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Proceedings

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SESSION 1: The Rules of Biofilm Behavior, *Matthew Fields, Session Chair*

Keynote Presentation

Mechanisms and consequences of biofilm formation

Presenter: **Fitnat Yildiz**, Professor
Affiliation: Microbiology and Environmental Toxicology, University of California Santa Cruz

Purpose of this Research:
Vibrio cholerae, the causative agent of cholera, is an aquatic bacterium whose ability to form biofilms—surface-associated communities enclosed in an extracellular matrix—supports environmental persistence and enhances transmission during outbreaks. Our objective is to uncover the molecular mechanisms underlying biofilm formation and biofilm-mediated hyperinfectivity.

Methods and Results:
Investigation of the *V. cholerae* matrix proteome revealed that the biofilm matrix contains a diverse set of proteins whose roles in biofilm matrix formation and assembly have not been thoroughly explored. We find that GluP, a glutamate-specific TRAP-TAXI protein, is a previously uncharacterized matrix component that plays a critical role in biofilm architecture.

Next Steps:
Future studies will investigate how the composition and biogenesis of the *V. cholerae* biofilm matrix are modulated by the different surfaces encountered during its infection cycle. These studies will explore how surface-specific matrix regulation contributes to biofilm-mediated hyperinfectivity and transmission.

Industrial Relevance:
Our studies provide conceptual frameworks for the development of intervention strategies to block biofilm formation by *V. cholerae* and other bacterial pathogens known to persist in biofilms.

Living within your means: From basic geometry to a bacterium's limited budget of surface area

Presenter: **Ross Carlson**, Professor
Affiliation: Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Bacterial phenotypes are constrained by their physical dimensions. A cell's length and width define the surface area available to interact with the environment and the volume available to store DNA or synthesize proteins. Cellular geometry is proposed to be a 'rule of life' and to provide theory to interpret a wide range of bacterial phenotypes including, potentially, antibiotic tolerance.

Methods and Results:
Cell geometry and its derivatives, surface area and volume, represent finite 'resource' pools, which were integrated into a systems biology study of bacteria phenotype. Analogous to a business carefully utilizing finite resources, a cell must also strategically utilize surface area and volume pools as poor investments can shift a survival trajectory from success to insolvency.

Next Steps:
To fully exploit the potential of geometry-facilitated control of microorganisms, a thorough accounting of membrane, and volume requirements for essential cellular functions, including pathogenicity factors, is necessary.

Industrial Relevance:
Understanding the intersection of cell geometry and cellular stresses could provide a blueprint for optimizing product formulations. For example, can a formulation be made more effective by adding inexpensive adjuvants that interfere with strategic use of cellular resources like membrane surface area or cellular volume?

Probing the life of gut microbes using next generation physiology tools

Presenter: **Roland Hatzenpichler**, Associate Professor
Affiliation: Chemistry and Biochemistry, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Characterize the activity of human gut and fecal microbes at (close to) in situ conditions at single cell resolution.

Methods and Results:
We are using a unique combination of stable isotope probing and Stimulated Raman Spectroscopy as well as substrate analog probing and fluorescence microscopy and fluorescence-activated cell sorting to determine which cells are metabolically active in the human microbiome.

Next Steps:
So far, we have used our approaches mainly on fecal samples (for benchmarking). Next, we will use human biopsy samples to study the activity of the gut microbiome at (sub)micron resolution in their native state.

Industrial Relevance:
We are able to screen the effects of any compound, including medication, on gut microbiome activity. We do this at single cell resolution, allowing us to address phenotypic heterogeneity, and high speed.

SESSION 2: Interactions at the Interface, *Chris Jones, Session Chair*

Evaluating a standard operating procedure for multidomain biofilm growth in the Industrial Surfaces Biofilm Reactor and demonstration of use cases

Presenter: **Kylie Bodle**, Postdoctoral Researcher
Affiliation: Center for Biofilm Engineering, Montana State University

Purpose of this Research:
We developed a standard operating procedure (SOP) for the Industrial Surfaces Biofilm Reactor (ISBR), a novel system that provides low shear stress, high gas transfer, and intermittent wetting. We evaluated the ruggedness, repeatability, and reproducibility of our SOP. Methods were also developed for growth of three unique multidomain biofilms (MDBs) in the ISBR.

Methods and Results:
After SOP development with a model species, 3 MDB consortia from cooling towers and military vehicles were cultivated. SOP ruggedness was assessed by varying stir, recycle, and influent feed rates. The SOP was rugged for varied stir and recycle rates, but not influent feed. Despite this, repeatability and reproducibility values were comparable to those for ASTM-standardized bioreactors. This method yields reproducible biofilms and establishes the ISBR as a robust biofilm cultivation platform.

Next Steps:
ISBRs will be used to cultivate MDBs on industrial coatings and assess biologically-driven coating defacement. Untargeted metabolomics will be applied to identify metabolites that increase with coating degradation, and transcriptomics will be used to identify genes linked with degradative metabolite production.

Industrial Relevance:
This study demonstrates the ISBR's ability to reproducibly cultivate complex MDBs from diverse environments. It is therefore a valuable tool for various biofilm-related industrial purposes, such as biocide testing, biofouling assessments, or biofilm-resistant surface testing, to name a few.

Investigating the environmental and genetic factors that influence early surface attachment in the foodborne pathogen *Vibrio vulnificus*

Presenter: **Tiffany Williams**, Staff Scientist
Affiliation: Growth and Innovation, Diversey – A Solenis Company

Purpose of this Research:
Vibrio vulnificus is an estuarine bacterium and the leading cause of seafood-related deaths in the U.S. This study investigated the genetic and physical factors that influence initial attachment to chitinous surfaces, aiming to clarify why E-genotype strains are more prevalent in shellfish and marine environments, while C-genotypes are more frequently associated with severe human infections.

Methods and Results:
We developed a chitin attachment assay using magnetic beads and compared wild-type and mutant strains under various environmental conditions. Gene expression was analyzed during early colonization. E-genotypes attached more efficiently to chitin, while C-genotypes showed greater stress resistance once attached. Both genotypes displayed distinct patterns of gene regulation related to attachment.

Next Steps:
These findings highlight how genotype and environment shape early surface colonization and biofilm development. Future work could utilize this type of knowledge to develop strategies for biofilm control and disruption.

Industrial Relevance:
To successfully address biofilm-related problems, it is critical to understand the processes by which biofilms form, thrive, and propagate. The research presented elucidates factors that allow pathogens to colonize surfaces. Such understanding is vital for devising improved strategies to prevent and eliminate biofilms.

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Selecting representative multi-domain microbial communities for surface-associated laboratory consortia

Presenter: **Ghazal Vahidi**, Postdoctoral Researcher
Affiliation: Center for Biofilm Engineering

Purpose of this Research:
Multi-domain biofilms (MDB) predominate in natural and engineered environments, yet most in vitro models rely on simplified single-domain biofilms due to challenges in cultivating diverse environmental MDBs in the lab. Here, we developed a streamlined screening approach to identify stable, ecologically relevant, surface-associated MDB consortia from environmental isolates before in-depth assessments.

Methods and Results:
Bacterial and fungal isolates from a military helicopter were screened in 96-well plates using OD, crystal violet, CFUs, and high-throughput domain-specific imaging. A ranked experimental design assessed growth and compatibility across isolates, intra-domain combinations and MDB consortia. The top MDB consortium with the strongest surface growth, was inoculated into an industrial surface biofilm reactor, with CFUs and microscopy confirming sustained multi-domain colonization on coated coupons.

Next Steps:
This approach links environmental complexity with in vitro control by down selecting defined, surface-associated MDBs from diverse communities. Ongoing work dissects how each bacteria and fungus domain contributes to coating biodeterioration at the biofilm-material interface using tools including sequencing, Coherent Anti-Stokes Raman Spectroscopy (CARS), and nanoindentation.

Industrial Relevance:
This work supports the systematic development of a stable, environmentally relevant MDB consortium, a prerequisite for in vitro studies evaluating biofilms across a range of industrial interfaces, including applications in material degradation and cleaning or mitigation strategies.

Testing the implications of micron-scale texture on microbial surface sensing and biofilm formation: An indwelling device model

Presenter: **Shawna Pratt**, Postdoctoral Researcher
Affiliation: Microbiology and Immunology, Geisel School of Medicine at Dartmouth College
*Young Investigator Awardee

Purpose of this Research:
This research aims to understand the role of micron-scale texture in microbial surface sensing at solid-liquid interfaces, focusing on the pathogenic, model biofilm-forming organism *Pseudomonas aeruginosa*. A key outcome will be the biologically informed improvement of surface design, a potential alternative for reducing biofouling on surfaces.

Methods and Results:
Initiating biofilm formation involves translating mechanical signals from extracellular appendages to cytoplasmic proteins via surface signaling molecules. My research utilizes *P. aeruginosa* strains carrying fluorescent reporters as a readout of these signaling molecules, combined with gene deletions in surface sensing components, for micropatterned microfluidic flow cell studies. This approach investigates how texture influences surface sensing and biofilm establishment.

Next Steps:
Subsequent work consists of incorporating more sophisticated computational models for the characterization of surface textures and testing additional microbes' diversity, such as the non-motile, gram-positive organisms like *Staphylococcus aureus*.

