Presentation & Poster Proceedings

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Darla Goeres, CBE Coordinator of Industrial Development

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SESSION 1: Biofilm as a System *Darla Goeres, Session Chair*

A fascination with phenazines

Presenter: **Dianne Newman**, Gordon M. Binder/Amgen Professor
Affiliation: Biology and Geobiology, Caltech, Pasadena, CA, USA.

Purpose of this Research:
My lab's interdisciplinary research focuses on elucidating mechanisms of bacterial energy conservation and survival when oxygen is scarce, with an emphasis on how redox-active extracellular electron shuttles sustain metabolically attenuated biofilms.

Methods and Results:
In this talk, I will explain how the opportunistic pathogen *Pseudomonas aeruginosa* fuels an extremely low power lifestyle by recycling self-generated redox-active antibiotics called phenazines.

Next Steps:
I will close with a discussion of how recent conceptual and technical advances poise us to better understand life in the slow lane.

Industrial Relevance:
Such an understanding is important to helping us combat chronic infections, designing more efficient metabolic engineering platforms, and estimating the contribution of microbes to global biogeochemical cycles.

Biofilms: The First Bio-Systems?

Presenter: **Matthew Fields**, Director, Professor
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Microbial biofilms will be discussed in terms of key components that comprise the higher-level system, and what these systems may be able to teach society about biology.

Methods and Results:
Microorganisms play major roles in global biogeochemical processes that are beneficial and detrimental in almost every habitat on the planet including human and engineered environments. The majority of microbiological diversity and biomass on the planet resides as attached growth at phase boundaries (i.e., biofilm), and biofilms were likely an early biological adaption.

Next Steps:
N/A

Industrial Relevance:
Biofilms are important both in terms of applied knowledge for utilizing natural and man-made systems for the good of society (e.g., wastewater treatment) but also for fundamental knowledge of how biological systems work and evolve.

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Systems Biology 101: From the basics to biofilms

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

Purpose of this Research:
The living world is a multiscale system spanning from angstroms to kilometers and comprised of many components and interactions. We will discuss some elementary concepts relevant to systems biology and examine some examples relevant to biofilms.

Methods and Results:
Naturally occurring consortia typically exist as biofilms and are often complex. We will discuss some tractable, synthetic consortia with defined components and interactions and will argue the lessons learned from synthetic consortia might be relevant to many settings.

Next Steps:
Apply the lessons learned from synthetic consortia to naturally occurring consortia.

Industrial Relevance:
If your company is experiencing biofilm challenges, these offending animalcules are part of a multiscale system. Knowing the basics of systems biology will help you communicate the challenge and devise informed intervention strategies.

PANEL DISCUSSION

Biofilm as a System

Panelists: Dianne Newman, Matthew Fields, Ross Carlson
Moderator: Darla Goeres

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SESSION 2: Kill, Remove, Prevent *Liz Sandvik, Session Chair*

Development of a test method to evaluate antibiofilm treatments of dental unit water lines

Presenter: **Christopher Jones**, PI, Standardized Biofilm Methods Laboratory
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Dental unit water lines are a frequent source of microbial contamination requiring consistent maintenance to manage biofilms. Here, we present the development of a method for growing biofilms in DUWL tubing to effectively compare and evaluate antibiofilm treatment approaches.

Methods and Results:
The model system developed in the SBML produces a representative worst-case scenario biofilm that mimics many of the key parameters involved in DUWL biofilms, including: organism, temperature, shear force, and duration. This model produces a repeatable biofilm that allows not only direct comparison of antibiofilm treatments, but also evaluation of clean-in-place protocols utilized to minimize biofilm development.

Next Steps:
This methodology is an excellent candidate for standardization due to its repeatable biofilm and sensitivity. We aim to continue development of the method and submit the method for publication as a Standard Test Method.

Industrial Relevance:
The method presented here will allow producers of DUWL cleaning solutions to submit data from a standardized method to regulatory agencies with the goal of adding antibiofilm claims to their products.

A method for evaluating the antimicrobial efficacy of residential laundry products

Presenter: **Laura Gage**, Senior Microbiology Manager
Affiliation: Arxada, Morristown, NJ, USA.

Purpose of this Research:
Demand for novel, eco-friendly laundry detergents and additives that provide efficient removal of soil and pathogen reduction on domestic textiles is increasing. Thus, reliable methodologies that can assess the antimicrobial performance under the conditions that are relevant to residential laundering are needed for both regulatory, research, and screening purposes.

Methods and Results:
We developed a modification of the EN 17658:2022 standard by utilizing a compact and more affordable, bench-top device along with some additional, procedural modifications that increased our throughput while maintaining the mechanical shear (tumbling) and ballast to liquor ratio consistent with the original standard. The data generated with the new method was more repeatable and statistically not different from the data generated in the international ring trial.

Next Steps:
Future studies should investigate other biocidal actives and formulations to ensure results between the two methods are translatable. The method can also be used to evaluate the removal and reduction of biofilms on textiles.

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Industrial Relevance:
The new method offers multiple benefits as a screening tool by providing increased capacity, reduction of labor and materials used. The tumbling device offers flexibility to evaluate a range of laundry parameters and can be easily tailored for other standardized methods.

Microbial survival and comparison of shutdown procedures for dormant water recovery system for spaceflight

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

Purpose of this Research:
Water recovery systems are critical components of life support for crewed spaceflight. Future systems will face an additional challenge of system dormancy during uncrewed operation during which time biofouling is a concern.

Methods and Results:
Here we present results from two studies comparing microbial survival after varying simulated shutdown procedures. Shutdown procedures included no flushing, potable water flushes, storage with biocides, and dry out conditions. Despite significant reductions in nutrient loads, microbial survival is similar across many conditions and the largest predictor of dormancy outcomes is the microbial load prior to system shutdown.

Next Steps:
Increased biocide concentrations and additional biocides are being considered to mitigate these systems prior to dormancy. Alternatively, if microbial loads are anticipated, procedures for restart may include a treatment prior to resumed standard operations.

Industrial Relevance:
While these studies focus on water systems in spaceflight, many residential, industrial, and commercial systems face similar challenges when systems are shut down for seasonal operations, maintenance, or repair. Understanding microbial activity during system dormancy and upon restart of systems has broad applications.

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SESSION 3: Innovative Techniques for Biofilm Analysis *Heidi Smith, Session Chair*

Label-free identification of microorganisms using spontaneous Raman spectroscopy

Presenter: **Bruce Boles**, PhD Student
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Civil Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
I am aiming to identify organisms in a label free manner using spontaneous Raman spectroscopy.

Methods and Results:
Utilizing discriminate analysis, K-means, and, permutational analysis of variants (PERMANOVA), we are able to accurately distinguish individual organisms on the genus level. Using K-means analysis and holding all other variables constant, we are able to differentiate cells under different environmental conditions 95% of the time.

Next Steps:
Through investigation of organisms from one of the saltiest bodies of water on Earth and using multiple statistical lines of evidence, Raman spectroscopy shows promise as a useful tool to differentiate between organisms in a label free manner.

Industrial Relevance:
This is very important for the identification and differentiation of microorganisms.

Imaging multispecies biofilms

Presenter: **Erika Espinosa Ortiz**, Assistant Professor
Affiliation: Biological Engineering, Utah State University, Logan, UT, USA.

Purpose of this Research:
This presentation will provide an overview of imaging approaches to characterize multispecies biofilms.

Methods and Results:
Advanced imaging approaches like optical coherence tomography, scanning electron microscopy and confocal laser microscopy will be discussed.

Next Steps:
The need for next-generation imaging in biofilm research will be discussed.

Industrial Relevance:
These imaging methods can provide insights into the social interactions and spatial arrangement of the species within biofilms relevant to industry.

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Using stimulated Raman spectroscopy (SRS) to study the human fecal microbiome

Presenter: **Jacob Schimetz**, PhD Student
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Traditional methods of studying the human gut microbiome rely on bulk DNA analysis to study the genetic make-up or metatranscriptomics to understand the activity of members of a microbial community, but provide an incomplete picture of cellular dynamics. To address this, we use SRS to provide insight into microbial metabolic activity at the single cell level.

