureus* cell growth showed no statistically significant differences amongst all coupon orientations and positions. Future research is needed to determine differences in biofilm volume due to coupon orientation and position.

CBE Poster #831

Date: 06/23

Title: **Biocementation using microbially induced calcium carbonate precipitation at 60°C and room temperature**

Authors: **J. L. Faber**[1,2], **L. C. Bala**[1,2], M. Willet[1,3], K. Bedey[1,2], A. Cunningham[1,2], A. Phillips[1,2], C. Kirkland[1,2]

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Microbially-induced calcium carbonate precipitation (MICP) can be applied as a biological cement, which has been researched for applications in the subsurface and to fill void spaces in fractured or porous media. It has the potential to seal leaks in oil and gas wells from shale fractures hundreds of meters below ground. The temperature increases as depth increases, so understanding mineral synthesis and resulting mineral properties of MICP at that depth can be challenging. This study aims to reveal the correlation between strength of material and operating temperature in the biocementation process. *Sporosarcina pasteurii* is commonly used in this process due to its ability to produce urease, which is a catalyst for urea hydrolysis. Using this process in the presence of calcium, calcium carbonate precipitates. In this experiment, *S. pasteurii*, urea and calcium were injected at 1 milliliter per minute into a 1 inch diameter and 2 inch long sand column at both room temperature and 60^{O}C until 20 injections were achieved. Influent and effluent samples were collected during each injection in order to perform Jung assay tests which quantify urea concentration. The tensile strength of the

mineralized columns were tested using the modified Brazilian method once the formation of the biological cement was complete.

CBE Poster #832

Date: 07/23

Title: **Algal adaptation to high salinity cultivation**

Authors: **Adrienne D. Arnold**[1], Sandra Rincon[1], Charles J. Holcomb[1], Robin Gerlach[1], Sridhar Viamajala[2], Ross P. Carlson[1]

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Algae are photosynthetic eukaryotes that play a major role in CO_2 cycling in the environment, carrying out about half of global CO_2 fixation. They are also industrially important, as they can be used to produce high value products like biodiesel and bioethanol. In both the environment and in industry, algae are subjected to high salinity stress: in the environment, high salinity can be caused by droughts that concentrate salts within water sources, and in industry, growing algae in seawater is one strategy to reduce costs associated with algal cultivation. The metabolic adaptations that algae use to acclimate to salt stress are difficult to predict. A better understanding of how algae adapt to high salinity cultivation will improve our knowledge of carbon cycling and may also improve techniques for industrial cultivation.

Chlorella sp. strain SLA-04 is a model salt- and alkali- tolerant microalga. To study the effects of osmotic stress on algae, SLA-04 was cultivated under varying concentrations of NaCl up to seawater levels of salinity. Growth was monitored across the conditions, and starch and lipid concentrations were measured. A genome-enabled metabolic model of SLA-04 metabolism was then used to interpret the experimental data. This work provides key insights into the flow of carbon and energy within microalgae grown under salt stress, which in turn can be applied to industrial cultivation and climate predictions.

CBE Poster #833

Date: 07/23

Title: **Using low-field nuclear magnetic resonance and x-ray computed microtomography imaging to explore potential of microbially-induced calcium carbonate precipitation treatment to seal shale fractures**

Authors: **Matthew R. Willett**[1,2], Kayla Bedey[2,3], Alfred B. Cunningham[2,3], Laura Dobeck[2,4], Dustin Crandall[5], Jonny Rutqvist[6], Joseph D. Seymour[1,2], Adrienne J. Phillips[2,3], Catherine M. Kirkland[2,3]

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Sponsored by: US Department of Energy, Office of Science, DOE Award No.: DE-SC0021324

Shale rock serves several roles in our energy portfolio, including as caprock for sequestered CO_2 and as a resource for natural gas. These applications highlight a need for methods to control permeability to ensure long-term safety and utility of these reservoirs. One promising approach is microbially-induced calcium carbonate precipitation (MICP), an emerging biotechnology which uses microbially produced urease enzymes to convert urea and calcium into solid calcium carbonate ($CaCO_3$) deposits. Studies have shown that MICP can seal fractures in shale, raising the possibility of applying this technology to restimulate fracking wells by plugging underperforming fractures or mitigate leakage from waste storage reservoirs. However, research is needed to determine how effectively MICP seals shale fractures under subsurface conditions. In this study, a 2.54 cm wide and 5.08 cm long Marcellus shale core with a single, ~1 mm wide fracture held open by sand “proppant” underwent MICP-treatment at 60°C until reaching three orders of magnitude

permeability reduction. Low-field nuclear magnetic resonance (LF-NMR) and X-Ray computed microtomography (μ-CT) techniques were used to assess the extent of biomineralization within the fracture. These tools revealed that while $CaCO_3$ precipitation occurred throughout the fracture, there was preferential precipitation around proppant, and the core sealed at the effluent end before filling most of the fracture. Results from this research will bring us one step closer to deploying MICP in the subsurface to control shale permeability, helping us prepare for the complex energy needs of the 21st century.