Industrial Relevance:
Biofilm formation on indwelling medical devices contributes to the onset of device-associated infections. Given the rise of antibiotic resistance and the reduced efficacy of antibiotics against biofilms, manipulating surface texture offers an under-explored strategy for preventing and reducing unwanted bacterial colonization.

SESSION 3: Multi-Species Pathology, *Kelly Kirker, Session Chair*

The role of physical forces in shaping polymicrobial communities

Presenter: **Patrick Secor**, Associate Professor
Affiliation: Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
Microbial communities often inhabit crowded, polymer-rich environments such as biofilms, mucus, and infected tissues. In these settings, physical forces arising from excluded volume effect drives microbes to spontaneously self-organize in ways that profoundly impact community structure, stress tolerance, and infection dynamics.

Methods and Results:
Using biophysical, microbiological, and genetic approaches, we explore depletion interactions (an entropically-driven attractive force) between polymers, bacteria with different cells shapes (rod vs. cocci) and filamentous phages. Our findings reveal that physical forces promote ordered bacterial aggregation, modulate material properties of biofilms, and govern competitive outcomes within microbial populations.

Next Steps:
Future work will aim to alter the host environment to weaken the polymer-driven aggregation forces that facilitate biofilm formation.

Industrial Relevance:
Understanding how physical forces promote biofilm formation will inform the development of new strategies for preventing chronic infections, improving biofilm control in industrial systems, and enhancing the design of anti-biofilm materials.

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Biofilm microorganism growth and community composition, and urogenital infection association in active-duty female soldiers using 2nd and 3rd generation Freshnette® female urinary diversion devices

Presenter: **Elizabeth Kostas-Polston**, Associate Professor; Deputy Director
Affiliation: Nursing Science Program, Uniformed Services University of the Health Sciences

Purpose of this Research:
To comprehensively evaluate the performance of Freshette® FUDD 2nd (F2) and 3rd generation (F3) devices in an austere environment, including education and training effectiveness, biofilm microorganism growth and community composition, urogenital infection (UGI) association, and user usage preference.

Methods and Results:
Female warfighters will be screened for enrollment into our crossover RCT, consented, and randomly assigned to 1 of 6 groups. Prior to and post 7-day use, participants will provide biosamples and turn in the used FUDD. Biosamples will be processed within 48-hours. Devices will be shipped to the Montana State University Center for Biofilm Engineering for testing.

Next Steps:
This study will be launched during fall 2025.

Industrial Relevance:
Determine the safety and efficacy of the Freshette® FUDD 2nd (F2) and 3rd generation (F3) devices.

Short-lived success: Recolonization of Carbapenem-resistant *Enterobacterales* in sink drains following repeated disinfection with a peroxide-peracetic acid-based foam

Presenter: **Amy Mathers**, Professor
Affiliation: Medicine and Pathology, University of Virginia Medical School

Purpose of this Research:
Sink drain biofilms in healthcare settings serve as persistent reservoirs for highly drug-resistant pathogens, contributing to healthcare-associated infections. While chemical disinfectants are widely used to reduce biofilm-associated pathogens, the optimal application frequency, efficacy over time and long-term effects on the microbiome remain unclear.

Methods and Results:
We assessed a peroxide, peracetic acid foaming disinfectant's impact on Carbapenem-resistant *Enterobacterales* (CRE), microbiome, and resistome with 3-, 5-, and 7-day intervals for product application in both a SinkLab environment and a hospital sinks. CRE and resistome loads initially declined but rebounded with an increase in *Enterobacterales* and decrease in diversity in both settings.

Next Steps:
Future research should focus on optimizing disinfection strategies that prevent rapid recolonization while preserving microbiome diversity.

Industrial Relevance:
Wide-scale use and deployment of disinfectant targeted for sink drain biofilms in healthcare settings requires rethinking. There is a great need to develop products with a frequency for application as well as microbiologic consequences over time to be considered when developing such products.

Cystic fibrosis: A paradigm biofilm infection in a new era

Presenter: **Philip S. Stewart**, Regents Professor
Affiliation: Chemical and Biological Engineering, Montana State University, CBE

Purpose of this Research:
The goal of this work was to develop a mathematical description of microbial dynamics in the cystic fibrosis lung and use it to predict the relative merits of alternative therapies with respect to infection control.

Methods and Results:
The model was able to capture, qualitatively, these clinically observed features: 1) clearance of bacteria from the healthy lung; 2) persistent infection in the CF lung; 3) transient reduction of bacteria in the CF lung when treated with antibiotics, followed by recurrence of persistent infection; 4) permanent reduction in bacteria in the CF lung treated with a modulator, but not clearance.

Next Steps:
The ability of the model to describe persistent infection depended on the assumption of a tolerant nidus deep in the lung. Thus, we hypothesize the existence of such an infection nidus and highlight the need to characterize the nidus anatomically and microbiologically.

Industrial Relevance:
A clinically relevant prediction of the model is that therapies targeting the tolerant nidus will add benefit to modulator treatment.

SESSION 4: Rapid Trios—The Matrix, *Laura Jennings, Session Chair*

Biofilm dispersal patterns revealed using far-red fluorogenic probes

Presenter: **Andrew Bridges**, Assistant Professor
Affiliation: Biological Sciences, Carnegie Mellon University
*Young Investigator Awardee

Purpose of this Research:
Biofilm dispersal is essential for bacteria to spread between niches, yet how the process is executed at the single-cell level remains mysterious due to the limitations of traditional fluorescent proteins, which lose functionality in large, oxygen-deprived biofilms. The purpose of our research was to develop new cell labeling approach(es) that overcome this challenge.

Methods and Results:
We developed a cell-labeling strategy utilizing fluorogen-activating proteins (FAPs) and cognate far-red dyes, which remain functional throughout biofilm development. Using this approach, we characterize dispersal at unprecedented resolution for the global pathogen *Vibrio cholerae*. Collectively, our findings provide fundamental insights into the mechanisms of biofilm dispersal and demonstrate the broad applicability of FAPs as a powerful tool for high-resolution studies of biofilm dynamics.

Next Steps:
Our probes could be used to tag proteins of interest, to label multiple organisms simultaneously, or to monitor gene expression in polymicrobial communities. Thus, we propose that extensions of the FAP technology employed in this work will enable a mechanistic understanding of microbial community development from the level of single species to the level of complex multispecies assemblages.

Industrial Relevance:
We anticipate that the FAP-fluorogen labeling approaches described here will enable research in diverse areas of industrial microbiology. The oxygen independence of FAP fluorescence combined with the far-red spectral properties could enable interrogation of industrially relevant microbial communities with unprecedented precision.

Random acts of resistance: Polysaccharide and epigenetic heterogeneity in *Pseudomonas aeruginosa* biofilms

Presenter: **Laura Jennings**, Assistant Professor
Affiliation: Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
This research investigates how structural and epigenetic heterogeneity within *Pseudomonas aeruginosa* biofilms contributes to antimicrobial resistance. Specifically, it examines the roles of Pel polysaccharide variability and phase-variable DNA methylation in shaping biofilm resilience.

Methods and Results:
We developed a method to directly visualize Pel in biofilms, revealing heterogeneous expression that contributes to variable tolerance to tobramycin. Additionally, we discovered that the HsdMSR methyltransferase system acts as a stochastic epigenetic switch, generating subpopulations with distinct DNA methylation profiles and variable resistance to polymyxin B. These findings highlight two distinct mechanisms of heterogeneity and drug resistance in biofilms.

Next Steps:
We plan to identify genes regulated by HsdMSR and define how these epigenetic changes influence pathogenesis. Additional work will explore how biofilm microenvironments select for specific subpopulations during infection and treatment.

Industrial Relevance:
This research will inform the design of next-generation therapeutics that disrupt biofilm heterogeneity and enhance antimicrobial efficacy. Insights into epigenetic regulation and polysaccharide variability have potential applications in developing anti-biofilm coatings, precision antibiotics, and infection diagnostics.

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Lab biofilm models, the brain ache of planning a breakup in real life

Presenter: **Alan House**, Senior Scientist
Affiliation: Industrial Cleaning, Novonesis

Purpose of this Research:
Armed with the adage that 'you get what you screen for,' a primary objective of our work is to build biofilms representative of those soiling industrial surfaces, with the ultimate goal of providing enzymes as ingredients in industrial cleaners designed to clean and remove organic soils prior to subsequent sanitization steps. As we struggle to build ever more relevant and reproducible lab biofilm models, we also explore different methods of enzyme screening and measuring performance.

Methods and Results:
Our enzyme screening methods are centered around 96 well plates, relying on either fluorescent stains and high throughput confocal microscopy; or crystal violet staining and absorbance to measure cleaning. As we scale up to validate enzyme blends, we rely on gravimetric or staining methods; but, all these results are predicated on building the right substrate. We rely mostly on visual properties, but also microscopy. Metagenomics or biotyping methods, together with FT-IR have also been used.

Next Steps:
The brain ache manifests when we deliberate about how detailed a soil characterization must take place within the time and resource constraints of a project looking to provide ingredients for a market of generally low-cost cleaners. The ache spills over into considerations of both the broad diversity of enzymes for selection and combination; and the broad diversity of methods available to measure cleaning performance, in both the lab at microtiter and bench scale, and also in the field. Evaluation and thought around these topics will continue as we work to alleviate our brain ache.