Methods and Results:
We incubated human fecal samples with four over-the-counter medications and five antibiotics in a medium containing heavy water. Metabolically active cells incorporate deuterium (D) into cellular components, creating a distinctive Raman signal that can be exploited for analysis. Combined with fluorescence in situ hybridization (FISH), we used SRS to identify microbial responses to the medications at the single-cell level, enhancing our understanding of their impact on the gut microbiome.

Next Steps:
We are continuing to target specific microbial populations to identify their response to the antibiotics and OTCs and will sort D-labeled cells for 16S rRNA gene or shotgun metagenomics sequencing.

Industrial Relevance:
SRS analysis of pure cultures, biofilms, or other environmental samples allows for a quick, label-free, and non-invasive method to identify active cells and macromolecules such as carotenoids, cytochromes, or pigments. SRS can further be applied to study clinical diagnostics, drug efficacy, and natural product production.

Microsensors and food processing

Presenter: **Stephan Warnat**, Associate Professor
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
The rising demand for maple syrup requires producers to enhance efficiency while keeping costs low. Despite quality control during boiling, microbial growth in harvesting infrastructure remains a challenge. Smart farming, including Wireless Sensor Networks, is crucial for monitoring and addressing microbial growth to ensure maple syrup quality.

Methods and Results:
This presentation shows biofilm formation in sap infrastructure during the harvesting season. Microbial characterization shows a diverse biome with a dominating *Pseudomonas* strain. In sterile sap, fabricated sensors allowed biofilm monitoring over 7 days.

Next Steps:
The MSU team plans to deploy the sensor technology at Michigan's Forestry Innovation Center in the 2025 harvesting season.

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Industrial Relevance:
In situ biofilm sensing techniques are a possible solution for biofilm mitigation.

Electrochemistry as a tool to understand biocorrosion

Presenter: **Giorgia Ghiara,** Visiting Faculty, Fulbright Scholar
Affiliation: Politecnico di Torino, Turin, Italy. Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Electrochemical techniques represent a valuable tool for providing insights on the mechanisms involved in microbiologically influenced corrosion (MIC) processes and can therefore assist in improving and expanding the existing MIC control and mitigation strategies.

Methods and Results:
The biogenically formed layers on metallic substrates and the redox reactions occurring at the metal-biofilm interface will be presented through cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) to illustrate the suitability of electrochemical methods for an improved understanding of the complex mechanisms involved in biocorrosion processes.

Next Steps:
The research will further focus on the investigation of the biofilm interaction with the metal surface, with the aim of identifying specific corrosion mechanisms related to microorganisms and their metabolites.

Industrial Relevance:
Improved understanding of MIC mechanisms is of primary importance to develop effective mitigation strategies (i.e. selection of materials, protective coatings, the use of biocides, and corrosion inhibitors). Regular monitoring and maintenance are also essential to control and prevent MIC in industrial and environmental settings.

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SESSION 4: Biofilms and Water Quality *Catherine Kirkland, Session Chair*

Monochloramine induces release of DNA and RNA from bacterial cells: Quantification, sequencing analyses, and implications

Presenter: Sakcham Bairoliya, Research Fellow
Affiliation: Civil and Environmental Engineering, Nanyang Technological University, Singapore. Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore.
***Young Investigator Awardee**

Purpose of this Research:
The origin of extracellular DNA (eDNA) in the drinking water distribution system (DWDS) is unknown. We hypothesize that monochloramine (MCA) can induce the release of nucleic acids from bacterial cells in DWDS. To test this hypothesis, we aim to quantify the influence of MCA on the release of nucleic acids from bacterial cells in both planktonic and biofilm modes.

Methods and Results:
MCA treatment induced massive release of DNA from both planktonic cells (flow cytometry and real time imaging) and biofilms (MCA effluent characterization). Intriguingly, massive release of RNA was also observed, and the extracellular RNA (eRNA) was found to persist in water for days. RNA sequencing showed that the eRNA contains non-coding RNA and mRNA, implying its role as a possible signalling molecule in environmental systems and a snapshot of the past metabolic state of the bacterial cells.

Next Steps:
We have already verified the presence of eDNA and eRNA in the actual drinking water system. eRNA was used as a reference for differential transcriptomics to determine the survival mechanisms utilized by microorganisms in drinking water. Further work includes designing lab scale experiments to validate the functionality of eRNA.

Industrial Relevance:
The potential applications of this research to the drinking water industry include increased accuracy of detection for pathogens based on RNA/DNA. Monitoring eRNA also allows the utilities to non-invasively determine how microorganisms react to changes in operational parameters in drinking water systems.

Biofilm dynamics in treatment wetlands

Presenter: **Ellen Lauchnor**, Associate Professor
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Civil Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Biofilms drive the removal of wastewater contaminants in treatment wetlands (TW). Research in the Treatment Wetland Group at MSU investigates TW from the laboratory to the field scale to understand and optimize the processes that drive their operation.

Methods and Results:
Spatiotemporal dynamics of biofilm communities in a field-scale TW that seasonally treats high-strength wastewater from a ski resort have been analyzed via 16S rRNA sequencing. The microbial results indicate that communities adapt over the several months of operation and have identified the

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likely microorganisms contributing to the high rates of nitrogen transformation observed at low temperatures.

Next Steps:
Ongoing work is investigating temporal shifts in microbial communities utilizing shot-gun metagenomic sequencing and assessing the presence of organisms capable of mitigating specific contaminants. We are investigating the use of media that enhances sorption and retention of nutrients in the TW, to facilitate biotic removal in TW treating water with high nutrient content.

Industrial Relevance:
TW are effective for treatment of a wide range of water qualities, including stormwater, and industrial and municipal wastewater, as well as for remediation of mining influenced water. Better understanding of the biofilm dynamics in these systems will improve the ability to optimize removal of diverse water constituents.

Pathogenic free-living amoeba: Interactions with biofilms in natural and engineered systems

Presenters: **John Shikany**, PhD Student
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Pathogenic free-living amoeba (FLA), specifically *Naegleria fowleri*, is expanding its geographical range in the United States and is found in natural and engineered water systems. The goal of this study was to map the distribution of *N. fowleri* in natural hot springs in the Western United States and determine by 16S rRNA gene sequencing which microbial communities are associated with the pathogen.

Methods and Results:
Water and biofilm samples were taken from hot springs between 2018 and 2023. FLA were detected by quantitative polymerase chain reaction and confirmed by Sanger sequencing. Naegleria species were detected in every National Park. Results from natural systems in the United States will be compared/contrasted with *N. fowleri* detections in drinking water distribution systems (DWDS) in Australia provided by collaborators from the Commonwealth Scientific and Industrial Research Organization.

Next Steps:
The next steps are to confirm the FLA detections and run Illumina 16S sequencing to determine associated microbial communities in hot spring biofilms. Further sampling will be completed to enrich for environmental isolates of FLA.

Industrial Relevance:
N. fowleri has been detected in drinking water distribution systems throughout the world and has recently caused a fatality through a nasal rinse in Florida. Improving our understanding of the interactions between free-living amoeba and biofilms will improve monitoring and treating this problem.

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Biofilms and public health

Presenter: **Joe Sexton**, Team Lead
Affiliation: Biofilm and Microbial Control Laboratory, Clinical and Environmental Microbiology Branch, CDC, Atlanta, GA, USA.

Purpose of this Research:
The Biofilm and Microbial Control laboratory at CDC performs applied research to inform the prevention and control of biofilm-associated infections. This includes work dedicated to water systems in healthcare settings such as distribution fixtures, drains and wastewater.

Methods and Results:
Methods include established laboratory models (e.g. CDC Biofilm Reactor) as well as the CDC Sink Gallery, which features experimental sinks that can more comprehensively reflect healthcare facilities. This talk will discuss previous and ongoing work with these models within the context of real-world observations in the healthcare setting.

Next Steps:
Future work will continue to study biofilms in healthcare water systems to improve and inform prevention and control strategies.

Industrial Relevance:
Biofilms in water systems are a known reservoir for antimicrobial resistant pathogens that pose risk to patients in healthcare settings. Products and solutions that improve biofilm control strategies can help reduce exposure and improve patient safety.

