Proceedings

JULY 12-14, 2022

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Bozeman, Montana

The Center for Biofilm Engineering and its Industrial Associates gratefully acknowledge Elsevier and Biofilm Journal for sponsoring the 2022 Montana Biofilm Meeting.

Table of Contents

22 **Microbial isolation and characterization from two flex lines from the urine processor assembly onboard the International Space Station**
Hang Nguyen, Microbiologist, JES Tech, NASA

23 **Preliminary results of the ISS-operated *Pseudomonas aeruginosa* 'Space Biofilms' experiment**
Pamela Flores, PhD Student, Molecular Cellular & Developmental Biology, University of Colorado, Boulder
Luis Zea, Assistant Research Professor, BioServ Technologies, Aerospace Engineering Sciences, University of Colorado, Boulder

Posters

24 794 **Determination of biosurfactant producing microbes for biotechnological applications**
Aspen Burke, Chemical & Biological Engineering, MSU, CBE

24 795 **Investigation of ice nucleation activity of microplastics**
Christy Teska, Chemical & Biological Engineering, MSU, CBE

24 796 **Phycosome activity during the diel cycle**
Isaac Miller, Microbiology & Cell Biology, MSU, CBE

25 797 **Influence of antimicrobial coatings on biofilm accumulation of multidomain ISS wastewater isolates**
Madelyn Mettler, Chemical & Biological Engineering, MSU, CBE

26 798 **Pharmaceutical effects on aerobic granular sludge morphology and treatment capacity**
Kylie Bodle, Civil Engineering, MSU, CBE

26 799 **Analyzing the performance of aerobic granular sludge to reduce PFAS and conventional nutrients from wastewater in sequencing batch reactors**
Tasnim Sultana Ritu, Civil Engineering, MSU, CBE

27 800 **Highlights of a recent publication on biofilm harvesting & disaggregation**
Kelli Buckingham-Meyer, Research Assistant III, MSU, CBE

27 801 **Structured hydrogel microcapsules for understanding cell physiology in a chronic wound biofilm community**
Shawna Pratt, Chemical & Biological Engineering, MSU, CBE

28 802 **Using synthetic ecology to decode complex consortial interactions**
Martina Du, Chemical & Biological Engineering, MSU, CBE

28 803 **Investigating material properties of subsurface rock formations modified by engineered mineral precipitation**
Kayla Bedey, Civil Engineering, MSU, CBE

29 804 **Durability of microbially produced calcium carbonate adhesives**
Kaylin Clark, MSU, CBE

30 805 **Characterizing aerobic granular sludge MRI contrast though artificial biofilm models**
Matthew R. Willett, Chemical Engineering, MSU, CBE

Lab Demos

SESSION 1: Defining a Biofilm, *Darla Goeres, Session Chair*

Biological films: Keeping the cart behind the horse

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

It is becoming increasingly clear that attached microbial growth (*i.e.*, biofilm) more closely resembles *in situ* conditions for many microorganisms in different environments and is likely a universal feature with unique physiology, ecology, and evolution that requires deeper investigation to improve overall understanding of the microbial world. Moreover, microorganisms and the biofilms they form are known to play vital roles in ecosystem functions, including global biogeochemical cycling, industrial processes, and human health that have profound implications for the grand societal challenges of water, food, energy, and health (both human and environmental). The ubiquity and uniqueness of biofilms is commonly attributed to the distinct physiological state of cells existing and growing in the biofilm growth mode as well as to the secreted matrix components that interact with surfaces and the external environment. For multiple years, many research projects have attempted to identify common biological attributes that could 'define' what it means to be biofilm across a diverse array of microorganisms, but despite progress with some selected microorganisms, a condensed definition and/or marker has remained elusive across biofilm microbiology. This notion is hindered by an increasing knowledge that shows the sheer complexity and heterogeneity inherent to microbial biofilms and the microorganisms that form them under various conditions and constraints. Therefore, given a re-evaluation in the biofilm field to not only better define biofilm but to re-think how, why, and what is being defined will help guide both fundamental and applied biofilm research. The presentation will attempt to provide a different perspective on microbial biofilms more in terms of the physical and chemical constraints to which the bio-system is responding instead of focusing on the biological responses *per se*, in essence, "keeping the horse in front of the cart."