Industrial Relevance:
Breaking up is hard to do. As an enzyme research and manufacturing company, we have a long history of using enzymes to break up soils and improve cleaning, mostly in household laundry and dish detergents. Enzymes have the potential to aid in the cleaning of industrial soils, too. Our challenge is to cut through, or embrace, the variability and complexity to prove efficacy, understand if the benefit is of sufficient value to drive change, and commercialize enzymes as one option in the quest to keep industrial lines clean.

SESSION 5: Rapid Trios—Engineered Systems, *Isaak Thornton, Ethan Viles, Session Chairs*

Scaling up and validating processes for biocement production for soil stabilization

Presenter: **Michael Carter**, Biomaterials Research Scientist
Affiliation: Air Force Research Laboratory

Purpose of this Research:
We developed and validated a scalable method for preserving biomass for biocement applications.

Methods and Results:
We used percolation and strength studies to add reconstituted preserved biomass to identify cementation efficacy. We also investigated process variables such as salt concentration, biomass and feedstock concentrations, and number of feedstock application iterations.

Next Steps:
We will continue to investigate larger size applications and improved throughput, yield, and cementation efficacy of biomass production.

Industrial Relevance:
Our method simplifies the application of biocement technology in construction of infrastructure, particularly in resource limited environment.

mRNA PNA-FISH for high resolution gene expression and the spatial ecology of *Legionella pneumophila* biofilms

Presenter: **Ana Barbosa**, PhD Student
Affiliation: Chemical & Biological Engineering, University of Porto

Purpose of this Research:
The main goal of this work is to explore the use of mRNA PNA-FISH to study the regulatory network involved in the biphasic life cycle of *Legionella pneumophila* clarifying the organization and functional development of biofilms.

Methods and Results:
Bioinformatics tools were used to design PNA probes targeting key genes involved in the biphasic life cycle of *L. pneumophila*. Probe performance was optimized in planktonic cells, and gene expression in both planktonic and biofilm states was quantified using RT-qPCR and compared to mRNA PNA-FISH signals. Strong correlations ($r \geq 0.9$) between the two methods validated probe accuracy, and preliminary biofilm analyses confirmed the method's suitability for in situ transcriptomic profiling.

Next Steps:
A multiplex approach is currently under development to enable the simultaneous detection of multiple gene targets in a single hybridization step. Additionally, we will investigate the spatial organization of *L. pneumophila* and its physiological state in a multispecies biofilm.

Industrial Relevance:
Ultimately, findings on the single-cell expression of gene transcripts will contribute to a better understanding of the ecology and survival of *L. pneumophila* in biofilms present in industrial water systems.

Biofilms in pharmaceutical and clinical water systems

Presenter: **Mark Pasmore**, Associate Director
Affiliation: R&D Sterility Assurance and Microbial Sciences, Vantive Healthcare

Purpose of this Research:
The work described in this presentation was performed to evaluate the disinfection and cleaning efficacy in treating biofilms in medically relevant water systems.

Methods and Results:
The presentation describes a combination of ASTM methodologies with simulated use testing to evaluate heat disinfection and biofilm removal chemistries. Heat disinfection was shown to be efficacious at treating biofilms in this study at temperatures exceeding 75 C and the removal testing was less effective with only a couple chemistries showing efficacy at removing biofilms under simulated use conditions.

Next Steps:
The work described here is complete, however. Vantive continues to test biofilms and explore ways to improve our cleaning and disinfection efficacy, while reducing risks associated with the use of disinfection.

Industrial Relevance:
This work is relevant to pharmaceutical and clinical water systems, in that it demonstrates disinfection against a heat tolerant biofilm, as well as describes a test method for evaluating biofilm removal in a simulated pharmaceutical manufacturing system.

SESSION 6: Life in the Extreme, *Liz Sandvik, Session Chair*

Material coatings and strategies for biofilm mitigation in spacecraft water systems

Presenter: **Madelyn Mettler**, Affiliate Postdoctoral Researcher
Affiliation: Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The goal of the research was to explore multiple strategies alone and in combination to control multidomain biofilms in the ISS wastewater tank. Strategies included a coating (Sher-Loxane 800), nutrient limitation (phosphorus), and biocide dose (silver fluoride).

Methods and Results:
CDC biofilm reactor tests indicated that none of the methods reduced biofilm accumulation by more than a couple orders of magnitude alone. But when combined, biofilm accumulation dropped to the limit of detection after 7 days in a CDC reactor. Additional tests were completed in a simulated microgravity reactor where no viable cells were recovered after 7 days when all three biofilm control strategies were implemented together.

Next Steps:
Next steps would include additional simulated microgravity tests which would include longer time scales and repeated inoculation to determine the longevity of the biofilm control.

Industrial Relevance:
This research can be applied to many industrial systems, especially industrial water systems where multiple biofilm control strategies can be implemented to control multidomain biofilms.

Material coatings and strategies for biofilm mitigation in spacecraft water systems

Presenter: **Christine Foreman**, Associate Dean; Professor
Affiliation: Norm Asbjornson College of Engineering; Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The survival mechanisms of extremophilic life on Earth can be studied to help find innovative solutions to industrial issues and more sustainable industry practices.

Methods and Results:
Low water activity, limited nutrients and energy sources, repeated freeze-thaw cycling and exposure to intense UV irradiation are a few of the primary stressors experienced by organisms in cold temperature environments. Psychrophiles have developed broad-ranging adaptations to survive these harsh conditions. One such adaptation is the production of biosurfactants (BS) –which are biologically produced metabolites with amphiphilic properties. BS can enhance microbial survival through the emulsification of recalcitrant nutrient sources, with benefits also shown through the promotion of cell motility, communication, and biofilm formation.

Next Steps:
We will present our investigation into the role of cold active biosurfactants in microbial survival under a variety of environmental stressors.

Closing Keynote Presentation

Polymicrobial biofilm growth and control during spaceflight

Presenter: **Bob McLean**, Regents Professor
Affiliation: Biology, Texas State University

Purpose of this Research:
Examine mixed culture biofilm growth, morphology and gene expression patterns during spaceflight and assess its control by AgF. Evaluate biofilm-induced corrosion on 316L stainless steel (Water Recovery System). Do any unique genome changes arise during spaceflight?

Methods and Results:
Culture investigations using BioCell flight hardware, fluorescently-labeled *E. coli* (cystitis isolate) and *Pseudomonas aeruginosa*. Examination with confocal and scanning electron microscopy with energy dispersive X-ray analysis. Computational analysis of microscopy data along with statistics. From a molecular perspective we are doing metatranscriptome measurements (for gene expression patterns of mixed cultures, in progress); and metagenomic investigations (to identify unique mutations).

Next Steps:
Completing molecular analysis (metatranscriptome and metagenomic) to identify unique gene expression patterns and unique mutations.

Industrial Relevance:
Main aspect is to identify potential biofilm-associated risks for long-term spaceflight.

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POSTER ABSTRACTS

Optimizing the lipid production of Hidden Lake algae for biofuel applications

Abby Novak, *Eco-Start Intern*
Microbiology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Certain microalgae are currently grown on an industrial scale to produce lipids which can be harvested and converted into biodiesel, however, improvements to the efficiency of production and harvesting are needed. Optimizing the lipid production of *Hidden Lake Algae* (*HLA*), a green alga isolated from Hidden Lake in Montana with potential for significant lipid production, is currently in progress.

Methods and Results:
Methodologies include determining preferred nutrients, growth in a hydrogel environment, and analysis of the endemic microbial community, known as the phycosome. Cell growth in a 3D-printed hydrogel is visualized through confocal microscopy. Phycosome analysis includes identifying possible microorganisms that interact with *HLA* through 16S rRNA sequencing, as well as investigating the influence of silica and diatoms on *HLA*, as Hidden Lake has a high concentration of dissolved silica.

Next Steps:
Optimizing microalgal growth in a hydrogel, as well as creating and maintaining a fully axenic microalgal culture for bacterial add-back experimentation, is in progress.

Industrial Relevance:
These findings advance the current understanding of algal growth methods and optimization, creating a more sustainable future for our fuel.

Integrating biomineralization with 3D printing for sustainable bio-based building materials

Adnina Rudaiba, *Graduate Student*
Civil Engineering, Montana State University, CBE

Purpose of this Research:
Bacterial biomineralization has been used to densify aggregates to manufacture building materials sufficient for certain applications. 3D printing may potentially increase the complexity of materials that can be manufactured using biomineralization. This research aims to develop sustainable, bio-based building materials by integrating bacterial biomineralization with 3D printing.

Methods and Results:
E. coli MJK2 (GFP and urease-producing) was cultured with PEGDA formulations varying in concentration (10–30%), additives (LAP, Tartrazine, mineralizing components), exposure time (1–4 hours) and incubation conditions (shaking vs. non-shaking). Viability remained high in 10% PEGDA with additives but declined at higher concentrations, particularly with both LAP and Tartrazine. Longer exposures further reduced viability, with shaking effects varying by PEGDA concentration and duration.

Next Steps:
The next steps involve utilizing DLP (Digital Light Processing) 3D printing technology to embed *E. coli* within PEGDA hydrogel structures, assessing the survival rate of the *E. coli* post-curing, and then figuring out how to introduce and facilitate biomineralization by the *E. coli* to produce biominerals.