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SESSION 5: Medicine and Healthcare *Kelly Kirker, Session Chair*

Revisiting the biofilm model of wound infection

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

Purpose of this Research:
The presence of microbial biofilms in many human chronic wounds led to the hypothesis that the amount of biofilm delays healing of these wounds. The overall objective of this study was to report associations between the amount of biofilm and other microbial metrics and the rate of healing of a defined subset of human chronic wounds.

Methods and Results:
Debridement specimens from a population of 117 older individuals with venous leg ulcers were analyzed for the amount of bacterial biomass by two independent methods: a microscopic approach that scored the relative size and number of bacterial aggregates, interpreted as a biofilm metric, and conventional enumeration by agar plating for viable bacteria. There was no statistically significant association between wound healing and the biofilm metric in any of the three analyses performed.

Next Steps:
A refinement of the model of chronic wound infection pathogenesis is proposed in which the contribution of metabolically inactive bacteria is to serve as a nidus for recurrence of the infection whereas impaired healing results from the outgrowth of metabolically active bacteria.

Industrial Relevance:
Understanding the mechanistic role of biofilm formation and bacterial metabolic activity is important for designing improved strategies for treating infected non-healing wounds.

Crafting polymicrobial wound biofilm models through microbiome analysis

Presenters: **Jontana Allkja**, Postdoctoral Researcher
Affiliation: Dental School and Hospital, University of Glasgow, Glasgow, Scotland.
***Young Investigator Awardee**

Purpose of this Research:
Biofilm wound infections are commonly polymicrobial in nature, making them difficult to treat and often evolving into chronic or non-healing wounds. The role of the microbiome in wound healing is becoming increasingly more evident. Here, we present two different approaches to developing different biofilm models (defined and undefined) using the wound microbiome as a starting point.

Methods and Results:
Three different five-species defined biofilm models have been designed: Gram-positive pathogen model, Gram-positive commensal model and Gram-negative pathogen model. The undefined biofilm model is grown using inocula grown from patient wound swabs. Species abundance will be assessed through qPCR analysis, and the composition of the undefined models will be further assessed through NanoPore sequencing. A debridement protocol will also be developed to mimic clinical practice.

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Next Steps:
In future work, the models will be used to test different treatment strategies such as Cold Atmospheric Plasma (CAP) and different antimicrobial compounds. Moreover, the addition of human cell lines to the models will be tested, to assess influence on host cells.

Industrial Relevance:
These models could serve as a tool to assess if various treatments can disperse/kill complex wound biofilms, as well as test how they affect the composition of the wound microbiome and whether we can direct growth towards a healing wound.

2-aminoimidazoles as antibiotic adjuvants: A piece to the AMR puzzle

Presenter: **Amethyst Demeritte**, PhD Student
Affiliation: Chemistry and Biochemistry, Montana State University, Bozeman, MT USA, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Poly-substituted 2-aminoimidazoles (2-AI) possess the ability to inhibit, disperse and re-sensitize multidrug-resistant bacterial strains to conventional antibiotics. The synthesis and biological evaluation of a sizeable library of novel 2-AIs and their highly active imidazo[1,2-a]pyrimidinium salt precursors has been assembled.

Methods and Results:
A refined synthetic pathway, with high efficiency, has been developed to prepare these compounds. Bulk preliminary screening, using Kirby-Bauer Disk Diffusion, exhibit a moderate to high activity against Methicillin-resistant *Staphylococcus aureus* for our initial sulfide linkers; whereas current derivatives have proven to be more active against *Pseudomonas aeruginosa*.
Next Steps:
Further synthetic analysis into dimerization and tetrahydroimidazo[1,2-a]pyrimidinium derivatives will be conducted. Subsequent biological validation and mechanistic studies will involve MIC, MBC and MBEC assays, along with re-sensitization studies; using synergy checkerboard assays and robust biofilm models, such as the Colony Drip Flow Biofilm Model to simulate chronic wound environments.

Industrial Relevance:
Overall, the discovery of new, highly potent antimicrobial agents presents a pertinent approach in the identification of adjuvants that potentiate the activity of current antibiotics.

One is not the loneliest number: Using new tools to study virus infections at a single cell level

Presenter: **Emma Loveday**, Assistant Professor
Affiliation: Microbiology and Cell Biology, Montana State University, Bozeman, MT USA, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Most viral infection research is performed with large population of cells, which hinders our ability to understand and evaluate of the heterogeneity of infection dynamics. Single cell infections using drop-based microfluidics can enable higher-resolution analysis of cellular and viral heterogeneity during viral infection.

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Methods and Results:
We used multiple drop-based microfluidic methods to study individual infected cells. Drop-based microfluidics was applied to the study of influenza A virus (IAV) and herpes simplex virus 1 infections (HSV-1).

Next Steps:
We discovered that only a small proportion of infected cells fully complete the viral lifecycle. The application of drop-based microfluidics in this work expands the capacity to propagate IAV viruses and perform high-throughput analyses of individually infected cells.

Industrial Relevance:
This work can be applied toward detection of many different infectious agents. In addition, our ability to work with many different viral agents can be beneficial for industrial partners that want to assess inactivation of these pathogens.

Bacterial hibernation: A mechanism for biofilm antibiotic tolerance

Presenter: **Mike Franklin**, Professor
Affiliation: Microbiology & Cell Biology, Montana State University, Bozeman, MT USA. Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Purpose of this Research:
Biofilms contain subpopulations of bacteria that are tolerant to the antibiotics that are normally effective at killing the metabolically active bacteria. Our goals are to identify molecular targets that may be used to inactivate the dormant subpopulations of biofilms.

Methods and Results:
A critical supply of ribosomes is required for bacteria to resuscitate from dormancy. We use approaches to anaylze ribosome abundances and integrity during bacterial dormancy. These approaches include ribosomal RNA and ribosomal protein analysis, bi-fluorescence molecular complementation, and cryo-electron microscopy.

Next Steps:
Our next steps are to perform both in vivo and in vitro analysis of ribosomes. For in vivo analysis, we analyzed ribosomes by combining bi-fluorescence molecular complementation with in vivo imaging of whole cells using confocal laser scanning microscopy. For in vitro analysis we analyze ribosomes at near atomic scale resolution, using cryo-electron microscopy and tomography.

Industrial Relevance:
Our research will provide both kinetics and molecular resolution of hibernating ribosomes, that are necessary to design inhibitors that may prevent biofilm bacteria from entering a hibernating, antibiotic-tolerant state.

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POSTER ABSTRACTS

Algae worth their salt: Osmotic stress in a biofuel-producing microalga

Adrienne Arnold, *PhD Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Algal cultivation requires large amounts of CO_2 and water, which increases costs and potentially depletes freshwater reserves. The goal of this work is to determine how high alkalinity and seawater cultivation of algae can reduce costs while producing useful compounds like biofuels and amino acids.

Methods and Results:
Chlorella sp. SLA-04 was grown in high alkalinity Bold's Basal Media with up to seawater levels of added sodium chloride. Growth rate decreased as salinity increased, but the proportion of biofuel precursors within biomass increased. Targeted metabolomics revealed that intracellular levels of proline were also elevated under high salinity.

Next Steps:
The growth and metabolomics data from this experiment will be incorporated into a metabolic model. Analysis with this model will help us determine how green algae respond to osmotic stress, information that can help guide industrial cultivation.

Industrial Relevance:
Algae are effective producers of triacylglycerols, which can be used as biofuel precursors, and of amino acids like proline, which are used as stabilizers or supplements in the medical field. This research will provide key insight into growing algae sustainably while minimizing costs.

Assessing the effects of *Burkholderia contaminans* on *Coniochaeta mutabilis*

Andrew May, *Undergraduate Researcher*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Assess how fungus *Coniochaeta mutabilis* grows when in the presence of *Burkholderia contaminans* which may have antifungal properties. Both organisms have been isolated from the International Space Station wastewater tank.

Methods and Results:
The microbes were grown in CDC biofilm reactors on Teflon or nickel-chromium alloy (Inconel) coupons. Each species was grown individually, then experiments were run with the *B. contaminans* inoculated 24 hours after *C. mutabilis* and both organisms inoculated simultaneously. There was little difference in the accumulation of *C. mutabilis* across all experiments, but they all showed more growth of *C. mutabilis* than when all organisms from the biofilm are grown together.