An industrial perspective on biofilm biology: Formation, maintenance, and mitigation

Presenter: **Christopher Jones, PhD**, Director of R&D
Affiliation: Sharklet Technologies, Inc. Aurora, CO, USA.

Biofilms are ubiquitous in nearly all industrial processes, with a total estimated annual cost to industries at over $3 trillion. From biofouling of jet fuel bladders and ship hulls affecting transportation, to biodegradation and corrosion of infrastructure, to contamination of industrial surfaces and fluids affecting machining, production, and longevity of goods, biofilms must be considered by all aspects of industry. Many approaches have been tried to control biofilm growth on industrial surfaces, such as cleaning agents, antimicrobials, mechanical methods, and repellent surfaces. However, no universal solution has been identified to solve biofilm. Further understanding is required of biofilm formation, maintenance, and mitigation to gain some control over biofilm-related contamination and the associated effects and costs.

Bacteria interact with surfaces to form a biofilm. This presentation will focus on the interface between the bacterium and surface, from initial contact to dispersal. Upon contact with a surface, bacteria initially adhere loosely, however a rapid response results in a transition from a motile to sessile state. The hallmark of this transition is the close association between the bacterium and the surface, facilitated by a variety of factors including pili and adhesins. Daughter cells of surface-attached bacteria are retained by a self-produced matrix consisting of a combination of proteins, nucleic acids, and polysaccharides. These communities of cells form a microcolony on the surface, which develops into a mature biofilm. The definition of a surface regarding biofilms will be discussed, as well as the contribution of the surface to the biofilm phenotype. Using this paradigm of biofilm formation, the importance and influence of surfaces will help to determine where along the continuum of biofilm development an adherent cell becomes a biofilm.

Finally, bacterial biofilm factors present interesting opportunities for biofilm mitigation in industrial settings. Several approaches to biofilm prevention, dissolution, and removal will be explored, with an emphasis on

areas of opportunity for widespread use in industrial processes. Due to the constant strain of biofilm-related effects and associated costs in industry, the area of biofilm prevention and mitigation is teeming with opportunities for creativity and innovation.

Important factors for defining medical biofilms

Presenter: **Garth James**, PI, Medical Biofilms Laboratory, Associate Research Professor
Affiliation: Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Medically relevant biofilms span a large range, from surfaces in treatment facilities to reusable medical devices, short- and long-term implanted devices, tissue/mucous associated, and intracellular biofilms. These biofilms can include a wide variety of microorganisms including bacteria, bacteriophages, fungi, and viruses as well as host components such as, fibrin, other proteins, polysaccharides, and DNA. Of primary concern is the tolerance of biofilms to antimicrobial treatments and host immune defenses. Biofilms are often defined as microorganisms attached to a surface, although non surface associated bacterial aggregates display similar antimicrobial tolerance. Bacterial aggregates have been documented in sputum from subjects with cystic fibrosis associated pneumonia as well as in synovial fluid from subjects with prosthetic joint infections. It has been suggested that *in vitro* biofilms tend to be "small" relative to typical *in vitro* models. However, "large" biofilms have been observed in chronic wound and long-term urinary catheter (LTUC) specimens. As with other biofilms, *in vivo* biofilms incorporate components from their environment (the host). An example of this are biofilms formed on intravenous catheters by *Staphylococcus aureus*, where coagulase production by the bacteria can accelerate fibrin deposition. Neutrophil lysis and the formation of neutrophil extracellular traps can contribute host DNA to the biofilm. In LTUC, pH increases due to biofilm metabolism can result in the formation of crystalline deposits from the urine that can occlude the catheter.

Regardless of our current understanding of medical biofilms, definitions remain a challenge. Are a few scattered bacteria within an endoscope a biofilm? There are no accepted clinical guidelines for diagnosing a biofilm infection, although some have been proposed. Traditionally, there has been a distinction between colonization (the mere presence of bacteria) and infection (the presence of bacteria with pathological affects). However, as we have learned more about subtle effects of biofilms in chronic infections this line has become blurry. Reaching consensuses on medical biofilm definitions will help improve healthcare.