CBE Poster #834

Date: 06/23

Title: **Thermally induced carbonate precipitation as a method to control hydraulic properties in enhanced geothermal systems**

Authors: **Kaelyn Harms**, Alexa Lord, Laura Dobeck, Al Cunningham, Adrienne Philips

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Sponsored by: US Department of Energy

To effectively cultivate geothermal energy from natural systems, engineered fractures are hydraulically implemented to improve access to water through a network of channels in the heated rock. The result of manipulating the permeability of a geothermal reservoir in this way is referred to as Enhanced Geothermal Systems (EGS). Through this collaborative research project, Montana State University and Berkeley National Laboratory aim to increase the understanding of thermally induced carbonate precipitation (TICP) in the application of influencing permeability in EGS. The implementation of microbially induced calcium carbonate precipitation (MICP) has been widely explored for sealing fractures in the subsurface, for example in leaking oil and gas wells. However, the temperatures present in EGS are too high to support most microbial metabolic functions, leading to the research of thermally induced calcium carbonate precipitation (TICP). Urea, when heated in solution above 100^{O}C can be thermally hydrolyzed to promote calcium carbonate precipitation. Mineral precipitation at hotter temperatures has an application to controlling permeability in the deeper subsurface and enhanced geothermal systems.

Presently, data has been collected through batch testing of the thermal kinetics of the reaction between urea and calcium chloride at various concentrations (1M and 3M), temperatures (150 to 195 degrees Celsius) and in the presence of granite (the host rock in EGS). Batch testing was performed by filling stainless steel reactors, heating them and pulling them from the oven at various time points to assess the remaining concentration of urea. The results of the batch experiments were used to design a flow-through, high-temperature granite core sealing test to model enhanced geothermal systems. One core experiment has successfully confirmed the ability of TICP to reduce the permeability of a fracture in granite, and future testing is anticipated to further provide analytical support to the hypothesis. This poster will describe the analysis of urea hydrolysis and the preliminary data from the core sealing experiment.

CBE Poster #835

Date: 06/23

Title: ***Mycoplasma ovipneumoniae* biofilms promote gentamicin resistance.**

Authors: **B. Tegner Jacobson**[1], Jessica DeWit[1], Sobha Sonar[1], LaShae Zanca[1], Katrina Lyon[1], Chris Corona[2], Michael Throolin[2], Noah Adams[3], Diane Bimczok[1]

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Sponsored by: USDA-NIFA, NIH ITHS

Mycoplasma ovipneumoniae (M. ovi) is a respiratory pathogen associated with polymicrobial pneumonia in domestic and bighorn sheep. *M. ovi* resists clearance and treatment and can have drastic effects on infected herds. Increased antibiotic resistance has been shown in sheep infected with *M. ovi*, but the mechanism of this resistance is not well characterized. One possible mechanism of interest is the formation of biofilms. A biofilm is generally defined as an aggregate of cells contained in an extracellular polymeric substance (EPS) utilized by pathogens to increase virulence within a host. The ability to form a biofilm is an innate characteristic in most bacteria, including many *Mycoplasma* species, but *M. ovi*

biofilm growth is poorly characterized. In our research, we aimed to optimize *M. ovi* biofilm growth under a variety of growing conditions to further evaluate and represent the ability of *M. ovi* to form biofilms and to investigate how biofilms may play a role in antibiotic resistance. Examination of biofilm formation for a reference strain (Y98) and field isolate (MSU-NW4) of *M. ovi* showed novel robust biofilm formation under optimized growing conditions comparable to that of *M. bovis*, a known biofilm forming *Mycoplasma* species. A crystal violet microplate assay was completed in a glass bottom 96-well plate and results suggest a robust biofilm forming ability for both strains of *M. ovi.* In investigating antibiotic susceptibility, *M. ovi* biofilms grown in glass bottom 96-well plates showed an MIC of 64 ug/mL to gentamicin as compared to previous data showing a 4 ug/mL MIC for planktonic populations. We also investigated the metabolic ability of *M. ovi* biofilms when exposed to sublethal levels of gentamicin and found that one of the strains had significantly reduced acid production in the presence of these sublethal levels of gentamicin. These data are significant as it is a novel look at the biofilm formation capabilities for *Mycoplasma ovipneumoniae* and will help to further understand sheep infections and the biofilm resistance mechanisms for future experimentation and treatment application.

CBE Poster #836

Date: 06/23

Title: **Impacts of mixing regimes over the diel cycle for axenic and xenic *Chlorella* cultures**