Industrial Relevance:
Achieving microbial viability in 3D-printed materials could enable sustainable, load-bearing structures and pave the way for self-repairing building materials. Biological manufacturing and repair processes may also lower CO_2 emissions compared to traditional concrete production.

Salt stress in algae: Insights into high alkalinity survival

Adrienne Arnold, *Graduate Research Assistant*
Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
Algae can convert the greenhouse gas CO_2 into valuable products like biofuels, but CO_2 cultivation is prohibitively expensive. High alkalinity cultivation is cheaper but imposes an osmotic stress on the algae. This project investigates how algae adapt to osmotic stress, with the goal of improving high alkalinity cultivation techniques.

Methods and Results
Chlorella sp. SLA-04 was grown in high alkalinity media with varying levels of salt. *SLA-04* grows more slowly under high osmotic pressure, but overall biomass yields on nitrogen are similar despite the salt stress. LC-MS shows that high salt conditions trigger production of useful compounds like proline.

Next Steps:
We will use metabolic modeling to evaluate possible responses to high osmotic pressure. Why does *SLA-04* make proline and not some other protective compound? Can we control which products algae make under osmotic stress?

Industrial Relevance:
While high osmotic pressure can slow growth, this study reveals that high salt conditions can steer algae towards synthesis of useful bioproducts, giving us new possibilities for cost-effective algal cultivation.

A prophage-encoded sRNA limits lytic phage infection of adherent-invasive *E. Coli*

Alex Joyce, *PhD Graduate Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Prophages are prevalent features of bacterial genomes that can reduce susceptibility to lytic phage infection, yet the mechanisms involved are often elusive. Here, we identify a small RNA (svsR) encoded by the lambdoid prophage NC-SV in adherent-invasive *Escherichia coli (AIEC) strain NC101* that confers resistance to lytic coliphages.

Methods and Results:
Comparative genomics and transcriptional analyses revealed that NC-SV-like prophages and their svsR homologs are conserved in *Enterobacteriaceae* and repress lamB, a phage receptor. Nutrient experiments showed that maltodextrin enhances, while glucose suppresses, lamB expression and phage susceptibility. In vivo, the NC-SV prophage reduced intestinal phage levels and promoted *E. coli* spread, highlighting a nutrient-responsive mechanism that supports bacterial survival during inflammation.

Next Steps:
Although svsR appears to protect against certain lytic phages, further research is needed to elucidate its regulatory network, underlying molecular mechanisms, and potential roles beyond phage resistance in bacterial physiology.

Industrial Relevance:
These findings suggest that targeting prophage-encoded regulators could be a strategy to metabolically reprogram bacterial biofilms and enhance their susceptibility to phage infection. Such approaches may improve the efficacy of phage-based therapies for treating infections and clearing biofilms from medical devices.

Bacterial-fungal interactions in multidomain biofilms

Amanda Haab, Research Technician
Center for Biofilm Engineering, Montana State University

Purpose of this Research
The Water Processor Assembly on the Internation Space Station (ISS) is threatened by biofouling that requires expensive repairs and replacement. In ground testing, bacterial colonization of filamentous fungi leads to increase biomass and biofouling. This series of experiments investigates two-species interactions between bacteria and fungus isolated from the ISS water recycling system.

Methods and Results:
Flask-based assays were used to study two-species growth and interactions between the fungus and fluorescently labelled ISS bacteria in synthetic ISS wastewater. Viable counts on selective agar were used to assess competition between organisms and suspended biofilm flocs were assessed for size, frequency with imaging using the FlowCam and colonization patterns were visualized with Thunder imaging. Several unique patterns of bacterial colonization patterns were observed.

Next Steps:
Additional tests to investigate the varied colonization patterns could be conducted using co-cultures with a killed hyphal fungus to analyze whether bacterial colonization is simple bacterial surface colonization or due to bacterial-fungal signaling. More distal interactions and changes in floc formation could be further probed through membrane separated co-cultures.

Industrial Relevance:
Biofilms rarely grow in monocultures. Studying interactions within multispecies biofilms allows us to further understand emergent properties such as increased biomass production and identify challenges in mitigating biofilms representative of environmental and industrial systems.

Investigating the production of flagella and visualizing motility by *Sporosarcina pasteurii*

Britta Meyer, *Eco-Start Intern*
Mechanical and Industrial Engineering, Montana State University, CBE

Purpose of this Research:
The goal of this research is to determine whether, and under which conditions, *Sporosarcina pasteurii* produces flagella and becomes motile. While this microorganism is well studied in the context of biomineralization, the conditions under which *S. pasteurii* produces flagella and becomes motile remain unclear.

Methods and Results:
Motility of *S. pasteurii* was confirmed by motility plate assays and direct observation by phase contrast and fluorescence microscopy of unstained and NanoOrange stained cultures. The direct visualization of flagella with microscopy has been unsuccessful using stained cultures or cultures partially immobilized on agar pads. There is evidence of flagella-like structures from scanning electron microscopy (SEM), but the potential of artifacts introduced during sample preparation cannot be ruled out.

Next Steps:
The next steps for this research include using a lower volume and concentration of culture, and testing alternative fixing solutions to image the flagella. These results will be used to support other experiments regarding the possible chemotaxis of *S. pasteurii*.

Industrial Relevance:
Sporoscarina pasteurii is frequently used in microbially induced calcium carbonate precipitation (MICP)—a process used in numerous engineering applications. Understanding its motility and how to control it will aid in optimizing it's applications in engineering, such as biocementation and self-healing concrete.

Patterning biomineralization through spatially controlling microorganisms

Cade Wichmann, *Undergraduate Student*
Mechanical and Industrial Engineering, Montana State University, CBE

Purpose of this Research
This research aims to find ways to adhere microbes such as *Sporosarcina pasteuri* and *Escherichia coli MJK2* onto patterned surfaces. Biomineralizing bacteria onto patterned surfaces would provide an ability to create complex objects from sustainable concrete production, with added abilities to modify surfaces and repair cracks.

Methods and Results:
S. pasteurii and *E. coli MJK2* both provide promising abilities to biomineralize, while also having strong negative charge. Because of these factors, the microbes can attract to positively charged amine groups found on silicon wafers dipped in a mixture of APMDES (3-aminopropylmethyldiethoxysilane) and ethanol (1:100) this process is called functionalization. There have been promising results in this patterning, with visibly more microbes on functionalized wafers than on non-functionalized ones.

Next Steps:
In the future, more experiments will be conducted to refine the functionalization and patterning processes before moving onto biomineralization on these surfaces. In the future, there are plans to see the effects of sonication on bacteria to determine if vibrations are harmful to viability. Additionally, future experiments on *E. coli MJK2* will be preformed to see its biomineralization properties.

Industrial Relevance:
The ability to adhere microbes onto surfaces as intended would be a giant step in bringing biomineralized concrete into industry. This research steps toward biomineralizing concrete into complex shapes, allowing for more complex construction techniques, cheaper crack repairs in existing concrete, and visually appealing art.

Roadblocks in the fungal highway: Controlling fungal growth by disrupting transport mechanisms

Campbell B. Putnam, *Graduate Research Assistant*
Chemical and Biological Engineering, Montana State University, CBE

Purpose of this Research:
Filamentous fungi [e.g., *Aspergillus niger*, *Candida albicans* (dimorphic), *Fusarium venenatum*] are remarkably resilient, often thriving under extreme environmental and chemical stress. This research investigates how internal convective transport within fungal filament networks supports their growth, survival, and stress tolerance.

Methods and Results:
Overnight cultures of *F. venenatum* were grown on thin agar pads and imaged using epifluorescence microscopy. Using fluorescent markers, we mapped (1) filament branching architecture and (2) convective transport rates across the network. These experimental data informed a computational model of the fungal network, where we simulated flow and systematically removed segments to determine which structural disruptions most effectively compromised transport.

Next Steps:
In real-world environments, ranging from soil to fermentation processes, to human tissue, filamentous fungi often coexist with bacteria. Ongoing work is focused on how fungal network architecture and transport dynamics change when *F. venenatum* is cocultured with mutualistic, commensal, or antagonistic bacteria.

Industrial Relevance:
Due to their exceptional durability, filamentous fungi are frequent culprits in bioprocess contamination, damage to paint/coatings, and opportunistic infections. This work identifies internal fluid transport as a potential vulnerability, opening new avenues for antifungal strategies in sanitation, manufacturing, and healthcare.

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Stymieing the slime: Viral engineering for eradicating mucoid *Pseudomonas aeruginosa*

Dominick Faith, *PhD Candidate*
Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
Bacteria have existed for over 3.5 billion years, spreading into every ecological niche despite constant threats like viral infections from bacteriophages, which outnumber them 10:1 and infect them an estimated 10^{23} times per second. But if phages are so adept at killing their bacterial hosts, then how is it that bacteria survive?

Methods and Results:
We have found that in the bacterium *Pseudomonas aeruginosa*, cells that overproduce the exopolysaccharide alginate are more tolerant to phage infection. In fact, cells that harbor mutations in the gene mucA have an increased survival rate and are selected for during phage infections. MucA inhibits the sigma factor, AlgU, which initiates transcription of genes involved in alginate production. Mutations in mucA lead to dysregulation of AlgU, and therefore, overproduction of alginate.