The importance of clearly defining biofilm in laboratory standard test methods

Presenter: **Darla Goeres**, PI, Standardized Biofilm Methods Lab, Research Professor of Regulatory Science
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Achieving consensus on a definition for biofilm is nontrivial. Rather than focusing on what is "typical," the temptation is to define biofilm based on a broad range of possibilities and/or exceptions, often justified by practical experience. In this session, we are embracing the idea that the various fields may define biofilm slightly differently based upon the questions being asked or problem being solved. By asking the question, how important an application is (or not) when defining biofilm, we hope to capture key features that transcend the application area, while simultaneously highlighting features that are fundamental to understanding why biofilm thrives in a particular setting.

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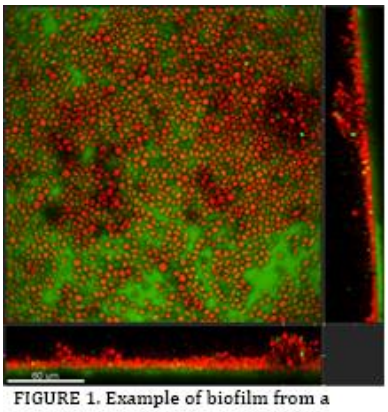

FIGURE 1. Example of biofilm from a contaminated beer line. L.A. Miller

Standard setting organizations and the regulatory bodies that develop guidance based on the methods they develop and validate endorse the need for consensus with regard to definitions. These definitions may be found in standalone documents and/or embedded in specific methods. A carefully developed standard methods will engineer a biofilm to have the desired characteristics based on fundamental engineering and microbiology principles. Ideally, how the method defines biofilm will align with the biofilm that is generated if all the steps are exactly followed. Standard methods are referenced in regulatory guidance and the results achieved are used for decision making. In this context, there are legal ramifications behind how a term is defined. If a label claim is being made that a product "kills biofilm," for instance, then it is critical to understand what biofilm means in this context. Exactly what is being killed?

This presentation will focus on how laboratory standard methods across multiple organizations define biofilm. An analysis of the frequency of key factors that are included in the definitions will be presented as well as the significance of what these factors mean in terms of justifying the significance and use of the method. Finally, the idea of defining biofilm based on a continuum rather than a set of absolute criteria will be explored.

Why evolution in biofilms is different, and some remarkable consequences

Presenter: **Vaughn Cooper**, Professor
Affiliation: Center for Evolutionary Biology and Medicine, University of Pittsburgh, PA.

A fundamental feature of biofilms is their genetic diversity. This diversity evolves more readily and rapidly in biofilms for two non-exclusive reasons. First, growth on surfaces or in aggregates subdivides the original population into many smaller populations, much like an archipelago in comparison to a single contiguous island. Just as island clusters are hotspots for biodiversity, subdivision in biofilms enables different genotypes to arise and persist in different regions because the environmental structure prevents admixture and competition. A predicted outcome of this process is that functionally equivalent genotypes, e.g. different mutations in the same gene, will become prevalent in different biofilm regions. This outcome is revealed in evolution experiments with *Acinetobacter baumannii* and *Pseudomonas aeruginosa* where populations grown under antibiotic pressure evolved multiple co-occurring mutations in regulators of efflux pumps in biofilms, but only a single mutation that became fixed in well-mixed populations. The second cause of increased diversity involves a wide range of ecological interactions between cells or clusters that are facilitated by structure, which changes the type and number of traits under selection. In some cases, selection can generate feedback on biofilm traits themselves, whereby early colonizing genotypes provide substrates or gaps for secondary attachment by other cells expressing different attachment polymers, pili, or fimbriae. Similarly, if neighboring aggregates consume nutrients or produce toxic by-products at different rates, selection may favor functional differentiation that can facilitate increased productivity of the biofilm community. I will discuss examples of structural and functional diversification arising even by single mutations in *P. aeruginosa* and *Burkholderia cenocepacia*, which pose challenges and opportunities for treating infections.