Authors: **J. Wood**, I.R. Miller, R. Gerlach, and M.W. Fields

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Open cultures commonly used for large-scale algal production employ active mixing to maintain high levels of productivity through facilitating gas exchange and minimizing prolonged shading. Coinciding with the diel cycle, mixing may not be needed during the dark phases and would reduce energy consumption. However, in the context of decreased gas flux (CO_2 and O_2) in the dark, the impacts on algal physiology are poorly understood with respect to overall biomass productivity and lipid accumulation (i.e. subsequent light and dark phases). In the described study, dissolved oxygen was tracked over time in both axenic and xenic cultures to determine potential impacts on algal and bacterial growth rates, algal lipid accumulation, chlorophyll levels, and nitrate-utilization under high-alkalinity conditions across the diel cycle. For the tested growth conditions, both axenic and xenic cultures were either shaken over the entire diel cycle, or only shaken during the light cycles. Axenic and xenic cultures followed similar pH trends, starting at pH of 10.3 and ending at pH>11.2. As expected, with shaking, dissolved oxygen levels remained high during the dark cycles. However, discontinuing shaking during the dark cycles, both axenic and xenic cultures exhibited declining oxygen levels during the dark cycle. The xenic samples showed a sharper decrease in dissolved oxygen levels compared to the axenic cultures, and these results suggest a role of the phycosome in oxygen utilization. Non-shaking axenic and xenic cultures reached nitrate depletion later than shaking samples, and shaking samples had higher lipid contents compared to non-shaking samples. Xenic shaking and non-shaking samples demonstrated opposite trends in chlorophyll concentration. Our data indicate that the phycosome can impact dissolved oxygen concentrations in an algal culture and that algal productivity can be altered with different mixing regimes across the diel cycle. Future work includes the elucidation of mechanisms as well as techno-economic analyses.

(Poster abstracts continue on next page)

CBE Poster #837

Date: 06/23

Title: **Assessing changes in mechanical properties of shale modified by engineered mineral precipitation**

Authors: **Kayla Bedey**[1,2], Matthew R. Willett[1,3], Laura Dobeck[4], Joe Eldring[5], Dustin Crandall[6], Jonny Rutqvist[7], Alfred B. Cunningham[1,2], Adrienne J. Phillips[1,2], Catherine M. Kirkland[1,2]

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Sponsored by: US Department of Energy (DOE), Office of Sciences, DOE Award No.: DE-SC0021324

Fractures in subsurface shale formations serve multiple purposes, for example, in the recovery of resources in hydraulic fracturing or as potential harmful leakage passages through caprocks that may contribute undesired fluids to the atmosphere or functional groundwater aquifers. A proposed method to seal or influence fracture properties is Ureolysis-Induced Calcium Carbonate Precipitation (UICP), a bio-mineralization technology driven by the enzymatic hydrolysis of urea, resulting in the formation of calcium carbonate. *Sporosarcina pasteurii* is a common microbe used as the source of the urease enzyme that catalyzes the chemical reaction. The resulting calcium carbonate can bridge the gaps in fractured shale and reduce fluid flow through fractures. However, there is little information on how this process affects the mechanical properties of the resulting biomineralized shale. The goal of this preliminary work is twofold: first, we aim to identify a repeatable method to test tensile strength along a core axis, and second, we seek to assess the effect of temperature on tensile strength on two types of intact, unfractured shale cores that will be compared with the tensile strength of fractured, UICP-treated cores. All cores in this study adhere to a 2:1 length to diameter ratio (5.08 cm (2 in) long, 2.54 cm (1 in) diameter). A modified Brazilian indirect tensile strength (BITS) test successfully measured splitting tensile strength of intact shale cores from Eagle Ford and Wolfcamp formations at room temperature and 60°C. Though 60°C may not mimic subsurface temperatures of all the shales used in this study, it was chosen due to limitations of the UICP process while still approaching temperatures of shallow shale formations. We observed a decrease in tensile strength as temperature increased. Wolfcamp cores expressed a wider variability in tensile strength values compared to Eagle Ford cores. The modified BITS method was implemented on fractured, UICP-treated shale cores from the Marcellus formation at 60°C to assess the strength of the composite cores. This study represents the first step toward determining the influence of UICP treatment on shale material and its subsequent mechanical strength properties.

CBE Poster #838

Date: 06/23

Title: **Mixed microbial biofilms for the degradation of lignocellulosic biomass: potential applications to closed-loop systems in space**

Authors: **Gabe Griffin**[1], Erika Espinosa-Ortiz[1], Robin Gerlach[1,2]

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Sponsored by: NASA, NSF – National Research Traineeship

As NASA prepares for its goal of long-term space exploration, requiring longer term human habitation of space, a supply of resources (*e.g.* food) will be needed. Plants will be an important source of food, oxygen, and raw materials for various purposes in Bioregenerative Life Support Systems (BLSS). Besides providing various resources, plants will also produce waste (*e.g.* leaves, roots, stems) in the form of lignocellulosic biomass (LCB). The next generation of BLSS will have to consider recovery of potential value-added products from waste, such as LCB. The main components of LCB are cellulose and hemicellulose which are carbohydrates, and lignin which is a complex aromatic polymer that serves as a

structural and protective component. These components can be transformed into value-added products such as fuels (e.g. bioethanol) or platform chemicals (e.g. levulinic acid and benzene). This research aims to investigate the potential use of mixed microbial biofilms for the conversion of LCB into value-added products and recycling of important nutrients in LCB for these next generation BLSS. Though there are chemical and biological methods for the degradation of LCB, they were not designed with space applications in mind. Current chemical methods generally involve heat, pressure, as well as corrosive and toxic chemicals; biological methods usually require longer degradation times, and both generally result in incomplete breakdown and utilization of LCB. Furthermore, until now, biological approaches often limit themselves to single model organisms, often focusing on white-rot-fungi for their ability to degrade lignin with extracellular oxidative enzymes. However, in nature, LCB is degraded by a complex consortium of fungi and bacteria. Our work looks to balance a combined mild physiochemical pretreatment with a biological, multi-species culture approach for the degradation and utilization LCB.