Next Steps:
We aim to engineer virulent phages to express alginate lyase (Alg2A) using a CRISPR-based strategy that replaces a target site in the phage genome with alg2A under a strong promoter via homologous recombination. We hypothesize that the resulting phages, will be able to degrade alginate and render alginate-overproducing cells more susceptible to phage infection.

Industrial Relevance:
Phages are promising tools for biofilm eradication due to their targeted bactericidal activity. This project aims to engineer phages to express enzymes that degrade bacterial exopolysaccharides, counteracting the unintended selection of biofilm-forming populations during phage infection.

APMDES surface functionalization of sand and fiber reinforcement within MICP bricks

Ethan Heyneman, *Graduate Research Assistant*
Chemical and Biological Engineering, Montana State University, CBE

Purpose of this Research:
The overarching goal is to develop sustainable building materials as concrete alternatives using biological processes. The question this experiment asks: how does Aminopropyl(diethoxy)methylsilane (APMDES) surface functionalization of aggregate and/or silica fiber reinforcement within microbially induced calcium carbonate precipitate (MICP) bricks impact their unconfined compressive strength?

Methods and Results:
Within a mold sand is packed with fibers before receiving MICP treatment with *Sporosarcina pasteurii* to bind the two together. Bricks are cured before undergoing an unconfined compressive load test. MICP bricks of solely APMDES sand had significantly larger ultimate strength than those of non-APMDES sand, on average 60% stronger. Only bricks composed of APMDES sand and reinforced with non-APMDES fibers had an ultimate strength not significantly lower than those composed solely of nonfunctionalized sand.

Next Steps:
The fiber reinforced samples did not see significantly larger ultimate strength than those unreinforced likely because of the type of fiber used. The silica fibers used resembled a wool like material that would not mix well with sand. Investigating fibers that can have APMDES bound to its surface and mixes well in sand may yield data showing significantly larger ultimate compressive strength.

Industrial Relevance:
Conventional concrete is responsible for ~8% of global CO_2 emissions through its manufacturing and curing. Concrete is often used where its large loading strength is not needed but its 'carbon cost' is still fully paid. For sidewalks or retaining walls MICP bricks can provide the needed strength without the external carbon cost.

Microbial biomineralization to enhance material properties of 3D printed cellulose composites

Ethan Viles, *PhD Candidate*
Mechanical Engineering, Montana State University, CBE

Purpose of this Research:
Cellulose composites have garnered attention for their sustainability benefits, impressive material properties, and their ability to be 3D printed. Here, we improve the material properties of 3D printed cellulose composites through microbial biomineralization. Additionally, we investigate the spatial distribution of biomineral and its affects on the material properties of this composite.

Methods and Results:
3D printed cellulose composites were biomineralized via *Sporosarcina pasteurii* for 1, 4, 8, 24, or 48 hours. We quantified mineral accumulation, mineral location, and material properties through thermogravimetric analyses, scanning electron microscopy, and 3-point bend testing respectively. Mineral accumulation improved the material properties of cellulose filaments for short biomineralization durations, but longer durations worsened the material properties compared with untreated filaments.

Next Steps:
In the future, we will investigate interfacial bonding between biomineral and cellulose using nanoindentation. Additionally, we will utilize analytical models that explore the affects of mineral size, interface bonding, and mineral distribution on the material properties of these filaments.

Industrial Relevance:
This research will inform the design of future sustainable structural materials by identifying optimal biomineral content and spatial distribution needed to enhance mechanical performance in 3D printed cellulose composites.

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Quantifying and controlling the motility of ureolytic bacteria for engineering applications in biomineralization

Felix Weinhardt, *Visiting Research Fellow*
Hemholtz Centre for Environmental Research (UFZ)

Purpose of this Research:
This research aims to identify potential chemoattractants and repellents that influence the motility of *Sporosarcina pasteurii*. A deeper understanding of its chemotactic behavior could enable targeted guidance of the bacterium to specific locations, thereby enhancing its use in biomineralization for engineering applications.

Methods and Results:
The initial approach involves established chemical plug assays on soft agar plates to screen for chemoattractants. For more detailed and dynamic studies, microfluidic chips will be used to enable real-time, single-cell analysis of bacterial movement.

Next Steps:
Future work includes designing robust and reproducible microfluidic protocols to study chemotaxis in detail. Additionally, integrating electrodes into the setup will make it possible to explore the ability to control movement in an electrical field.

Industrial Relevance:
The findings have direct implications for environmental engineering, particularly in areas like construction materials and groundwater remediation. Moreover, the development of novel microfluidic platforms and protocols may contribute to innovations in microfluidic sensor technologies.

Understanding the influence of thermochemical pretreatments on metal-zeolite catalysts synthesized from chlorides

Hannah O'Connell, *Undergraduate Researcher*
Chemical Engineering, Montana State University, CBE

Purpose of this Research:
The purpose of this research is to determine how to remove chlorine from the zeolite while still using the chloride metal precursors. My overall research is producing nickel zeolites that can be used in carbon capture via a reverse urea hydrolysis reaction.

Methods and Results:
The synthesis method utilizes solid-state ion exchange, high heat treatment of the zeolite mixtures, and application of other treatments if needed applied after heating. My most recent research step determined that heat treatment, and heat treatment with washing are the two effective methods to distribute nickel with low amounts of chlorine on the sample while maintaining the zeolite's crystal structure.

Next Steps:
The next steps in my research are to run the reverse urea and forward urea reactions in a batch reactor using my synthesized zeolites of different metal loadings as catalysts and analyze the findings.

Industrial Relevance:
Because zeolites are inorganic crystal materials, they can handle a broader range of temperature and pressure than enzymes. This means that companies can utilize these zeolites to capture carbon they omit in their processes and convert it to the valuable resource of urea.

Engineering microbialites for infrastructure materials

Hossein Khadivar, *Graduate Research Assistant*
Chemical and Biological Engineering, Montana State University

Purpose of this Research:
This research aims to investigate how the synergistic relationship between photoautotrophs (such as algae and cyanobacteria) and heterotrophs (such as fungi) can be engineered to grow structural materials.

Methods and Results:
The photobionts as the main driver of biomineralization are being screened in terms of pH tolerance and their ability to induce a pH shift indicative of biomineralization potential followed by characterizing the biomineralization outcomes for each photobiont. Next, co-cultures of photobionts and mycobionts will be assessed in terms of biomineralization capabilities using calcium carbonate precipitation as the main factor.

Next Steps:
After finalizing the screening experiments for photobionts, each photobiont will be used to assess their ability to establish stable co-cultures with a fungus and the associated biomineralization outcomes. This will allow us to choose the top performers among the co-cultures in terms of biomineralization.

Industrial Relevance:
The materials being developed could make construction and infrastructural materials more sustainable, contribute to better environmental remediation technologies, and the biofabrication of new materials, which could be capable of self-repair and maintenance.

Building biofilms: 3D printing hydrogel constructs and scaffolds for microbial engineering

Isaak Thornton, *Postdoctoral Researcher*
Center for Biofilm Engineering

Purpose of this Research:
This research explores the use of 3D printing as a versatile tool to create and study artificial biofilms and engineered living materials. By selecting and integrating various materials, printing methods, structural designs, microorganisms, and environmental conditions, we aim to advance both the understanding of microbial behaviors and the development of engineered living materials.

Methods and Results:
We use a range of 3D printing methods—including digital light processing (DLP) for hydrogel constructs and fused deposition modeling (FDM) for scaffolds—to fabricate structures with embedded or surface-adhered microbes. We create lab-scale models to explore areas of research including microbial interaction, bioremediation, and biomineralization.

Next Steps:
We plan to expand upon this work by incorporating complex porous geometries, multiple species in co-culture, and varied environmental conditions. Future research will focus on developing living materials for tailored applications in pollution remediation, biofuel production, and the creation of biomineralized living building materials.

Industrial Relevance:
3D printing provides a reproducible and customizable approach to study specific microbial interactions and behaviors. This supports the development of targeted strategies to address real-world challenges in areas like biofilm control, bioremediation, and engineered living materials.

Stable isotope labeling of algae

J.P. Kaffer, *Graduate Student*
Microbiology, Montana State University, CBE

Purpose of this Research:
Identification of recently made proteins and lipids produced during specific growth phases in the high pH/high alkalinity adapted alga *Chlorella sp. strain SLA-04* using stable isotopes and mass spectrometry.

Methods and Results:
Previous work has shown that the transcriptome of *SLA-04* undergoes significant change after running out of available nitrogen. Here, we are using 13C-bicarbonate to see how lipids and proteins are altered as a result of regulatory changes and resulting changes in metabolism.

Next Steps:
Initial labeling attempts were unsuccessful, prompting further testing into higher 13C-bicarbonate percentages and expanded testing outside of biomass to understand the fate of 13C-bicarbonate in the system.

Industrial Relevance:
Algae are a potential alternative to petroleum hydrocarbons and a leading option for biofuel and biochemical production. Isotopic labeling allows for a more focused view of the biochemical activity of algae, which can give more insight into how to increase productivity.

Chlorella sp. SLA-04 growth in simulated suboptimal microenvironments of industrial raceway ponds

Jessica (Bear) Wood, *PhD Candidate*
Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
Industrial outdoor microalgae cultivation employs active mixing to maximize gas exchange, nutrient availability, and light exposure. Although mixing may be continuous, there can be formation of microenvironments (light, temperature, aeration, etc.) that harm overall microalgae biomass productivity and are poorly understood.