PANEL DISCUSSION

Defining a Biofilm

Panelists: Vaughn Cooper[1], Professor; Matthew Fields[2,3], Director, Professor; Garth James[2,4], PI, Associate Research Professor; Chris Jones, Director, R&D[5]
Moderator: Darla Goeres[2]
Affiliation: [1]Microbiology & Molecular Genetics, University of Pittsburgh, PA, USA. [2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [3]Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA. [4]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA. [5]Sharklet Technologies, Inc. Aurora, CO, USA.

SESSION 2: CBE Imaging and Analysis Capabilities, *Heidi Smith, Session Chair*

Microscopy Core

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

The CBE Bioimaging Facility consists of numerous imaging technologies specially configured to accommodate diverse sample types and span different spatial scales. The facility houses light/epifluorescent microscopes, stereoscopes, confocal microscopes, a laser micro-dissection microscope, an optical coherence tomography system, and a confocal Raman microscope. We have recently updated the facility with ~$1.8M of new state-of-the-art instrumentation. Of note are the facility's two new Confocal Scanning Laser Microscopes (CSLM).

The first addition is an Inverted Leica DMI8 Stellaris CSLM with 405, 488, 561, and 633 nm laser excitation lines. This microscope is equipped with a digital light sheet module which is ideally suited for sensitive 3D imaging of intact and complex samples. The CSLM is also fully enclosed and contains an environmental chamber to control temperature, humidity, CO_2/Air or hypoxic/hyperoxia. Additionally, this system was specifically configured to allow for stimulated Raman imaging to be added at a later date.

The second CSLM is an Upright DM6 Multiphoton CSLM, equipped with a white light laser. The white light laser enables light gating to be applied to any excitation line from 460-660 nm in combination with single photon imaging. This system is capable of lifetime imaging (FLIM-FRET). Its Multi-Photon Hyperspectral imaging (4-Tune) system will impact biofilm imaging by enabling increased penetration (>500 μm) and allow for label free imaging of mixed microbial samples. An Extended IR laser enables MP excitation from 680 nm to 1300 nm and MP imaging of red-shifted fluorophores (e.g., Alexa 568, 594). This upright CSLM is fully enclosed and contains an environmental control chamber to control temperature and humidity.

Combined, these instrumentations will transform how we are able to image biofilms by: i) increasing image acquisition speeds nearly eight-fold a prerequisite for the imaging of fast processes (e.g., diffusion, cell growth, and cell motility); ii) directly interfacing with modern microfluidic and sensor approaches; iii) reducing phototoxicity to maintain cell viability during live imaging; iv) enable high resolution hyperspectral imaging for label-free identification of cells and; v) increase penetration depths producing images more representative of intact biofilms. Examples of relevance to industrial applications include the ability to visualize intact biofilms to understand the mechanism of action for a product or processes, or the visualization of substrates with curvature (e.g., tubing from beverage lines or catheters) and thicker materials (e.g., textiles, membranes and wipes). We anticipate that the new instrumentation will significantly advance work with the treatment flow cell due to reduced phototoxicity, environmental controls, and fast image acquisition speeds.

CBE Analytical capabilities

Presenter: **Kristen Brileya**, Technical Operations Manager
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The CBE Analytical Lab is a high-volume research driven teaching lab, where undergraduates, graduates, staff, faculty and visitors to the CBE can be trained and/or use a variety of analytical instruments. Users receive tailored training that suits their experience and comfort level, from experimental design and sample collection plans, through instrument operation, data analysis, troubleshooting and method development. For example, there are capabilities for basic and advanced spectrophotometric measurements in microwell plates, cuvettes and tubes, which are used by many for assays including growth curves, spectral scans of plates, and bioluminescence-based assays.

In addition to spectrophotometry, the primary analysis tools are chromatographs for separation, identification, and quantification of components in liquid and gas samples. Three gas chromatographs and three liquid chromatographs with a variety of detectors can be configured to analyze a broad range of analytes. These types of analyses can be used to determine if specific compounds of interest are being consumed or produced in a pure or mixed culture or potentially an environmental sample. A dedicated carbon analyzer can measure either non-purgeable organic carbon or dissolved inorganic carbon, essentially TOC or DIC.