Next Steps:
Similar experiments will be performed with *Ralstonia insidiosa* (another ISS wastewater tank isolate) rather than *B. comtaminans* to assess the change in growth of *C. mutabilis*. Imaging of the biofilm will take place at multiple phases of growth to better understand biofilm development.

Industrial Relevance:
This research can be used to better understand and decrease biofilm buildup in other systems such as wastewater and industrial pumping systems.

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The role of biosurfactants in enhancing microbial survival

Breuklyn Opp, *PhD Student*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Many cold-tolerant organisms have biosurfactant production capabilities. My research aims to understand the role of biosurfactants in improving survivability of these organisms in their environment.

Methods and Results:
I have been studying a chosen model microorganism's behavior under stress conditions such as high/low pH and high salinity both with and without added biosurfactant. Growth has been observed both in terms of optical density growth curves and respirometry experiments.

Next Steps:
After determining limits of life for the organism, further respirometry experiments are needed to fully understand the role of the biosurfactant in more extreme stress conditions.

Industrial Relevance:
Biosurfactants are industrially relevant in most spaces that traditional surfactants are used, such as oil emulsification and ice packing. The study of the microorganism in addition to the biosurfactants may provide insight into crude oil degredation.

Detection and analysis of chronic wound pathogens via isothermal microcalorimetry

Campbell Putnam, *PhD Student*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The presence of bacterial biofilms is a recurrent comorbidity in chronic wounds. To better address the increasing healthcare burden imposed by chronic wounds, this study implements isothermal microcalorimetry to analyze a community of chronic wound bacterial isolates *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods and Results:
All biological systems produce heat as a byproduct of their metabolic activity: isothermal microcalorimetry exploits this phenomenon to measure metabolic heat as a proxy for cell growth. Application of this method to *S. aureus* and *P. aeruginosa* cultures produces unique, characteristic heat flux profiles from each species. The species-characteristic nature of these heat flux profiles allows either species to be identified even when grown in community with each other.

Next Steps:
First, we will align metabolite flux data with the heat flux profiles so as to derive a more comprehensive understanding of the *S. aureus*/*P. aeruginosa* community metabolism. Second, *Candida albicans* is a fungal pathogen routinely isolated from chronic wounds; we therefore will analyze *S. aureus*, *P. aeruginosa*, and *C. albicans* communities using isothermal microcalorimetry.

Industrial Relevance:
This study aims (1) to demonstrate the diagnostic potential offered by isothermal microcalorimetry and (2) to improve understanding of metabolism in chronic wound bacterial communities. With regards to the latter aim, improved knowledge of metabolic interactions offers novel targets for anti-bacterial therapy.

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Correlating NMR and MICP to measure the extent of MICP in beach sand

Cara Butler, *Undergraduate Researcher*
Civil Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The goal of my research is to use well-controlled laboratory conditions to correlate NMR T2 relaxation and the extent of biomineralization as a function of relative humidity. This data will be used to support NMR data interpretation during a full-scale field demonstration in the spring of 2025 at Camp LeJeune, NC.

Methods and Results:
Reactors were developed for the columns that were compatible with the automated pulse system, top-down flow and the NMR Rock Core Analyzer (Magritek, Wellington NZ). Columns are biomineralized using pulses of *S. Pasteri* bacteria followed by a urea and calcium chloride solution. Top-down pulses are used to mimic the application method used in the beach test. An already developed automated pulse system made by my colleague, Sabine Olds, was used to administer pulses consecutively and at constant intervals.

Next Steps:
Next, these columns will be subjected to varying quantities of pulses to develop different levels of biomineralization. Influent and effluent testing will be carried out to monitor microbe activity. Finally, the columns will be exposed to different relative humidity and then measured using NMR.

Industrial Relevance:
It is proposed to use microbially induced calcite precipitation (MICP) as the biotechnology to prevent and reduce problematic beach erosion. To determine the amount of biomineralization, nuclear magnetic resonance (NMR) can be used as an in-situ, non-invasive monitoring technology.

Ammonium byproduct management via zeolites and struvite precipitation

Cora Rose Hannigan, *Undergraduate Researcher*
Civil Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
MICP is a process where bacteria induce calcite precipitation from environmental calcium and carbonate ions, but it generates ammonium byproducts. Our study aims to evaluate zeolite sorption and struvite precipitation to address this issue.

Methods and Results:
In batch studies, using an ammonium enriched microbial culture and powdered and granular forms of zeolite, it was found that zeolite decreased ammonium concentrations by ~ 80% and struvite by ~90%. In a column study, three conditions were assessed: MICP, struvite column (MISP), and a combination column (MICP+MISP). Results indicate that incorporation of zeolites or struvite can decrease the concentration of ammonium byproducts in biocementation waste.

Next Steps:
Batch studies will evaluate zeolite adsorption capacity for ammonium across various concentrations and conditions, including biotic and abiotic conditions. The most effective techniques will progress to column studies, where breakthrough curves will be generated to quantify adsorptive capacity under simulated environmental conditions, particularly focusing on high-strength wastewater effluents.

Industrial Relevance:
High strength wastewater can come from many sources, including agricultural sources such as animal feeding operations (pig farms) or from the MICP process. Zeolite sorption and struvite precipitation are promising techniques to manage high strength wastewater.

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Investigation of bio-mineralization in the presence of surfaces functionalized with APMDES

Ethan Heyneman, *PhD Student*
Material Sciences, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The main purpose of my research is to investigate the potential benefits of surface functionalization with APMDES in calcium carbonate bio-mineralized materials.

Methods and Results:
The main methodology revolves around attaching *S. pasteurii* to the surface of aggregates before inducing calcium carbonate mineralization with calcium mineralization media. Both aggregates with and without APMDES attached are undergo the mineralization process and are analyzed with SEM, EDX, XRD, and Raman instruments.

Next Steps:
The next steps will be to collect live images under Raman as the mineralization process occurs and to investigate patterning the APMDES on a surface to see if the mineralization process can also be patterned.

Industrial Relevance:
If a bio-mineralized building material that is capable of being interchanged with traditional concrete can be developed it would provide a low carbon, low energy option construction material.

Mycelium as a scaffold for biomineralized engineered living materials

Ethan Viles, *PhD Student*
Mechanical and Industrial Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Current biomineralized engineered living materials (ELMs) lack the required strength and microbial viability to serve as living structural materials. We used fungal mycelium to design a biomineralized ELM with increased microbial viability (>4 weeks) and a designed interior microarchitecture to increase the range of available designs for biomineralized ELMs.

Methods and Results:
Two biomineralized ELMs were constructed: one with a living mycelium scaffold that self-mineralizes, and one with a non-viable fungal scaffold that is bacterially-mineralized. The viability of living components from both ELMs was high after 4-weeks of drying. Biomineralization efficiency was greater for bacterially-mineralized ELMs than fungally-mineralized ELMs. A bacterially-mineralized ELM to construct a bone-biomemetic structure.

Next Steps:
Different interior microarchitectures and how they affect the mechanical properties of our biomineralized ELM will be explored in further research.

Industrial Relevance:
The use of mycelium to increase microbial viability and the control of interior microarchitecure within biomineralized ELMs provides new pathways for living structural materials with additional functionalities, such as self-healing or environmental sensing.

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Developing methods to screen and analyze fungal-bacterial co-cultures to enhance the bioconversion of lignin

Gabriel Griffin, *PhD Student*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Develop methods to assist the establishment of mixed-use microbial (i.e., fungal-bacterial) cocultures to efficiently depolymerize lignin and convert lignin-derived molecules into value-added products.

Methods and Results:
We are developing two methods to address challenges in using fungal-bacterial cocultures: (1) high-throughput screening for determining optimal cocultures by using single-walled Carbon nanotube (SWCNT) fluorescent sensors wrapped in lignin to detect enhanced ligninolytic enzyme production, and (2) analyzing fungal growth by using ergosterol, unique to fungi, as a proxy. These methods aim to streamline the selection of effective cocultures and provide accurate measurements of fungal growth.

Next Steps:
The next steps involve utilizing the screening method to select optimal cocultures from a set of model organisms. These selected cocultures will then be analyzed for their ability to depolymerize lignin and convert the lignin-derived molecules into value-added products.

Industrial Relevance:
Lignin, an underutilized renewable resource, has great potential for conversion into high-value industrial products such as cis-cis muconate, lipids, and polyhydroxyalkanoates (PHAs).