This research investigates the potential synergistic interactions between known LCB-degrading microbes, the fungus *Phanerochaete chrysosporium*, and the bacterium *Pseudomonas putida* KT2440 when cultured as biofilms on an LCB substrate. Biofilms were grown in drip flow reactors (DFR), for 13 days at 30°C on coupons comprised of pretreated tomato plant waste as the LCB substratum with growth medium initially at a pH of 6.5. The tomato plant waste was blended to reduce the size (<2mm) followed by leaching in water for 48 hours at ambient temperature. To assess LCB degradation by the monoculture and co-culture biofilms, LCB coupons were analyzed at the end of the incubation period for composition changes using methods developed by the National Renewable Energy Laboratory (NREL). Microbial growth was assessed, and the biofilms grown on LCB coupons were characterized by confocal and scanning electron microscopy (SEM). Ultimately, this work will provide insights into how we can engineer and optimize such biological systems to degrade, convert, and recycle lignocellulosic biomass.

CBE Poster #839

Date: 06/23

Title: **Achieving a fundamental understanding of flow dynamics in a biofilm reactor**

Authors: **Keenan Vincent**[1], Sarah Morris[1], Erick Johnson[1], Darla Goeres[2], and Yaofa Li[1]

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Sponsored by: Center for Faculty Excellence

Biofilm reactors play a crucial role in biofilm related research, and specifically, in the daily research activities within our Center for Biofilm Engineering (CBE). Although it is widely accepted that biofilm growth is extremely susceptible to various flow conditions, the fluid dynamics aspects of the reactors are not well understood and rarely studied. To this end, an innovative flow apparatus has been designed and built based on a real biofilm reactor. The flow apparatus not only allows us to reproduce the flow within a real biofilm reactor under various well controlled conditions, but also provides valuable optical access that is necessary for optical flow diagnosis. The particle image velocimetry (PIV) technique will be employed to perform innovative flow and shear stress characterization. The goal of this research is to further our understanding of the fundamental flow dynamics in biofilm reactors and potentially provide guidance for better reactor usage and designs.

CBE Poster #840

Date: 07/23

Title: **Impedance spectroscopy sensors to detect biofilm in maple sap**

Authors: **Ruby Jackson**, Matthew McGlennen, Haley Ketteler, Markus Dieser, Seth Walk, Christine Foreman, Stephan Warnat

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilm, an assemblage of surface-associated microbial cells enclosed in an extracellular polymeric matrix (EPS), are known to have pervasive effects in the food processing industry. Specifically, unwanted biofilm growth occurring in

maple sap lines reduces the economic syrup value. One technique to monitor biofilm growth in real time is with the use of microfabricated electrochemical impedance spectroscopy (EIS) sensors. EIS is a method which involves applying sinusoidal perturbations over a range of frequencies across an interface and the responses are recorded. We integrated these sensors into sap lines to continuously measure biofilm growth, temperature, and microbial-specific concentrations, allowing producers to accurately track sap quality in real-time and make decisions on sanitation practices to improve maple syrup quality and economic value. Experiments were performed both abiotically and biotically over a 72-hour time frame in a laboratory-controlled environment, where elemental conditions were closely regulated. In order to observe the effect of humidity and temperature on the sensors, similar experiments were performed outdoors, in an uncontrolled environment. For biotic experiments, EIS data followed trends that suggest microbial growth and confocal microscopy confirmed a biofilm monolayer on the surface of the sensor. Our study suggests that microfabricated EIS sensors can establish a reliable *in situ* quality control system and effectively aid in the mitigation of biofilm growth in sap lines.

CBE Poster #841

Date: 07/23

Title: **Use of epifluorescence widefield deconvolution microscopy for imaging and three-dimensional rendering of biofilms and extracellular matrix material**

Authors: **Heidi J. Smith**[1,2], **Michael J. Franklin**[1,2]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.

Sponsored by: National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474

Microbial biofilms form on surfaces in most aqueous environments and play key roles in many biological processes, ranging from nutrient cycling in the environment to persistence of infectious diseases. Microscopic imaging is a core technique used to help understand microbial biofilms, including their structure and development, location of gene expression, extracellular matrix production, and community composition. Due to the speed of imaging, epifluorescence widefield microscopy is well suited for high throughput image analysis and screening of biofilm samples. However, epifluorescence microscopy is not ideal for obtaining high resolution images of three-dimensional biofilm structures, due to the high background signal from out-of-focus light, which compromises the lateral and axial resolution of the biofilms. Here, we demonstrate the application of epifluorescence widefield deconvolution microscopy (WF-DCM) for imaging *Pseudomonas aeruginosa* biofilms. Using fluorescent stains for *P. aeruginosa* extracellular matrix components, we demonstrate that WF-DCM allows quantification of biofilm components, including cell numbers and biovolumes of matrix materials. We show that environmental conditions, such as calcium addition, affects *P. aeruginosa* PAO1 biofilm structure, and determine the effects of extracellular polysaccharide production on early *P. aeruginosa* biofilm development. The results demonstrate that WF-DCM provides an imaging strategy based on epifluorescence microscopy for obtaining clear in-focus images of three-dimensional biofilms. The technique may be used to quantify biofilm parameters, including the effects of environmental conditions on biofilm structure, and it may be used as a mutant screening strategy that is based on biofilm imaging.