Methods and Results:
In this study, we employed growth of *Chlorella sp. SLA_04* high alkalinity (HA), low alkalinity (LA), axenic, xenic, and shaking during the entire cycle or stationery during the dark period. HA cultures deplete nitrate faster and have higher productivity than LA cultures. HA Shaking and Non-shaking, Axenic and Xenic, cultures have similar growth results where Nile Red accumulation, starch content, and AFDW were all highest in HA shaking cultures compared to HA non-shaking.

Next Steps:
To continue to provide industrially relevant data, cost-benefit analysis of energy-saving methods (no shaking/mixing) and biomass growth will be investigated.

Industrial Relevance:
The continuous development of microalgae cultivation practices will advance the renewable fuels field and strive to make microalgae biofuel competitive with petroleum-based fuels.

Synthesizing 3D bioprinting and microscopy to design, observe, and quantify synthetic biofilms

Kathryn Zimlich, *PhD Candidate*
Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
Biofilms are ubiquitous on Earth, and at the microscopic scale, are extraordinary examples of the intricate structure-function relationship which exists in all living communities. Investigating the reciprocal connection between biofilm structure and function is crucial for understanding biofilm development in diverse contexts and harnessing their biotechnological potential.

Methods and Results:
We have developed light-based 3D printing methodology to fabricate biofilms, vary initial characteristics, and quantify development with confocal microscopy. The initial concentration of bacteria in 3D printed biofilms was systematically varied, spanning approximately three orders of magnitude, and cellular growth and aggregate development was followed over six days. Steady-state measurements of colony size, colony number, and characteristic length scale depend on initial cell concentration.

Next Steps:
We are currently transitioning synthetic biofilm fabrication to flow cell environments and exploring the effects of systematically changing mass transport on synthetic biofilm development and resource processing. Future work will also involve using 3D bioprinting to systematically vary the spatial mixing of co-culture populations and observing the effects of spatial mixing on biofilm development.

Industrial Relevance:
This integrated engineering approach opens new possibilities for both innovation in biofilm research and technology as well as improved understanding of underlying biofilm fundamentals.

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Dry biofilms contribute to bacterial persistence on environmental surfaces

Kelli Buckingham-Meyer, *Research Lab Manager*
Center for Biofilm Engineering

Purpose of this Research:
A comprehensive definition of dried biofilm has yet to be determined, however, it is recognized that dried biofilms survive longer than 12 months and are causative agents of healthcare-acquired infections (HAIs) and food contamination events. We hypothesize that microbial biofilms have different tolerance to drying, which affects their ability to persist in various environments.

Methods and Results:
A panel of twenty HAI and food contamination microorganism biofilms were formed in microtiter plates, the media was removed at the end of the growth period and dried in a humidity-controlled environment for 1, 4, 28 days and 4 months. To determine viability of the biofilm, media was added after drying and bacterial growth was monitored for 24 hours by optical density. High, intermediate and low desiccation tolerance was observed among strains over time.

Next Steps:
Future work will investigate the mechanism of antimicrobial tolerance by a variety of dry biofilms.

Industrial Relevance:
Dried biofilms are relevant across many industries where they can serve as a reservoir for microbes, affecting the ability to effectively clean and sanitize surfaces. Here, we have established methodologies for screening strains for biofilm desiccation tolerance and determining the effect on antimicrobial sensitivity.

Assessing cross-species protection to hydrogen peroxide in multi-domain cultures of ISS water system isolates

Kyle Walde, Undergraduate Researcher
Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The main goal of this research is to evaluate how different microbial species respond to hydrogen peroxide treatment by measuring their viability and ability to degrade peroxide. Additionally, this project investigates the potential for cross-species protection and assesses the efficacy of hydrogen peroxide as a biocide for microbial control in space-based water recycling systems.

Methods and Results:
A batch flask assay was used in which bacterial and fungal cultures were grown in mono or coculture with hydrogen peroxide dosing after 24 hours of growth in synthetic wastewater. Residual peroxide was monitored with an ABTS/HRP assay, and viability was monitored by plating on selective media for multiple days after treatment. Key findings include differences in hydrogen peroxide tolerance between species, evidence of cross-species protection, and a rebound of microbial growth after treatment.

Next Steps:
The next steps of this research include repeating previous experiments, testing with different hydrogen peroxide concentrations, and eventually testing repeated dosing in a continuous flow system rather than a batch system.

Industrial Relevance:
This research investigates biofilm mitigation in wastewater systems, specifically on the ISS. Hydrogen peroxide can potentially prevent biofouling in wastewater systems, stopping clogging and damage to the systems.

Fungal hyphae as natural scaffolds for enhancing biocement properties

Martina Du, *Graduate Research Assistant*
Chemical and Biological Engineering, Montana State University, CBE

Purpose of this Research:
Our research investigates how fungal-bacterial cocultures can enhance biocement, addressing key challenges in mechanical strength, microbial viability, and cost-efficiency. By leveraging the ureolytic properties of *Sporosarcina pasteurii* and filamentous fungi, we aim to optimize microbially induced calcite precipitation (MICP) for sustainable infrastructure applications.

Methods and Results:
Biocement columns were formed using alternating microbial and biocementation solutions, with unconfined compressive strength (UCS) assessed at multiple points. Columns pre-seeded with fungi and later inoculated with *Sporosarcina pasteurii* exhibited the highest UCS, surpassing bacteria-only samples. Ongoing research focuses on scaling biocement structures (2-inch cubes) and spatial chemical analyses to refine material uniformity.

Next Steps:
Next steps include engineering fungal-bacterial consortia to enhance mineral distribution, microbial longevity, and self-healing potential. Further work will focus on cost reductions by optimizing fungi's ability to thrive on low-cost substrates and strategically coculturing with bacterial partners other than *Sporosarcina pasteurii*.

Industrial Relevance:
Biocement offers a sustainable, reduced-carbon alternative to traditional concrete, helping to lower CO_2 emissions while providing self-repair capabilities. This work aims to advance biocement as a durable, self-healing, and sustainable construction material by leveraging the synergistic properties of fungal-bacterial systems.

Visualizing MICP-induced flow modifications in shale fractures using magnetic resonance velocimetry and micro-CT

Matthew Willett, *Graduate Research Assistant*
Chemical Engineering, Montana State University, CBE

Purpose of this Research:
The main goal of this research was to evaluate how microbially-induced calcium carbonate precipitation (MICP), a biofilm-based sealing strategy, alters fluid flow in shale fractures, with the broader aim of enhancing subsurface sealing for carbon-neutral energy applications.

Methods and Results:
We used magnetic resonance velocimetry (MRV) to non-invasively measure 3D flow fields and propagators in fractured shale cores before and after MICP treatment. Complementary micro-CT imaging was used for local cubic law (LCL) simulations of flow. The results showed that MICP

significantly altered preferential flow paths and reduced permeability, confirming that MRV is a powerful tool for tracking sealing progress in subsurface rock fractures.

Next Steps:
The next steps are to refine MRV protocols for temporal tracking during active MICP injection. These efforts will support the development of predictive models for optimizing biomineralization-based sealing strategies in subsurface energy systems.

Industrial Relevance:
This research supports industrial efforts to enhance subsurface integrity for carbon storage, hydrogen geo-storage, and geothermal energy by providing tools to monitor and optimize fracture sealing.

Ribosome hibernation in *Pseudomonas aeruginosa*; in vivo interactions between ribosomes and hibernation promoting factors

Mert Kanik, *Postdoctoral Researcher*
Center for Biofilm Engineering

Purpose of this Research:
Pseudomonas aeruginosa is a major human pathogen capable of going dormant which allows it to escape host immunity as well as antibiotic treatments. We studied hibernation factors which bind the ribosome to induce dormancy and their binding dynamics with each other and the ribosome itself.

Methods and Results:
We developed a fluorescence-based BiFC assay optimized for *Pseudomonas aeruginosa* to study hibernation factors in vivo. This enabled us to visualize protein-protein interactions within the cell. Surprisingly, some hibernation factors exhibited behavior consistent with ribosome biogenesis factors, challenging the current model.

Next Steps:
We are currently using our established BiFC system to uncover the role of the signaling pathways in dormancy, such as the stringent response.

Industrial Relevance:
Hibernation factors and the pathways that regulate them are promising targets for therapeutic approaches.

Vibrioids: Nitric oxide dependent biofilm aggregates of *Vibrio cholerae*

Michelle Cherne, *Research Scientist*
Microbiology and Cell Biology, Montana State University

Purpose of this Research:
Vibrio cholerae cultured with human colonic epithelial cells in the presence of nitric oxide form large aggregates, exhibiting a biofilm 'shell' which encapsulates motile planktonic bacteria. Our goal was to characterize the formation and composition of these aggregates.

Methods and Results:
Vibrioids formed after 14-20 hours of treatment with nitric oxide, and the planktonic bacteria within the vibrioids became immobile after 27 hours. Confocal microscopy revealed vibrioid shells were composed of extracellular DNA and carbohydrates, suggesting a biofilm-like composition, while the planktonic bacteria within were negative for these components. Knockout of the nitric oxide sensors H-Nox and NosP reduced aggregate size but did not significantly block formation.