Staphylococcus aureus SaeR/S regulated factors overcome human complement mediated inhibition of aggregation to evade neutrophil killing

Gauri Gaur, *PhD Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Identify pathogen immune evasion strategies during early *S. aureus* biofilm neutrophil interactions.

Methods and Results:
Time-lapse confocal microscopy was employed to quantify interactions between *S. aureus* biofilm aggregates and human neutrophils in vitro using bacterial isogenic deletion mutants and sera affected in various complement proteins. *S. aureus* forms neutrophil resistant biofilm aggregates in human serum. Human serum inhibits bacterial aggregation: Complement Dependent Aggregation Interference (CDAI) while *S. aureus* SaeR/S regulated factors inhibit CDAI to facilitate neutrophil evasion.

Next Steps:
Next steps will look into describing the specific mechanism by which complement C3 and fB inhibit bacterial aggregation, whether by direct action of the alternative convertases, activation of an additional serum protein, or another mechanism.

Industrial Relevance:
This research could help the design of novel therapeutics aimed at disrupting bacterial aggregation, a potent defense mechanism against host innate immune defenses

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Microfluidic devices for single-cell qPCR: Enhancing gene expression analysis

Grace Ducharme, *Undergraduate Researcher*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:

This project aimed to simplify and execute on-chip, in-drop quantitative polymerase chain reaction (qPCR) for single-cell gene expression analysis, overcoming current limitations of qPCR within microfluidic drops to evaluate gene expression heterogeneity at the single-cell level.

Methods and Results:
The main challenge was addressing the instability of aqueous microfluidic drops during the temperature fluctuations necessary for qPCR. To combat this, devices will be constructed with materials including 3D printed resin, glass, and SU-8 instead of the traditional PDMS, which destabilizes at high temperatures. The project involves using CAD to design various device geometries, fabricating the devices, and evaluating their performance.

Next Steps:
The next steps involve testing droplet packing, incorporating PCR reagents, and ensuring droplet stability under temperature fluctuations.

Industrial Relevance:
The successful development of these devices will enable precise nucleic acid quantification from single cells, enhancing monitoring of heterogeneous cell lines and benefiting diagnostics, personalized medicine, and cellular research.

Live image analysis of microbially induced calcium carbonate precipitation

Grace Roemig, *Undergraduate Researcher*
Industrial and Mechanical Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The goal of this research is to understand where and how calcium carbonate precipitates due to *S. pasteurii* ureolysis. Additionally, a positive charge will be induced on a surface to attract the negatively charged bacteria, called functionalization, and the difference in precipitation kinetics on functionalized vs. nonfunctionalized surfaces will be investigated.

Methods and Results:
A culture of *S. pasteurii* is grown up and attached to glass beads, and then calcium chloride dihydrate is introduced to the system. The resulting precipitation of calcium carbonate is recorded using an eclipse microscope over the course of the next 6 hours. This video is then analyzed in MATLAB, specifically investigating the quantitative growth of *S. pasteurii* over time, along with other variables.

Next Steps:
Next, this imaging of calcium carbonate precipitation will be translated to silicon wafers. It is possible functionalization of surfaces will also be used to attempt to attach the calcium carbonate in a particular pattern to the surface.

Industrial Relevance:
Microbially precipitated calcium carbonate can be used as a building material or to repair cracks in a material. This understanding of how it forms and possible patterning of it could be used to design a material with more desirable mechanical properties for various building applications.

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Investigating co-cultures to produce self-assembling and self-repairing building materials

Hossein Khadivar, *PhD Student*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Our primary goals are (1) designing self-sustaining co-cultures capable of biomineralization, (2) understanding and controlling the biomineralization pathways of each community member, and (3) optimizing the material properties of the biomineralized products.

Methods and Results:
Co-cultures are grown in conditions which promote their growth and biomineralization capabilities. By manipulating environmental parameters such as pH, nutrient availability (incl. CO_2), and temperature, we aim to understand and ultimately control the mechanisms through which these organisms contribute to biomineralization and the creation of structures.

Next Steps:
The produced biomaterials will be examined in terms of hardness and strength using compression testing and Nano-indentation. Scanning Electron Microscopy combined with Energy Dispersive X-ray Spectroscopy and X-Ray Diffraction techniques will assist with material characterization such as assessing the composition and structure of the produced material.

Industrial Relevance:
Our insights will contribute to the development of novel materials that meet specific structural and environmental requirements, paving the way for their application in sustainable construction and beyond.

Inoculation culture condition effect on SLA-04 growth and lipid production

J.P. Kaffer, *PhD Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Determining the effect of altering alkalinity and pH inoculation conditions have on algal growth, lipid production, and transcriptomes.

Methods and Results:
High pH high alkalinity and low pH low alkalinity bioreactors were both inoculated with algal species strain Chlorella sp. SLA-04 from a high alkalinity culture and grown to post nitrogen depletion. Several analyses were taken over a week with biomass samples being taken from day 4 onward for GCMS. Growth was similar under both conditions with cell counts and the rate of nitrogen depletion being the same, but they differed with chlorophyll A and unsaturated lipid content.

Next Steps:
The next steps for my research are completing the RNA extraction and sequencing of different time points to gain a better understanding of how the transcriptome changes depending on inoculation condition and predict what proteins could be involved.

Industrial Relevance:
Algae has a large potential as an alternate hydrocarbon source but one of the larger associated costs is providing enough CO2, this can be solved under high alkaline conditions. This research will provide background on how algae are able to survive under these conditions and give ideas for further optimization of algal growth.

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Stimulated Raman Spectroscopy (SRS) as a tool to study the human microbiome

Jacob Schimetz, *PhD Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Traditional methods of studying the human gut microbiome rely on bulk DNA analysis to study the genetic make-up or metatranscriptomics to understand the activity of members of a microbial community, but provide an incomplete picture of cellular dynamics. To address this, we use SRS to provide insight into microbial metabolic activity at the single cell level.

Methods and Results:
We incubated human fecal samples with four commonly used over-the-counter (OTC) medication and five commonly used antibiotics in a medium containing heavy water (D2O). Metabolically active cells incorporate deuterium (D) into cellular components, creating a distinctive Raman signal that can be exploited for analysis. We used SRS to identify microbial responses to the medications at the single-cell level, enhancing our understanding of their impact on the gut microbiome.

Next Steps:
We are continuing to target specific microbial populations to identify their response to the antibiotics and OTCs and will sort D-labeled cells for 16S rRNA gene or shotgun metagenomics sequencing.

Industrial Relevance:
SRS analysis of pure cultures, biofilms, or other environmental samples allows for a quick, label-free, and non-invasive method to identify active cells and/or macromolecules such as carotenoids, cytochromes, or pigments. SRS can further be applied to study clinical diagnostics, drug efficacy, and natural product production.

Phycosomal bio-flocculation as a xenic culture

Jessica (Bear) Wood, *PhD Student*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Cellular aggregation can be common in xenic microalgal cultures, yet the physiological significance of aggregate growth is poorly understood, nor has it been routinely quantified even though aggregation can contribute to biomass collection and dewatering.

Methods and Results:
We show that aggregation is a quantifiable phenotype, and the extent of aggregation is dependent upon taxonomic composition that differs under low and high alkalinity. FlowCam particle analysis was used to track aggregate size during SLA-04 growth, and the mean particle size ranged between 30 to 187 μm2. The mean particle size (μm2) followed the aggregation efficiency trend and was directly correlated to aggregation efficiency, R2=0.7 and R2=0.8, for low and high alkalinity, respectively.

Next Steps:
For the highly productive, alkalitolerant SLA-04, the results demonstrated that growth in aggregates does not impact productivity but could lead to improved harvesting outcomes via phycosomal bio-flocculation.

Industrial Relevance:
When co-culturing highly productive microalgae with single microbial isolates, it can be challenging to identify industrially relevant, temporally consistent phenotypes, and moreover, most industrial cultivations will be open systems that are not simple co-cultures.

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Determining the pH-dependence of the zeta potential of APMDES-functionalized surfaces for microbial biomineralization

Kathrine LeBrun, *Undergraduate Research Assistant*
Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Functionalizing surfaces by attaching a layer of positively-charged amine groups, such as APMDES, can improve the efficiency of biomineralization by trapping negatively-charged microorganisms near the surface. The ratio of charged amines to neutral amines is expected to be pH-dependent, but experimental determination of the pH dependence of the zeta potential has not been performed.