CBE Poster #842

Date: 07/23

Title: **Using controlled growth to characterize carbon metabolism in bacteria**

Authors: **Katerina Bruhl**, Sara Altenburg, Heidi Smith, Matthew W. Fields

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: CBE Physiology and Ecology Lab, ENIGMA Group

Members of a microbial community are characterized by metabolic needs determined by their spatial and temporal placement within an ecosystem. Knowledge regarding the digestive processes that differentiate individuals can be utilized to monitor environments of concern, such as those contaminated by inorganic pollutants. While methods exist to aid in creating a metabolic profile for members of a bacterial consortium, they are often expensive or time-intensive.

However, this process can be streamlined using robust growth curve analysis to create the framework for a metabolic profile. This study measured the growth of two species from the families *Acidovorax* and *Rhodanobacter* isolated from a field site contaminated with high levels of various inorganic pollutants. These species were individually and collaboratively exposed to various individual carbon sources under anaerobic and aerobic conditions. Any resulting growth was then compared across carbon sources to create predictions as to the species' genetic composition. Ultimately, both *Rhodanobacter* and *Acidovorax* illustrated a preferential utilization of serine depending on oxygen presence within the environment. Also, when growing collaboratively, these species grew most rapidly at the expense of the other. Both isolates demonstrate a genetic potential to shift metabolism to selectively catabolize serine to maximize growth potential that countered the success of surrounding microbial partners. Thus, the conservation of this metabolic pathway amongst various bacterial species invites further investigation into the genes underlying the existence of a fitness advantage conferred by rapid serine catabolism.

External Poster 1

Date: 07/23

Title: **Data-driven sanitation chemistry selection: Does it work against biofilms?**

Authors: **Josie Greve-Peterson**[1], Al Parker[2]

Affiliation: [1]PSSI Food Safety Solutions, Kieler, WI, USA.

[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: PSSI Food Safety Solutions

Acknowledging that biofilms will always be a challenge for food producers and manufacturers, how do we best remediate their existence in the food production environment? PSSI conducts the 8-Steps of Sanitation to remove biofilms from the environment to ensure the safe production of food. The 8-Steps of Sanitation starts with sanitation preparation, including disassembly of equipment and dry and wet pick-up. The next step is the first rinse. Then detergent is applied, and surfaces are hand-scrubbed. The fourth step is a second rinse and inspection. If inspection of the area is acceptable, equipment is reassembled, and any condensation is removed. A pre-operational inspection is completed by PSSI and the customer in partnership. After a successful inspection, a final sanitizer is applied, and then the eighth and final step is to document the process. At step four an optional disinfection step is sometimes warranted.

One piece of biofilm control is to opt for sanitation chemistries that are effective against biofilms, which includes those used for cleaning (step 3), disinfection (optional step 4), and sanitization (step 7). The purpose of this project was to gather data from standardized ASTM testing to support the selection of sanitation chemistry against biofilms in food facilities. PSSI partnered with the Standardized Biofilm Methods Laboratory to test various sanitation chemicals using the MBEC Assay (ASTM E2799) with biofilm grown in low shear conditions. Differences in biofilm recovery were seen between two chlorinated alkaline cleaners, Product A and Product B at 2X concentration. Larger biofilm reductions were seen among chemistries in their disinfectant range compared to their respective sanitizer range. Further testing with other ASTM biofilm methods would provide additional effectiveness information on biofilm grown under high shear, which is applicable to various fluid systems common in food production. Moreover, these data will help promote using test data to choose a particular sanitation chemistry over another in the drive towards biofilm control.

(Poster abstracts continue on next page)

External Poster 2

Date: 07/23

Title: **Single-cell encapsulation, detection, and sorting of *Pseudomonas syringae* using drop-based microfluidics**

Authors: **Maureen Pelissero**[1], Travis Lindsay[2,3], Carter W. Hoffman[1,2,3], Connie B. Chang[1]

Affiliation: [1]Department of Biomedical Engineering and Physiology, Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: National Institutes of Health and United States Department of Agriculture

Pseudomonas syringae is a bacterium that causes 'Halo Blight' in legumes, resulting in significant crop yield reductions of up to 43%. The disease spreads through plant-to-plant contact, irrigation waters, and field workers. Currently, copper bactericides are used to control the disease. However, *P. syringae* infections can harbor a subpopulation of dormant, antibiotic-tolerant cells called persisters, which can reactivate and initiate new infections upon the removal of stress. To gain insight into the heterogeneous response of the bacterial population to stress, we employ drop-based microfluidics, where single bacterial cells are encapsulated in water-in-oil drops. Drops containing bacteria can be sorted and isolated from the population, enabling the screening of persister cells for downstream analyses. We utilize drop-based microfluidics and employ a Laser-Induced Fluorescence Detection (LIFD) method to detect fluorescence signals within individual drops. In this work, we aim to advance current methodologies by developing a portable microfluidic unit integrated with fiber optic technologies. This device will enable rapid kHz detection of bacterial growth in drops using scattering and fluorescence detection. Additionally, the device will incorporate droplet sorting via dielectrophoresis to isolate persister cells for further analysis. Our research aims to uncover the mechanisms underlying persistence and will contribute to a deeper understanding of this phenomenon in microbial systems.