The CBE analytical lab includes a molecular biology area which is equipped for nucleic acid extraction, quantification, quality checks, and sequencing via Illumina MiSeq or Oxford Nanopore MinION. This talk will focus on specific research questions that our CBE faculty and students have asked in the last year, and how they have used the analytical facilities to answer their questions.

Statistical considerations in image analysis

Presenter: **Albert Parker**[1,2], Biostatistician, Associate Research Professor
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Mathematical Sciences, Montana State University, Bozeman, MT, USA.

Microscopy is an essential tool for any biofilm researcher as it allows non-invasive, quantitative measurements of various biofilm characteristics over space and time. Sometimes, one wishes to quantify some characteristic of the biofilm that is seen in the image, for example, by biovolume, surface area, surface coverage, or the spatial heterogeneity of microbes. CBE has libraries of confocal microscope images of different microbial species subjected to different treatments at different phases of growth.

This library of imaging data can be used to predict variability and design future imaging experiments so that biofilm characteristics can be estimated with a specified level of precision and statistical confidence. Important aspects to consider for the subsequent quantitative image analysis are:

- capture rate over time for time lapse imaging - depends on whether imaging an early forming biofilm or a biofilm in steady state or a biofilm subjected to a treatment
- limit of detection (LOD) – the higher the magnification, the worse the LOD
- number of biological replicates – include as many as possible
- numbers of fields of view (FOV) – only a few FOVs are needed if biofilm is present, many FOVs are needed if there are very few microbes
- pixilation – the high pixilation that is commonly used for beautiful images is detrimental to the capture rate over time and also is detrimental to statistical precision
- thickness – thick biofilms present unique challenges
- thresholding – don't let a human subjectively choose a threshold
- variability – a function of growth phase and treatment efficacy

MONT Highlight: Montana Microfabrication Facility (MMF) and the Imaging and Chemical Analysis Laboratory (ICAL)

Presenter: **Sara Zacher**[1], Lab Manager
Co-authors: David Mogk[1], Assistant Director
Affiliation: [1]Imaging and Chemical Analysis Laboratory, Montana State University, Bozeman, MT, USA.

The Imaging and Chemical Analysis Laboratory (ICAL) is a user-oriented core facility that supports basic and applied research in all science and engineering disciplines at MSU. We welcome collaborative research from the academic, governmental, and industrial sectors. We can either run samples for new users or we offer short courses to train new users to safely and effectively conduct their own experiments. Our philosophy is to work closely with our users to clearly define the research question, help design the best experimental protocols to answer that question, and help our users to interpret their results. We work closely with the MSU Center for Biofilm Engineering, the Montana Microfabrication Facility, the Proteomics, Metabolomics and Mass Spectroscopy Facility and Cryo-Electron Microscopy Facility through the MONT project, a node of the NSF-supported National Nanotechnology Coordinated Infrastructure Program. Details of ICAL instrumentation, scheduling, training courses, and related policies can be found at: https://physics.montana.edu/ical/index.html.

ICAL is host to numerous advanced imaging and analytical instruments that are routinely used to characterize material properties such as morphology (size, shape, aspect ratio), distribution, texture (e.g., overgrowths or intergrowths), phase identification, "bulk" composition, compositional variation (zoning, distribution of trace elements), elemental mapping, surface chemistry (top few atomic layers; including maps of surface components and atomic-scale depth profiling), determination of chemical state, crystal structure refinement and crystallographic orientation. The following is an overview of instrumentation available in ICAL and typical applications.