Next Steps:
To further understand this unusual bacterial behavior, we will next perform live imaging experiments to assess formation dynamics and potential clonality. Additionally, we will investigate the role of aggregate formation in *V. cholerae* virulence within the nitric oxide-rich human intestine using human intestinal cell lines and organoids.

Piloting treatment wetland technology from raw wastewater to effluent polishing applications

Mohammad H. Mozaffari, *PhD Candidate*
Civil Engineering, Montana State University, CBE

Purpose of this Research:
This research aims to test three pilot vertical flow treatment wetlands, each designed for a specific use: raw influent treatment (lagoon replacement), ammonia removal from lagoon effluent, and nutrient polishing of Bozeman's high-quality effluent.

Methods and Results:
Three pilot-scale vertical flow treatment wetlands were constructed and operated at the Bozeman Water Reclamation Facility, each tailored to a different treatment goal. Weekly sampling showed that the two-stage system treating raw wastewater removed over 81% COD and 42% ammonium in the primary stage, with complete nitrification in the secondary stage. The tertiary wetland further polished Bozeman's effluent, achieving up to 48% phosphorus and 32% total nitrogen removal.

Next Steps:
The next steps include continuing pilot system operation through seasonal changes to assess long-term performance and resilience. Results will inform design guidelines for implementing vertical flow wetlands across Montana's diverse wastewater treatment needs.

Industrial Relevance:
Vertical flow wetlands offer a low-cost, low-energy solution for ammonia and nutrient removal, ideal for small communities and aging infrastructure.

Unraveling oxidative stress pathways in *Psychrobacter cryohalolentis*: Insights from silica induced metabolic changes

Nicole (Nicki) Krysiak, *Graduate Student*
Biochemistry, Montana State University, CBE

Purpose of this Research:
In this research, I investigate the ability of *Psychrobacter cryohalolentis*, isolated from Earth's polar regions, to grow under simulated Enceladus-like chemical conditions. These microorganisms are

exposed to different concentrations of silica that may be found on Enceladus to determine under which set of conditions life could be possible.

Methods and Results:
Bacterial activity is determined through the incorporation of isotopic labels, specifically heavy water (D2O), using Raman Spectroscopy. Metabolic adaptations are measured through comparative metabolic analyses using Liquid Chromatography Mass Spectrometry (LCMS).

Next Steps:
Next steps are to study the effects of the addition of reactive oxygen species quenchers to determine if metabolic pathways that were inhibited could be activated again.

Industrial Relevance:
This research aims to provide supporting evidence that life is possible under the geochemical conditions present on Enceladus and to understand metabolic adaptations related to extreme environments.

Screening and assessing extremophilic microalgae for potential use in industrial bioproducts

Patrick Thomas, *Postdoctoral Researcher*
Center for Biofilm Engineering

Purpose of this Research:
Microalgae have the potential to make sustainable food, animal feed, bioplastics, and biofuels at impactful scales. Our research goal is to test new ways of using extreme (i.e., high pH and high alkalinity) environments to make algae farming more reliable, productive, and economical in the future.

Methods and Results:
To achieve this, we use a combination of high-throughput bioprospecting as well as testing and validation of known extremophile algae strains. Laboratory-scale tests have shown common algae from natural aquatic environments have the potential to be used for industrial production of algal bioproducts, that high pH and high alkalinity culturing can exclude several potentially harmful algae species, and that algal microbiomes could potentially alter algal sensitivities to antibiotic stress.

Next Steps:
Ongoing work will test how heterotrophic bacteria influence the growth of these extremophilic algae. I will use high-throughput tests to assess how hundreds of carbon substrates affect algal growth and will test new process engineering approaches for using carbon compounds excreted by algae to induce PHA bioplastic production by bacteria.

Industrial Relevance:
This work will help the algae industry by testing and validating novel approaches for producing value-added bioproducts from algae, and will provide insights for any industry sector in which the ecology and physiology of extremophilic microbes may play an important role.

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Bacteriophage-mediated biofilm dynamics in *Leptospira*: Environmental persistence to Leptospirosis pathogenesis

Reetika Chaurasia, *Assistant Research Professor*
Microbiology and Cell Biology, Montana State University, CBE

Purpose of this Research:
This study investigates how bacteriophage induction influences *Leptospira* biofilm dispersal, persistence, and pathogenicity. By combining genomic, molecular, and in vivo approaches, we aim to reveal a novel phage-biofilm interaction that may inform new therapeutic and diagnostic strategies for leptospirosis.

Methods and Results:
Leptospira biofilms were visualized using confocal microscopy after fluorescent staining, and prophage regions were identified through PHASTER/PHASTEST analysis. Phage induction by Mitomycin C will be examined using plaque assays, qPCR, TEM, and RNA-seq, while in vivo models will monitor phage activity, biofilm dynamics, and kidney pathology.

Next Steps:
We aim to test the hypothesis that phage induction, triggered by environmental or host-related stressors, promotes biofilm dispersal and enhances pathogen transmission. Further studies will explore phage-biofilm-host interactions to uncover novel mechanisms of persistence and transition between hosts and environments.

Industrial Relevance:
Insights into phage-mediated biofilm control may enable novel anti-virulence therapies for leptospirosis. Prophage and biofilm markers also hold promise for enhanced diagnostics, with broad industrial relevance in healthcare, agriculture, and environmental monitoring.

Safe drinking water thanks to bacteria

Roos Goedhart, *Visiting PhD Candidate*
Water Management, Delft University of Technology

Purpose of this Research:
Can bacteria help treat arsenic (As) contaminated groundwater to produce safe drinking water? To find out, we studied the microbial community in groundwater sand filters, the most common treatment method to produce drinking water worldwide.

Methods and Results:
In a lab setup, sand filters were fed with As-enriched water. We followed the kinetics of As oxidation, as well as the composition and activity of the biofilm, over time. Biological As oxidation turned out to be 14 times more efficient than the current most used chemical oxidation method. Additionally, the biofilm activity and composition did not change significantly upon As enrichment, indicating that a common sand filter biofilm is capable of doing the job.

Next Steps:
As a next step, we are investigating how these bacteria respond to different minerals present in groundwater, such as iron and manganese.

Industrial Relevance:
Applying biological As oxidation in sand filters will reduce the need for chemical oxidants and enhance overall arsenic removal. This is especially essential in As-rich areas in Asia, where decentralized systems often lack chemical infrastructure, while ± 90-210 million people are potentially exposed to high As concentrations.

Isolation and identification of bacteria sourced from high pH/high alkalinity algae

Sofia Irem Tasdemir, *Undergraduate Student*
Chemical and Biological Engineering Engineering, Montana State University, CBE

Purpose of this Research
Many algae live in a rich and diverse microbiome. This study focuses on isolating bacteria from algal cultures from high pH/high alkalinity cultures, which were derived from industrial-scale, laboratory-scale and natural environments. The goal of this study is to identify algae-associated strains which have a natural function and/or have the potential to promote algal productivity.

Methods and Results:
Microbiome members have been isolated using repeated streak plating on various media types. DNA from each successfully isolated microbiome member has been extracted, and the 16S rRNA gene was sequenced to determine bacterial identity. Currently, studies are being conducted to observe the effects that microbiome members can have on the growth of xenic and axenic algal cultures on microwell plates.

Next Steps:
Following the identification process of the microbiome members, the effects of the identified species on algal growth rates will be reassessed and confirmed through shake-flask experiments. Additionally, effects such as growth enhancement, growth inhibition, and other possible functions will be pursued for further understanding.

Industrial Relevance:
These findings could become the basis for the optimization of industrial scale algal cultivation strategies, resulting in significant improvements in the production of fuels, feed, food fertilizer and biochemicals, as well as building up the foundation for the discovery of biological preservatives that could be useful in industry.

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Visitor Poster Abstracts

We need a biofilm imaging library!

Bettina Buttaro, **Joseph Picone**, **Heidi Smith**, **Al Parker**
LKSOM Temple University; Center for Biofilm Engineering

Purpose of this Research:
To accurately explain behavior of complex microbial biofilm communities one needs to combine mathematical simulation models to predict bacterial behavior coupled with machine learning to recognize structures and their organization. The Biofilm Imaging Library is a collaboration to curate data sets of 3D structural, spatial and temporal arrangements.

Methods and Results:
We propose five potential avenues for our initial efforts: (1) building a predictive tool for biofilm protection from toxic assaults, (2) quantifying the arrangement of interdependent mixed species biofilm communities, (3) the quantitation of fungal hyphae within multispecies biofilms, (4) analyzing 3D colony biofilm morphologies to predict clinical outcomes and (5) scaling biofilms that are not evenly distributed over surfaces.

Next Steps:
The purpose of this poster is to hear your feedback and suggestions to understand the needs of the research community for a biofilm imaging library. These discussions will allow us to prioritize initial projects as well as to identify possible collaborators and users willing to contribute images.

Industrial Relevance:
These different models of biofilm behavior have implications for industrial biofilm-based biofouling, treatment of pathogenic biofilms, and diagnostic microbiology.

Novel low corrosion disinfectant with superior antimicrobial efficacy against *Candida auris* biofilms

Carine Nkemngong, *Director*
Microbiology and Disinfectants, AvantGuard DBA Halomine Inc.