External Poster 3

Date: 06/23

Title: **Opto-mechanical removal of biofilms**

Authors: **Devatha Nair**[1], GM Kehe[1], MJ Schurr[1]

Affiliation: [1]University of Colorado Anschutz Medical Campus, Aurora, CO, USA.

Sponsored by: NIH NIDCR K25DE027418, University of Colorado SPARK/REACH National Institutes of Health

Visible-light-responsive azobenzene molecules that can cyclically transition between their *trans* and *cis* states are harnessed to disrupt mature *Pseudomonas aeruginosa* biofilms from the surface of substrates. Our results show 100% biofilm removal of established biofilms and the bacteria dispersed from the biofilms are susceptible to minimal concentrations of antibiotics. The azobenzene polymer coating described in this work can be covalently tethered on to different surfaces and introduces a novel approach to biofilm removal.

External Poster 4

Date: 07/23

Title: **Multispecies biofilm formation of *E. coli* O157:H7 and *L. monocytogenes* on various equipment surfaces formed at different hydrodynamic conditions and their removal by chlorine**

Authors: **Grishma Prabhukhot**[1,2], Ashley Boomer [2], Charles Eggleton[1], Jitendra Patel[2]

Affiliation: [1]Department of Mechanical Engineering, University of Maryland Baltimore County, Catonsville, MD, USA. [2]US Department of Agriculture, Agricultural Research Service, Environmental and Microbial Food Safety Laboratory, Beltsville, MD, USA.

The extent of biofilm growth on a surface can be influenced by surface's topographical features, hydrodynamic shear stress, sanitizer contact time and bacterial species. Stainless steel (SS 316L), PTFE and EPDM coupons were used to

grow *E. coli* O157:H7 and *L. monocytogenes* biofilms alone as a single species or with *R. insidiosa* for 48 hours in a CDC bioreactor containing 10% TSB, under two different hydrodynamic conditions of 100 rpm and 300 rpm. The rpm speeds of baffle plates were converted to shear stresses of 0.368 and 2.462 N/m^2 by incorporating the surface roughness of coupon materials. A concentration of 500 ppm chlorine was supplied as a disinfectant to coupons in an In-line bioreactor for 1 and 4 minutes. Bacterial populations were determined by spiral plating *E. coli* O157: H7 on SMAC, *L. monocytogenes* on MOX, and *R. insidiosa* on TSA. Three individual replicates were performed, and results were analyzed to determine significant differences due to coupon surface materials, chlorine treatment time and bacterial species. Topography of coupon surfaces were examined using profilometer and scanning electron microscope.

A significant interaction effect of biofilm type (single or multi) and type of surface material was observed in removal of *E. coli* O157:H7 biofilm. Following chlorine treatment, multispecies *E. coli* O157:H7 biofilm populations (1.98 log CFU/cm^2 reduction) were significantly different ($p < 0.05$) from EPDM material compared to single species *E. coli* O157:H7 (3.24 log CFU/cm^2 reduction). The type of surface, biofilm, and shear stresses used for biofilm formation exhibited significant interaction effect on *L. monocytogenes* biofilm removal by chlorine. The efficacy of chlorine in single species *L. monocytogenes* biofilm removal was significant on PTFE (3.26 log CFU/cm^2 reduction) and SS (4.13 log CFU/cm^2 reduction) for biofilms formed at 2.462 N/m^2 compared to multispecies *L. monocytogenes* environment (PTFE had 2.19 and SS had 1.37 log CFU/cm^2 log reductions); similar observations were recorded from EPDM material at this shear stress, however the difference was not significant ($p>0.05$).This study highlights that for single species *L. monocytogenes* biofilms formed under higher shear stress, it may be easier to remove them from SS and PTFE surfaces, compared to multi-species environments. *E. coli* O157:H7 biofilms can be easier to remove from stainless steel compared to EPDM for both single and multi-species environments. Existing treatments may not remove multispecies biofilms adequately.

(Agenda starts on next page.)