1. Field Emission Scanning Electron Microscopy (FESEM) has spatial resolution down to ~5 nm. It is equipped with a cryostage that permits imaging of microbes without introduction of artefacts from sample preparation procedures. It is equipped with an energy dispersive spectrometer (EDS) for acquisition of spot elemental analyses and elemental mapping, a back-scattered electron detector (BSE) for discriminating material variation by mean atomic number and/or density, and an electron back-scattered diffraction (EBSD) detector for *in situ* phase identification and crystallographic orientation. A second FESEM will be installed summer of 2022, and in addition to EDS and BSE detectors, will have a cathodoluminescence (CL) detector with spectrometer which will be able to map compositional heterogeneity, material defects and distribution of fluorescent dyes on a sub-micron scale.
2. Time of Flight Secondary Ion Mass Spectrometry (ToFSIMS) is a surface-sensitive technique that is commonly used to identify organic compounds on material surfaces (nm-scale); a mass range from 1–10,000 amu, mass resolution of 10^{-4} amu, sub-micron spatial resolution, and depth profiling capabilities provide mass spectra and images of molecular species and their distributions on material surfaces; (we have recently done these experiments with amino acids).
3. Auger Electron Spectroscopy (AES) is another surface sensitive method used to inventory elemental composition on surfaces, map their distribution, and is equipped with EDS and EBSD.
4. Atomic Force Microscopy (AFM) has nm-scale resolution of the 3D morphology of surfaces, allows *in situ* measurements and real time imaging of biological and chemical processes in liquid or air.

Through our NSF MONT/NNCI grant, we have proof-of-concept grant funding available to provide access to our facilities for new users. Please contact us if you have an interest in using ICAL to support your research initiatives.

Correlative microscopy links identity, morphology, biochemistry, and activity of uncultured microbes

Presenter: **Anthony Kohtz**[1], PhD Student
Co-authors: Roland Hatzenpichler[1,2,5], George Schaible[1], John Clif[45], Jeffrey Marlow[4], Rachel Spietz[5], Peter Girguis[6]
Affiliation: [1]Department of Chemistry and Biochemistry, Center for Biofilm Engineering, Thermal Biology Institute, Montana State University, Bozeman, MT, USA. [2]Montana Nanotechnology Facility, Montana State University, Bozeman, MT, USA. [3]Pacific Northwest National Laboratories, Richland, WA, USA. [4]Boston University, Boston, Massachusetts, USA. [5]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA. [6]Harvard University, Cambridge, Massachusetts, USA.

To gain a more holistic understanding of microbes in their native environment and determine how cells physically and metabolically interact with each other, we employed techniques to stain protein-synthesis active cells in their native habitat and visualized cells with complimentary multi-modal correlative microscopy techniques. We have established a workflow capable of providing fluorescence microscopy (activity), electron microscopy (EM; morphology), and energy-dispersive x-ray spectroscopy (EDX; mineralogy) information of the same sample and applied this workflow to characterize the *in situ* activity of a sediment microbiome and its interaction with different mineral species along a vertical sediment profile. We also have developed a complementary approach that allows us to sequentially determine the anabolic activity (via stable isotope probing and Raman spectroscopy), taxonomic identity (FISH), morphology (EM), and elemental composition (EDX and nano-scale secondary ion mass spectrometry) of any cultured or uncultured cell. After benchmarking this new approach with a mock archaeal-bacterial community we have successfully applied it to study the *in situ* physiology of multicellular associations of bacteria in a salt marsh.

CBE Open House: Poster Session and Lab Demonstrations

SESSION 3: Medical Biofilms, *Garth James, Session Chair*

Is there a universal biofilm defense?

Presenter: **Philip S. Stewart**[1], Regents Professor
Co-authors: Kerry S. Williamson[1], Laura Boegli[1], Timothy Hamerly[1], Ben White [1], Liam Scott[1], Xiao Hu[1], Brendan M. Mumey[1], Michael J. Franklin[1], Brian Bothner[1], Francisco G. Vital-Lopez[3], Anders Wallqvist[3], Garth A. James[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA. [3]Defense Biotechnology High Performance Computing Software Applications Institute, USA.