Methods and Results:
Zeta potential measurements are conducted using a Zeta-Meter System 4.0. We used tap water and adjusted the pH to values between 6 and 11 using hydrochloric acid and sodium bicarbonate and recorded the pH (Thermo Scientific Orion PH111). Zeta potential measurements were conducted in triplicate, and the average values and standard deviations were recorded.

Next Steps:
The majority of biomineralization does not happen in tap water, though for simplicity and a baseline understanding it is a good medium to use. Going forward, extending this research to media used in biomineralization experiments will improve the relevance of the data to mineralization experiments.

Industrial Relevance:
The ability to make a surface attract microbes efficiently is important for uses such as microbe filters, materials utilizing microbes, and many microbe-focused experiments. Increasing our understanding of the behavior of functionalized surfaces will lead to better utilization of biomineralization for basic and applied research.

Developing living building materials with ureolytic fungus and bacteria

Lauren Basye, *Undergraduate Researcher*
Civil Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Our goal is to create a more sustainable material for construction. We are using a fungal-bacteria consortia to create strong scaffolds and potentially a material with self healing properties.

Methods and Results:
We have developed a media in which both consortia members can grow to maximize ureolytic activity and biomass production. Ureolytic activity is important in calcium carbonate generation which is how concrete is formed. We tested *F. venentatum* and *S. pasteurii* ability to tolerate varying levels of urea to carry out hydrolysis most efficiently. *F. venanatum* can tolerate extremely high levels of pH as it grows which is promising for the long term goal of creating a self healing material.

Next Steps:
Next we will be using this consortia to create bricks out of a reusable mold that is less expensive than the current applications of 3D printed molds. We will investigate different ratios of consortia members to optimize brick strength.

Industrial Relevance:
We want to create more sustainable building materials to be used in mainstream practice. This will be done by developing a material that is equally tolerant to stress as current materials while possessing self healing properties.

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Silica-fume rich mixes to extend the applications and sustainability of hempcrete

Leah Davidson, *PhD Student*
Mechanical and Industrial Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Building-related operations, such as heating and cooling, account for 28% of anthropogenic carbon emissions, alongside the carbon cost of the materials themselves. This research aims to enhance the utility of hempcrete, a carbon-neutral insulation with poor load-bearing properties, via biomineralization and to explore how cement additives enhance microbial viability in hempcrete.

Methods and Results:
Using a design of experiment, we found that we could reduce the pH of hempcrete paste to ~10 using increased binder replacement. However, we found that higher cement replacement has an inverse effect on strength, and this relationship depends on the amount of silica and metakaolin shown by curvature within the response surface. Preliminary viability results demonstrated increased viability immediately after mixing from ~10^4 to ~10^6 MPN/g paste for high cement replacement mixes.

Next Steps:
The next steps would be to characterize the location and quality of biomineralization within hempcrete cylinders and to determine the impact of MICP on the strength and insulative properties of hempcrete.

Industrial Relevance:
This work is relevant for understanding how microbes can be incorporated into traditional building materials. Additionally, it shows how common cement additives can be leveraged to reduce pH, which could be advantageous for environments where high pH leaching is detrimental, such as aquatic ecosystems and waste repositories.

The salty science of brinicle formation

Madeline Garner, *PhD Student*
Chemistry and Biochemistry, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The goal of this research is to examine the impact that salt compositions have on brinicle growth rate, morphology, and stability. Specifically, this work aims to study brinicle growth and stability in diverse salt environments relevant to terrestrial and extraterrestrial systems.

Methods and Results:
Brinicles were grown in a controlled subzero environment using a ten-gallon tank and a peristaltic pump to introduce brine solutions of sodium chloride (NaCl), calcium chloride (CaCl), Don Juan Pond analog (DJP), and Enceladus analog (ENC) at a concentration of 200 PPT. Time-lapse photography and edge detection analysis were used to monitor growth. This research found that brinicles grown in CaCl dominated solutions exhibited significantly faster growth rates compared to those in NaCl.

Next Steps:
Future research will explore the habitability of brinicles. Specifically, we aim to study how differing salts impact the habitability of these structures in terrestrial and extraterrestrial environments.

Industrial Relevance:
This work could be used to inform antifreeze technologies and salt-based ice management. Additionally studying brinicle habitability could provide insights into the formation and stability of biofilms in extreme conditions.

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Cellular economics of metabolite synthesis determine ratios of microbial trading partners

Martina Du, *PhD Student*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Synthetic consortia with engineered interactions can help decode competitive interactions in natural multispecies biofilms by reducing unknowns and establishing rules to design, engineer, and control consortia with desired properties. Here, we used a synthetic consortium to study the cellular economics of metabolite exchange between two obligate cross-feeding *E. coli* strains.

Methods and Results:
The engineered consortium comprised of: an arginine secreting strain that cannot catabolize lactose (L-R+), and an arginine auxotroph that can catabolize lactose and secrete byproducts including pyruvate (L+R-). Strain ratios varied significantly between planktonic and biofilm growth, and within different biofilm regions, as determined by laser microdissection and qPCR. The ratios were analyzed with computational models and linked to the cellular economics of cross-fed metabolites.

Next Steps:
Systems biology tools were used to analyze experimental data, revealing that differences in O_2 flux substantially alter the metabolic cost of producing pyruvate but not arginine, which is reflected in the cell ratios. The predictions will be further tested using isothermal microcalorimetry experiments that vary the availability of electron donor (lactose) and electron acceptor (O_2).

Industrial Relevance:
Metabolite cross feeding is central to the stability, resilience, and productivity of microbial communities spanning from chronic wounds to industrial ecosystems. The predictable changes in cross fed metabolite costs highlights the potential to design and fine tune cross feeding consortia for enhanced biocontrol.

Analysis of the design and fluid dynamics of simulated microgravity biofilm reactors

Matthew Culp, *PhD Student*
Mechanical and Industrial Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Simulated Microgravity Biofilm Reactors (SMBRs) are novel tools that allow for ground-based research into the biofilms that cause fouling issues in spacecraft wastewater systems. This novel design was analyzed using multiple numerical simulation methods along with particle imaging velocimetry to better understand the fluid dynamics experienced within the SMBR system.

Methods and Results:
Computational fluid dynamics (CFD), finite difference numerical models and particle imaging velocimetry (PIV) were used to evaluate the fluid dynamics of the SMBR system. Design studies were conducted using CFD and numerical simulations to evaluate various design factors such as system rotational speed, sensor integration and reactor diameter. PIV was used to validate a CFD model by comparing the observed and predicted fluid behavior.

Next Steps:
Continued analysis of the PIV results and refinement of the CFD/numerical models should allow for improved simulation quality and system optimization.

Industrial Relevance:
SMBRs provide a novel solution for simulating spacecraft wastewater systems to test for biofouling. Additionally, the sensor integration methods explored in this system could be utilized to add biofilm sensing into fluid and pipe systems for a variety of industrial systems.

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Biomass quantification methods for the study of microbial fouling in the international space station water processor assembly

Micah Hickethier, *Undergraduate Researcher*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
This project compared biomass quantification methods of total carbohydrate, total protein, optical density, and FlowCam analysis as biomass proxies during growth of the ISS filamentous fungal isolate *L. mutabilis*, a yeast-like fungal isolate, *A. pullulans*, and the ISS bacterial isolates *R. insidiosa*, *B. cepacia*, *C. metallidurans*, and *M. organophilum*.

Methods and Results:
All organisms were grown in an ISS synthetic wastewater, Microbial Ersatz MTN. Biomass quantification methods were compared for all organisms during monoculture growth curves as well as in a multidomain co-culture of the four bacteria and *L. mutabilis*.

Next Steps:
Next steps entail completing one more trial and data analysis.

Industrial Relevance:
L. mutabilis is a filamentous fungus isolated from fouled components in the ISS wastewater processing assembly (WPA). While microbial growth is frequently quantified using colony forming units per milliliter (CFU/mL), this measure does not account for the extent of biomass, particularly for this fungus.