Montana Biofilm Science & Technology Meeting and Workshops

MONTANA BIOFILM MEETING

Featuring: a day of Engineered Living Materials (ELM)

July 11-13, 2023

Hilton Garden Inn Bozeman

Draft AGENDA

6/28/2023 4:29 PM

Monday July 10

6:00–8:30 pm
Registration & Welcome Reception Larkspur Foyer

Tuesday July 11

7:00-8:00 am CBE & IA Members Coffee Talk Boardroom

7:30–8:00 am
Registration & continental breakfast Larkspur Foyer

Meeting: Larkspur Ballroom

8:00-8:10
Opening Remarks
Matthew Fields, Director, CBE; Professor, Microbiology & Cell Biology, MSU
Darla Goeres, Industrial Coordinator, CBE

SESSION 1: Multispecies Biofilms

8:10-8:15
Session Introduction
Chris Jones, PI, Standardized Biofilm Methods Laboratory, CBE

8:15-8:45
War and Peace: Polymicrobial interactions during cystic fibrosis airway disease
Dominique Limoli, Assistant Professor, Microbiology & Immunology, University of Iowa

8:45-9:15
A biocide study for microbial control in the International Space Station Wastewater Recovery System
Liz Sandvik, Research Engineer, CBE

9:15-9:45
Using a fluorescent probe staining method and COMSAT to assess cell viability in *Aspergillus niger* biofilms treated with antimicrobial agents
Aswathy Shailaja, Postdoctoral Associate, Pediatrics, Duke University Medical Center
Young Investigator Award

9:45-10:15
Food fights and conflict avoidance: A systems biology analysis of contrarian *Pseudomonas aeruginosa* substrate preferences
Ross Carlson, Professor, Chemical & Biological Engineering, MSU, CBE

10:15-10:45 Break

10:45-11:45
Panel: Challenges & rewards of using a multispecies biofilm in the lab
Ross Carlson
Dominique Limoli
Liz Sandvik
Aswathy Shailaja
Moderator: Chris Jones

11:45-12:00
Lightning presentations
CBE Student Researchers

12:00-1:00 Lunch

SESSION 2: Measuring Biofilm

1:00-1:30
Session Introduction
Micro-sensor technology
Stephan Warnat, Assistant Professor, Mechanical & Industrial Engineering, MSU, CBE

1:30-1:55
Imaging for biofilm characterization
Heidi Smith, Manager, Bioimaging Facility, CBE; Asst. Research Professor, Microbiology & Cell Biology, MSU

1:55-2:20
Investigating microbial biofilms as indicators of heavy metals in the Clark Fork Basin, Montana
Elliott Barnhart, Research Hydrologist, USGS

2:20-2:45
16S Ratios method for analysis of active microbial communities
Hannah Goemann, PhD student, Microbiology & Cell Biology, MSU, CBE

2:45
Transport to CBE laboratories

CBE Open House: Poster session and lab demonstrations

3:00–5:00
3rd Floor Barnard Hall, MSU
Schedule available onsite

Wednesday July 12

7:00-8:00 am CBE & IA Members Coffee Talk Boardroom

7:30–8:00 am
Registration & continental breakfast Larkspur Foyer

Meeting: Larkspur Ballroom

8:00-8:05
Opening remarks
Matthew Fields, Darla Goeres

SESSION 3: Medical Biofilms & The Hospital Environment

8:05-8:15
Session Introduction
Kelly Kirker, Assistant Research Professor, Chemical & Biological Eng., MSU, CBE

(Continues on next page)

8:15-8:45
Hydrogen peroxide vs biofilms
Phil Stewart, Regents Professor, Chemical & Biological Engineering, MSU, CBE

8:45-9:15
Bacterial ingress through valves used for venous access
Garth James, PI, Medical Biofilms Laboratory, CBE; Associate Research Professor, Chemical & Biological Engineering, MSU

9:15-9:45
Electrochemical bandage
Haluk Beyenal, Professor, The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

9:45-10:15 Break

10:15-10:45
Metabolic niche composition affects bacteriophage replication in adherent-invasive *Escherichia coli* biofilms
Robert Brzozowski, Postdoctoral Researcher, Biological Sciences, University of Montana
Young Investigator Award

10:45-11:15
Microbial biofilm and sinus infections
James Wilking, Research Scientist, Soft & Biological Materials, Mayo Clinic

State of the CBE
11:15-11:45
Matthew Fields

11:45-1:00 Lunch

SESSION 4: Biofilm & Surfaces Interactions

1:00-1:10
Session Introduction
Madelyn Mettler, PhD Student, Chemical & Biological Engineering, MSU, CBE

Keynote Presentation
1:10-1:55
What's a surface?: Surface sensing in *Pseudomonas aeruginosa*
Matthew Parsek, Professor, Microbiology, University of Washington

1:55-2:30
Effects of surface roughness on Microbiologically Influenced Corrosion (MIC) of copper 101 by *Oleidesulfovibrio alaskensis* G20
Amit Acharjee, PhD Student, Materials Science, MSU, CBE

Yagmur Keskin, PhD Student, Chemical & Biological Engineering, MSU, CBE

2:30-3:00
Mixed domain biofilm responses to coated surfaces
Madelyn Mettler

3:00-3:30 Break

Strategic Planning Meeting for CBE Members
3:30-5:00
Hilton Garden Inn

BBQ Dinner
6:00
Big Yellow Barn, Bozeman

Thursday July 13

7:30–8:00 am
Registration & continental breakfast Larkspur Foyer

Meeting: Larkspur Ballroom

SESSION 5: Engineered Living Materials

8:00-8:30
Session Introduction
Matthew Fields
Chelsea Heveran, Assistant Professor, Mechanical & Industrial Eng., MSU, CBE