Purpose of this Research:
The goal of this study was to compare the anti *Candida auris* biofilm efficacy of AVA-003 formulated by AvantGuard to select US EPA list P products with planktonic *C. auris* claims.

Methods and Results:
We established 48 h wet *C. auris* biofilms on borosilicate glass coupons using the CDC biofilm reactor. Coupons were treated for 10 min. We found that AVA-003 (1.2% Cl) demonstrated higher anti *C. auris* biofilm efficacy than select EPA list P products.

Next Steps:
We will be evaluating AVA-003 for decolonizing *C. auris* using a mice model.

Industrial Relevance:
Our research will result in the commercialization of a low corrosion product with demonstrable efficacy against *C. auris* biofilms.

Table of Contents

Stretching biofilm: How biofilms mechanics change in response to environmental variations

Kōnane Bay, *Assistant Professor*
Chemical and Biological Engineering, University of Colorado Boulder

Purpose of this Research:
Determining how to remove harmful biofilms or how to engineer mechanically stable biofilms requires a fundamental understanding of biofilm mechanics. Methods have been developed to quantify the bulk shear stress response and the local surface stress response. However, there have been limited studies on the bulk tensile mechanics of biofilms.

Methods and Results:
We use a recently developed method to measure the complete uniaxial stress-strain relationship of *Bacillus subtilis pellicles*. We hold a pellicle (grown outside the instrument) on liquid media between a cantilever and a movable rigid boundary, measuring force-displacement from the cantilever deflection. We measure that the addition of metal ions to the liquid media after pellicle formation increases the elastic modulus and yield stress and decreases the failure strain of the pellicle.

Next Steps:
We are planning to explore how other environmental factors, antibiotics, biocides, etc. influence the tensile properties. Additionally, we are currently developing a method to transfer biofilms grown on solid surfaces into our instrument expanding our biofilms we can investigate.

Industrial Relevance:
We expect that using our method we will be able to investigate how different biocides and other liquid treatments change industrial biofouling biofilm strength and stiffness. Thus, providing a high throughput method to identify the necessary treatment for biofouling biofilm removal.

Montana Biofilm Science & Technology Meeting and Workshop

July 9-11, 2025

Hilton Garden Inn Bozeman

Final AGENDA

7/23/2025 3:57 PM

Tuesday July 8

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

Wednesday July 9

7:00–8:00 am Coffee Talk
Optional discussion hour for members & CBE leadership
Goldenrod Boardroom

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

8:00–3:10
Meeting Larkspur Ballroom

8:00-8:15
Opening Remarks
Matthew Fields, Director, CBE; Professor, Microbiology & Cell Biology, MSU
Darla Goeres, Coordinator of Industrial Development, CBE

8:15-8:45
Introductions

SESSION 1: The Rules of Biofilm Behavior

8:45-8:55
Session Introduction
Matthew Fields

Keynote Presentation

8:55-9:40
Mechanisms and consequences of biofilm formation
Fitnat Yildiz, Professor, Microbiology and Environmental Toxicology, University of California Santa Cruz

9:40-10:10 Networking Break

10:10-10:40
Living within your means: From basic geometry to a bacterium's limited budget of surface area
Ross Carlson, Professor, Chemical & Biological Eng., MSU, CBE

10:40-11:10
Probing the life of gut microbes using next generation physiology tools
Roland Hatzenpichler, Associate Professor, Chemistry & Biochemistry, MSU, CBE

11:10-11:55
Panel:
Fitnat Yildiz
Roland Hatzenpichler
Ross Carlson
Moderator: Matthew Fields

11:55-1:25 Networking Lunch

SESSION 2: Interactions at the Interface

1:25-1:30
Session Introduction
Chris Jones, PI, Standardized Biofilm Methods Lab, CBE

1:30-1:50
Evaluating a standard operating procedure for multidomain biofilm growth in the Industrial Surfaces Biofilm Reactor and demonstration of use cases
Kylie Bodle, Postdoctoral Researcher, CBE

1:50-2:10
Investigating the environmental and genetic factors that influence early surface attachment in the foodborne pathogen *Vibrio vulnificus*
Tiffany Williams, Senior Innovation Scientist–Microbiology, Diversey

2:10-2:30
Selecting representative multi-domain microbial communities for surface-associated laboratory consortia
Ghazal Vahidi, Postdoctoral Researcher, CBE

2:30-2:50
Testing the implications of micron-scale texture on microbial surface sensing and biofilm formation: An indwelling-device model
Shawna Pratt, Postdoctoral Researcher, Microbiology & Immunology, Geisel School of Medicine at Dartmouth College
*Young Investigator Awardee

2:50
Depart for Poster Session (on own)

CBE Poster Session

3:30–5:30
Inspiration Hall, MSU
Schedule & lab tour sign-up available onsite

Thursday July 10

7:00–8:00 am Coffee Talk
Optional discussion hour for members & CBE leadership
Goldenrod Boardroom

8:00–9:00 am
Cont. breakfast Larkspur Foyer

8:00-9:00 CBE Connect
Optional networking hour for attendees and CBE researchers
Larkspur meeting area

9:00–4:00
Meeting Larkspur Ballroom

9:00-9:05
Opening remarks
Matthew Fields, Darla Goeres

SESSION 3: Multi-Species Pathology

9:05-9:10
Session Introduction
Kelly Kirker, Assistant Research Professor, Chemical & Biological Eng., MSU, CBE

(Continues on next page)

9:10-9:40
The role of physical forces in shaping polymicrobial communities
Pat Secor, Associate Professor, Microbiology & Cell Biology, MSU, CBE

9:40-10:10
Biofilm microorganism growth and community composition, and urogenital infection association in active-duty female soldiers using 2nd and 3rd generation Freshette® female urinary diversion devices
Elizabeth Kostas-Polston, Assoc. Professor & Deputy Director, Nursing Science Program, Uniformed Services University of the Health Sciences

10:10-10:40 Networking Break

10:40-11:10
Short-lived success: Recolonization of Carbapenem-resistant *Enterobacterales* in sink drains following repeated disinfection with a peroxide-peracetic acid-based foam
Amy Mathers, Professor, Medicine and Pathology, University of Virginia Medical School

11:10-11:40
Cystic fibrosis: A paradigm biofilm infection in a new era
Phil Stewart, Regents Professor, Chemical & Biological Eng., MSU, CBE

11:40-12:05
CBE Awards & Milestones

12:05-1:20 Lunch

SESSION 4: Rapid Trios—The Matrix

1:20-1:25
Session Introduction
Laura Jennings, Assistant Professor, Microbiology & Cell Biology, MSU, CBE

1:25-1:45
Biofilm dispersal patterns revealed using far-red fluorogenic probes
Andrew Bridges, Assistant Professor, Biological Sciences, Carnegie Mellon University
*Young Investigator Awardee

1:45-2:05
Random acts of resistance: Polysaccharide and epigenetic heterogeneity in *Pseudomonas aeruginosa* biofilms
Laura Jennings

2:05-2:25
Lab biofilm models, the brain ache of planning a breakup IRL
Alan House, Senior Scientist, Industrial Cleaning, Novonesis

2:25-2:55 Networking Break

SESSION 5: Rapid Trios—Engineered Systems

2:55-3:00
Session Introduction
Isaak Thornton, Postdoctoral Researcher, CBE;
Ethan Viles, PhD Student, Mechanical & Industrial Eng., MSU, CBE

3:00-3:20
Scaling up and validating processes for biocement production for soil stabilization
Michael Carter, Biomaterials Research Scientist, Air Force Research Laboratory

3:20-3:40
mRNA PNA-FISH for high resolution gene expression and the spatial ecology of *Legionella pneumophila* biofilms
Ana Barbosa, Visiting PhD Student, Chemical & Biological Eng., University of Porto

3:40-4:00
Biofilms in pharmaceutical and clinical water systems
Mark Pasmore, Associate Director, R&D, Vantive

4:00-4:10 Short Break

Strategic Planning Meeting for CBE Members

4:10-5:10
Hilton Garden Inn

BBQ Dinner

6:00-9:00 pm
Big Yellow Barn, Bozeman

Friday July 11

8:00–8:30 am
Cont. breakfast Larkspur Foyer

8:30–11:50 am
Meeting Larkspur Ballroom

8:30-8:35
Opening Remarks
Matthew Fields, Darla Goeres

8:35-9:20
Career Insights Panel: STEM in Industry
AvantGuard
ICU Medical
Sherwin-Williams Company
STERIS
Moderator: Chris Jones

SESSION 6: Life in the Extreme

9:20-9:25
Session Introduction
Liz Sandvik, Research Engineer, CBE

9:25-9:55
Material coatings and strategies for biofilm mitigation in spacecraft water systems
Madelyn Mettler, Affiliate Postdoctoral Researcher, CBE

9:55-10:25 Networking Break

10:25-10:55
Chill Solutions: Cold-adapted biosurfactants for industrial innovation
Christine Foreman, Assoc. Dean, Norm Asbjornson College of Eng.; Professor, Chemical & Biological Eng., MSU, CBE

Closing Keynote Presentation

10:55-11:40
Polymicrobial biofilm growth and control during spaceflight
Bob McLean, Regents' Professor, Biology, Texas State University

11:40-11:50
Closing Remarks
Matthew Fields, Darla Goeres

CBE gratefully acknowledges the support from these meeting sponsors:

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