Determining if microorganisms could survive in ocean world environments

Nicole (Nicki) Krysiak, *Undergraduate Researcher*
Chemistry & Biochemistry, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Many scientists wonder if life is possible beyond Earth; this research uses data collected from the Cassini satellite (NASA) to investigate if it is possible for life to survive under environmental conditions believed to exist on Enceladus. *Psychrobacter arcticus* (*P. arcticus*) was exposed to three different concentrations of salt, phosphorus, and silica that may be present on Enceladus.

Methods and Results:
Growth curves are used to research microbial survival under the different Enceladus-like environment conditions. To investigate if *P. arcticus* is active after exposure to the Enceladus conditions, the uptake of D2O by individual cells is measured using Raman Spectroscopy.

Next Steps:
Moving forward, reactive oxygen species quenchers will be added to solutions containing silica to measure the effects of silica on biofilm formation. *P. arcticus* will also be exposed to different types of salt solutions that also may be present on Enceladus and Don Juan Pond (another astrobiological analogue site located in Antarctica).

Industrial Relevance:
This information may help scientists determine if life may be possible on Enceladus and help push science towards searching for life on Ocean Worlds.

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Growth of *Cutibacterium* and *Staphylococcus* in a defined, skin-relevant medium

Tess Kirkpatrick, *Undergraduate Researcher*
Chemistry and Biochemistry, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The goal of this project is to develop a defined medium that mimics the skin condition, i.e., with the addition of sebum-like compounds, to better characterize *S. epidermidis* and *C. acnes* metabolism under relevant skin conditions. Ultimately, we aim to develop a standardized coculture growth system for the two bacteria (and perhaps other skin microbes) for future biofilm interaction studies.

Methods and Results:
A defined medium was formulated and subsequently utilized to cultivate *C. acnes* and *S. epidermidis* under various growth conditions. Results indicated that the defined medium can be used to conduct growth experiments, and further analysis can be used to determine amino acid and carbon preferences of both bacteria. In coculture, growth is not inhibited by either organism.

Next Steps:
Future work aims to explore the differences in microbial interactions via coculture growth with varying carbon sources, as well as designing experiments that have a pH and oxygen concentration that more closely mimics true conditions of the skin.

Industrial Relevance:
S. epidermidis and *C. acnes* have been dubbed sentinels of the skin microbiota. As the cosmetic industry moves toward a microbiome-based research approach, characterization and application of these microbes will be key to developing new and innovative skin care products.

Disinfectant efficacy testing of fluorescent protein modified *P. aeruginosa* strains using the MBEC assay

Victoria Trivitt, *Undergraduate Researcher*
Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Utilization of fluorescently-tagged strains would facilitate many approaches including microscopy, however it is unknown whether the mutations affect their susceptibility profile. Here, we seek to determine whether there are differences in susceptibility between the fluorescent strains and the parental strain to facilitate biochemical and image-based analysis of biofilm treatments.

Methods and Results:
Strains were tested against several antimicrobials using the MBEC assay. The fluorescently-modified strains followed the same trend of susceptibility as the parental *P. aeruginosa* strain with each of the tested treatments. The modified strains were most similar in susceptibility to the parental strain in the 5000 mg/L acidified NaOCl. The 200 mg/L NaOCl, Cavicide, and Lysol treatments followed a similar trend, however the variability was higher due to the intermediate efficacy.

Next Steps:
Future work will expand on this foundation by continuing to test strains with additional antimicrobial agents with varied mechanisms of action such as peracetic acid, quaternary ammonium compounds, ozone, ethanol, and propylene glycol. We will then generate the fluorescently-tagged strain *P. aeruginosa* ATCC 15442, which is required by the EPA for anti-biofilm claims for a product.

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Industrial Relevance:
Fluorescent strains will allow novel and more streamlined approaches to antimicrobial testing and biofilm imaging, reducing cost and improving the efficiency of real-time analysis of living cells in biofilms via fluorescence assays of confocal microscopy.

Transport across scales: Application of microfluidic platforms to simulate a contaminated aquifer

James Marquis, *PhD Candidate*
Microbiology and Cell Biology, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Our goal is to develop laboratory techniques to effectively simulate bacterial transport through the contaminated Bear Creek Aquifer and utilize findings to accurately predict species partitioning at the field scale. We place particular emphasis on the Subsurface Observatory (SSO), a series of nine wells on site that are equipped with real-time hydrologic and geochemical monitoring devices.

Methods and Results:
We have developed silica oxide microfluidic devices which accurately emulate the pore spacing of field sediments and these are currently being used to determine attachment and detachment rates of key environmental isolates. To maintain field relevance, we have been utilizing data gleaned from the SSO to develop media formulations that accurately depict subsurface conditions.

Next Steps:
We will utilize X-Ray computed tomography to develop micromodels that recapitulate the pore structure of the SSO and develop fluorescent strains of field isolates to enhance visualization of bacterial partitioning. Additionally, we will monitor changes in hydrologic and geochemical parameters during a rainfall event to determine the linkages between environmental perturbation and partitioning.

Industrial Relevance:
This work is relevant both to the oil and gas industry as well as the remediation industry. We expect that this work may improve the viability of enhanced oil recovery as well as certain bioremediation techniques.

Efforts towards biofilm monitoring in spacecraft water recovery systems

Haley Ketteler, *PhD Student*
Mechanical and Industrial Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
To facilitate future human space exploration, better control of biofilms in the water recovery systems of spacecraft must be addressed. This work explores the initial steps towards ground-based microgravity simulation and biofilm detection using electrochemical impedance spectroscopy sensors.

Methods and Results:
To explore the application of the simulated microgravity reactor for biofilm research, a new sampling method was explored and it was demonstrated that similar biofilm growth can be achieved across the diameter of the reactor allowing for the integration of biofilm sensors in the reactor. The sensors were tested in a custom setup to allow for timelapse microscopy which allows for correlation between biofilm accumulation and sensor signal.

Next Steps:
To further explore the sensor behavior in relevant systems, the sensors will be retested in the same microscopy setup shown and will then be integrated into the simulated microgravity biofilm reactor to confirm correct function of the sensors in a relevant environment.

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Industrial Relevance:
While this specific application of the sensors is not applicable for most industrial applications, the sensors have demonstrated functionality in many mediums in previous work. The investigation being done to understand the sensor behavior in a spacecraft water system will help strengthen the sensor applications in other fields.

Exploring methods to enhance engineered mineral precipitation in shale fractures

Matthew Willett, *PhD Candidate*
Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
Engineered mineral precipitation through microbially-induced calcium carbonate precipitation (MICP), a biofilm-based technology, has been demonstrated to reduce permeability in shale fractures under subsurface conditions. However, this process is time-consuming, requiring 13-26 repeat injections of MICP-treatment, and precipitation also tends to be inhomogeneous.

Methods and Results:
Studies show that polymer additives have potential to speed up and enhance MICP-treatment of porous media. Additionally, another study showed that when MICP solutions were modified with surfactants, improved calcite distribution as well as more efficient precipitation rates were achieved. This research will present the results of polymer-modified MICP-treatment of shale fractures and surfactant-modified MICP-treatment of packed beds of shale rocks using nuclear magnetic resonance (NMR) tools.

Next Steps:
As a next step, delivery methods for polymer-modified MICP-treatment need to be established. Additionally, surfactant-modified MICP-treatment needs to be tested on shale fractures at temperature.

Industrial Relevance:
Engineered mineral precipitation has potential to enhance the reliability of shale reservoirs for geo-engineering applications that can help combat the climate crisis. Plugging fractures created in hydraulic fracturing wells can also prevent contamination of aquifers and allow wells to be restimulated to enhance natural gas recovery.

A novel multiplex mRNA PNA-FISH for studying gene expression and spatial organization in biofilms

Ana Barbosa, *PhD Student*
Chemical Engineering, University of Porto, Center for Biofilm Engineering, Montana State University

Purpose of this Research:
The main goal of this work will be to explore the use of mRNA PNA-FISH to study the regulatory network involved in the biphasic life cycle of *L. pneumophila* helping to clarify the organization and functional development of biofilms combining spatial and functional information from cells in situ.

Methods and Results:
A multiplex mRNA PNA-FISH will be developed in order to provide essential data on gene expression in individual cells, both in the planktonic and sessile states, while also recording functionality and spatial information.