8:30-9:00
Programming bacteria to grow into macroscopic, tailored materials
Caroline Ajo-Franklin, Professor, Biosciences, Rice University

9:00-9:30
Engineered Living Building Materials: From concept to commercialization
Wil Srubar, Associate Professor, Civil & Architectural Eng., University of Colorado

9:30-10:15
Industry Panel

10:15-10:45 Break

10:45-11:15
3D printing of engineered bacteria for the production of biofilms-on-a-chip
Anne Meyer, Associate Professor, Biology, University of Rochester

11:15-11:45
Polymer platforms for 3D printing engineered living materials
Alshakim Nelson, Professor, Chemistry, University of Washington

11:45-12:15
Biologically fabricated materials from engineered microbes
Neel Joshi, Associate Professor, Chemistry & Chemical Biology, Northeastern University

12:15-1:15 Lunch

1:15-2:00
Small Group Discussion

2:00-2:45
Moderated Whole Group Discussion

2:45-3:15 Break

3:15-4:00
Government Agency Panel

4:00-4:45
Moderated Whole Group Discussion, Part 2

4:45-5:00
Closing Remarks

***Thank you** for attending the Montana Biofilm Science & Technology Meeting!*

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Save the date!
CBE Pathways to Product Development Meeting
February 2024
Washington, D.C.

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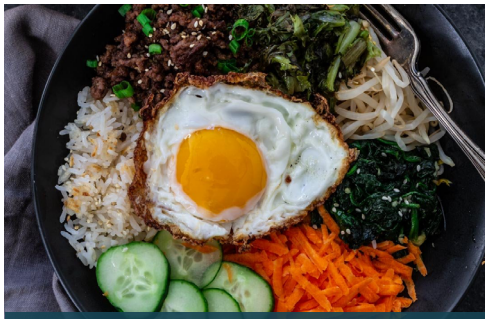
The Center for Biofilm Engineering gratefully acknowledges Biofilm Journal and its publisher Elsevier for its sponsorship.

Things to do in and around the BZN

Main Street Overeasy • Downtown

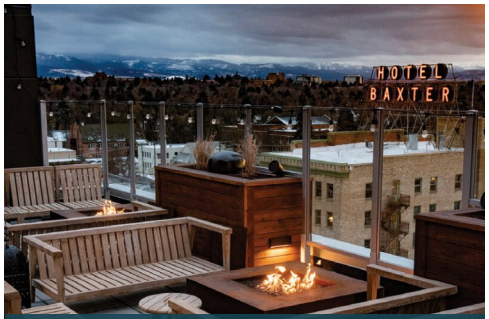
Breakfast

Main Street Overeasy If you enjoy your breakfast prepared exceptionally well, not to mention a creative eggs benedict menu, you'll find Main Street Overeasy to be worth the line that reliably forms mid-morning. Another bonus of arriving early: They have the best cinnamon rolls this side of the Continental Divide.


Ihos' Korean Grill • Downtown

Lunch, Dinner

Iho's Korean Grill is a Bozeman mainstay. If your familiarity of Korean cuisine is limited to sour, ferminted cabbage (kimchi), the broader offerings might surprise you. A sizzling stone bowl of rice, fresh veggies, and a fried egg (bibimbap) is a flagship menu item, as is tasty bulgogi (grilled or stirfried thin slices of beef).



Sky Shed • Downtown

Socializing

The Sky Shed atop the Armory Hotel offers panoramic views of the mountains surrounding our beautiful valley. The food offerings may be unremarkable. But the craft cocktails and sunset views are outstanding. Don't let the photo above mislead you, most of the venue is indoors with table seating, a bar, and sofa areas.

Montana Whitewater Rafting and Zipline Tours

Located in an extraordinarily beautiful canyon bisected by the Gallatin River, Montana Whitewater, Rafting, and Ziplines is an easy onramp for enjoying Montana's great outdoors. While it's obvious that the whitewater rafting allows rafters to interact with one of the three rivers that converge to form the Missouri River, zipliners will also interact with it – soaring over it!

WWW.MONTANAWHITEWATER.COM

Museum of the Rockies

Boasting one of the world's most notable dinosaur fossil collections, a planetarium, and commendable traveling exhibits, the Museum of the Rockies is a slam-dunk crowdpleaser. Much of the success can be attributed to former lead palentologist Jack Horner, who served as scientific advisor to the *Jurassic Park* movie franchise.

WWW.MUSEUMOFTHE ROCKIES.ORG

Alpacas of Montana

These charming cuties are raised for their luxurious wool. So, after your immersive experience petting these friendly animals, stop by the store to peruse their pricy (but worth it!) wardrobe wares.

ALPACASOFMONTANA.COM

Are you planning to explore our vast hiking trails and wilderness areas? Be safe! We have bear spray available for you to borrow! Email skipanderson@montana.edu to request a loaner cannister.