Next Steps:
After optimizing the planktonic state, we will test the specificity of the PNA probes against *P. aeruginosa* and *K. pneumoniae*. Then, we will investigate the spatial organization of *L. pneumophila* and its physiological state in different niches within multispecies biofilms using a biofilm reactor developed at the CDC (ASTM Method E2562).

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Industrial Relevance:
Contribute to a better understanding of the ecology and survival of *L. pneumophila* in biofilms present in industrial water systems.

Investigation of biofilm growth by intermittent flow paths in a microfluidic cell

Kerem Bozkurt, *PhD Student*
Institute for Modelling Hydraulic and Environmental Systems, Hydromechanics and Modelling of Hydrosystems, University of Stuttgart, Montana State University

Purpose of this Research:
The aim of this study is to investigate how biofilm growth is varied under different flow conditions and shear forces as a result of constant flow velocity.

Methods and Results:
It can be observed that under certain conditions intermittent flow paths form with a dynamic, but quasi-steady interaction of growth, detachment, and re-attachment. We can conclude that the regimes for intermittent flow path development is determined by a ratio of shear stress versus the biofilm's ability to resist to shear forces.

Next Steps:
A detailed comparison with numerical results. Perform additional experiments under constant pressure fluxes.

Industrial Relevance:
Biofilms are widespread in different porous media, including wastewater treatment systems, soil stabilization and bioremediation. The emergence of preferential flow paths with fluid flow can substantially influence the movement of nutrients and contaminants within porous media.

A screening method for evaluating the antimicrobial efficacy of residential laundry products, an adaptation of BSI EN 17658:2022

KortneJo Anello, *Microbiologist*
Department of Microbial Control Solutions (MCS), Arxada

Purpose of this Research:
A method was developed to screen experimental formulations before conducting the full EN17658 study. Our poster will elaborate on the steps taken to validate our screening method compared to EN17658.

Methods and Results:
The developed screening method conserves critical parameters of the EN17658 residential laundry test. Statistical analysis shows that this method is suitable as a screening tool when compared to the standard.

Next Steps:
Next step would be to explore reduction and removal of biofilms on textiles. The new screening method can also be modified to mimic other standard laundry tests ie. ASTM 2406.

Industrial Relevance:
Unless multiple analysts are used, the EN17658 methodology can only assess the efficacy of one formulation per test day. This screening method allows for 6 to 12 formulations to be tested in one sitting.

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Comparison of quantification methods for an endoscope lumen biofilm model

Sarah James, *Scientist*
Engineering Research, STERIS

Purpose of this Research:
The objective of this research was to evaluate biofilm quantification methods for ISO 15883:5 Annex F model to determine their ability to accurately and precisely assess biofilm reduction.

Methods and Results:
Five methods were compared to quantify biofilm within lumens representative of endoscope channels: protein using Pierce micro bicinchoninic acid (BCA) assay, protein using an orthophtalaldehyde (OPA) fluorescence assay, crystal violet assay, Total Organic Carbon (TOC), and CFUs. The results showed that TOC and protein methods (μBCA and OPA) provided the most consistant results when differentiating between tube sizes, making them suitable for assessing biofilm removal.

Next Steps:
N/A

Industrial Relevance:
Automated endoscope reprocessor manufacturers can optimize cleaning and disinfection of reusable medical devices by accurately assessing biofilm reduction, aligning with ISO 15883-5 standards.

Microfluidic and computational approach to modeling biomineralization in living materials

Brooke Filanoski, *PhD Student*
Biomedical Engineering, Cornell

Purpose of this Research:
We have developed a novel agent-based model for biomineralization that incorporates biofilm growth, urease secretion, chemicals diffusion, and calcium carbonate precipitation. Here, we demonstrate a microfluidic approach to analyze the bacterial microenvironment and validate our computational modeling. This modeling will aid in the understanding of living materials.

Methods and Results:
In parallel with image acquisition, we are acquiring batch reaction kinetics from benchtop flask studies as well as the effluent from the microfluidic devices. Urea, ammonium, soluble calcium, pH, and calcium carbonate precipitate were measured over time to calculate precipitation kinetics, optimizing the computational model to enable predictive growth and precipitation when varying parameters. We are excited to numerically simulate the complexities of self-fortifying living materials.

Next Steps:
We would like to add a machine learning portion to this model, allowing the model to be used to optimize parameters through large simulations.

Industrial Relevance:
This model could be used in industrial biocement applications, like in cases of binding porous media for pollution rumination or in living building materials. These findings could be used in building better future materials.

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Structural and spatial organization of biofilm communities

Bettina (Tina) Buttaro, *Associate Professor*
Microbiology, Immunology and Inflammation, LKSOM Temple University

Purpose of this Research:
Our research combines biological experiments, mathematical modeling, and machine learning to understand how 3D spatial organization of biofilms influences molecule exchange and biological behavior. Models are the interdependent organization of Cyanobacteria and heterotroph subaerial biofilms on stone monuments and how bacterial aggregation in heterogeous biofilms confer resistance to antibiotics.

Methods and Results:
Both projects combine 3D LSCM images with mathematical modeling and machine learning. Subaerial biofilms: This project examines water activity influences biofilm thickness and how Cyanobacteria fix carbon, excreting excess carbon as organic acids, which heterotrophs use for growth maintaining the pH balance. Enterococcus faecalis: A LSCM image based mathematical model was developed to examine the how aggregatives can provide protected microenvironment from antibiotics.

Next Steps:
Subaerial Biofilms: Use machine learning to identify continguous communities coupled with hybridization of 16S rRNA gene probes and predicted metabolic activity to identify potential interactions. *Enterococcus faecalis*: use machine learning coupled with an agent-based model to examine the spatial organization and size of aggregates needed to provide protected microenvironments.

Industrial Relevance:
Aggregative structures may confer resistance to various stressors, such biofouling prevention products. In *Enterococcus faecalis*, the endogenous hydrogen peroxide production is the likely stressor initiating the formation these structures. Aggregative species may also protect non-aggregative species in mixed-species biofilms.

Establishing a reproducible model to evaluate markers associated with biofilms on reusable medical devices

Gregory Anderson, *Senior Staff Fellow*
Center for Devices and Radiological Health, US FDA

Purpose of this Research:
The purpose of this study was to establish a reproducible biofilm model that could be used to identify markers of biofilms formed on medical device materials.

Methods and Results:
Biofilms of two bacterial species that often cause reusable device-related infections were formed in a drip flow reactor system. Analysis of colony forming units and confocal microscopy revealed that biofilms developed to consistent and repeatable levels on the coupons across experimental replicates. An exhaustive extraction further indicated that a single extraction removed greater than 93% of bacteria from the coupons.

Next Steps:
In additional experiments, the biofilm extractions could be tested in analytic assays to quantify biofilm molecules. These efforts are necessary to detect and quantify residual biofilms present after reprocessing reusable medical devices.

Industrial Relevance:
Methods to reproducibly develop biofilms on medical device material surfaces will be important for manufacturers wanting to validate biofilm related claims in their labeling and to ensure that properly executed reprocessing instructions will render these medical devices safe for subsequent use.

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Assessment and quantification of markers associated with biofilms on reusable medical device surfaces

Ruchi Pandey, *Staff Fellow*
Center for Devices and Radiological Health, US FDA

Purpose of this Research:
Identification and quantification of the appropriate markers associated with biofilms on medical device surfaces, using them to establish validation criteria to support manufacturer labeling claims related to cleaning or disinfection efficacy against biofilm.

Methods and Results:
We grew the Gram-negative bacterial biofilm using drip flow reactor and quantified as colony forming units, metabolic activity, endotoxin units, protein, peptidoglycan, and ATP concentration. The results suggested that the signal from each of these markers was commensurate with the bacterial/biofilm density, thus suggesting that the markers can be used to quantify residual biofilm on device surfaces.

Next Steps:
To determine endpoints for these markers that can be used to demonstrate that biofilm has been adequately reduced to allow for subsequent reprocessing steps.

Industrial Relevance:
Through our research, we aim to assist stakeholders in evaluating safety and effectiveness of technologies to reduce medical device associated infections, specifically through standardizing methodologies to evaluate claims in reprocessing instructions for medical devices pertaining to reduction of biofilms.

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