Is there a universal genetically programmed defense providing tolerance to antibiotics when bacteria grow as biofilms? A comparison between biofilms of three different bacterial species by transcriptomic and metabolomic approaches uncovered no evidence of one. Single-species biofilms of three bacterial species (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acinetobacter baumannii*) were grown *in vitro* for three days then challenged with respective antibiotics (ciprofloxacin, daptomycin, tigecycline) for an additional 24 h. All three microorganisms displayed reduced susceptibility in biofilms compared to planktonic cultures. Global transcriptomic profiling of gene expression comparing biofilm to planktonic and antibiotic-treated biofilm to untreated biofilm was performed. Extracellular metabolites were measured to characterize the utilization of carbon sources between biofilms, treated biofilms, and planktonic cells. While all three bacteria exhibited a species-specific signature of stationary phase, no conserved gene, gene set, or common functional pathway could be identified that changed consistently across the three microorganisms. Across the three species, glucose consumption was increased in biofilms compared to planktonic cells and alanine and aspartic

acid utilization were decreased in biofilms compared to planktonic cells. The reasons for these changes were not readily apparent in the transcriptomes. No common shift in the utilization pattern of carbon sources was discerned when comparing untreated to antibiotic-exposed biofilms. Overall, our measurements do not support the existence of a common genetic or biochemical basis for biofilm tolerance against antibiotics. Rather, there are likely myriad genes, proteins, and metabolic pathways that influence the physiological state of individual microorganisms in biofilms and contribute to antibiotic tolerance.

Table 1. Gene sets expressed at higher levels in biofilm compared to planktonic conditions (top six for each microorganism).

P. aeruginosa	***A. baumannii***	***S. aureus***
Stationary phase	Stationary phase	Stationary phase
NO_3^-/NO_2^-/NO metabolism	Phenylacetic acid	Iron acquisition
Phenazine biosynthesis	Antibiotic resistance	Host binding/virulence
HSL quorum sensing	Valine degradation	Pyruvate/Ser/Ala fermentation
Pyochelin biosynthesis (iron)	PNAG synthesis	Superantigen-like proteins
Bacteriophage Pf1	Trehalose synthesis	Zn transport

Using organoid models to analyze gastrointestinal bacterial infections

Presenter: **Diane Bimczok**[1,2], Associate Professor
Co-authors: T. Andy Sebrell[2], Barkan Sidar[3], Katrina N. Lyon[1,2], Michelle D. Cherne[2], James N. Wilking[1,3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA. [3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Organoids are 3-dimensional primary cell cultures maintained in a hydrogel matrix that replicate the physiological complexity of mammalian tissues. Organoid cultures can be derived from inducible pluripotent or tissue-specific stem cells and can be propagated *in vitro* for several months by using a combination of growth factors that support stem cell self-renewal. Since organoids are composed of non-transformed cells and can be generated from multiple individuals, organoids are superior to traditional cell culture models that utilize clonal cells derived from tumor tissues.

We are harnessing human gastric organoids to study infection with *Helicobacter pylori,* a gastric pathogen that causes gastritis, peptic ulcer disease and gastric cancer. We have derived >30 gastric organoid lines from human gastric tissues samples (Fig. 1A) and have demonstrated that gastric organoids show spontaneous mucus secretion and inducible acid secretion, consistent with the physiological function of the gastric epithelium. Gene expression analysis and microscopy confirmed that relevant cell types including mucus-acid- and enzyme-secreting cells are represented in the organoids. To analyze the impact of *H. pylori* on the gastric epithelium, we have established a protocol to infect the organoids by microinjecting bacteria into the organoid lumen. Following microinjection into gastric organoids, both motile planktonic bacteria and immobile biofilm-like bacterial aggregates of *H. pylori* (Fig. 1B) were detected after 48 h using confocal microscopy. We also demonstrate that gastric organoids can sustain active *H. pylori* infection for at least 2 weeks over multiple passages (Fig. 1C). To investigate mucosal immune responses to *H. pylori*, dendritic cells (DCs), which are innate immune cells that are centrally involved in immunosurveillance and antigen presentation, were incorporated into the gastric organoid cultures. DCs spontaneously migrated towards the organoid and established direct interactions with the epithelial cells (Fig. 1D), a process that was dependent on epithelial chemokines and that was significantly enhanced upon *H. pylori* infection (Fig. 1E). In summary, our work has demonstrated the feasibility of modeling infectious disease using complex organoid co-cultures and now enables us to investigate host pathogen interactions in the human gut.

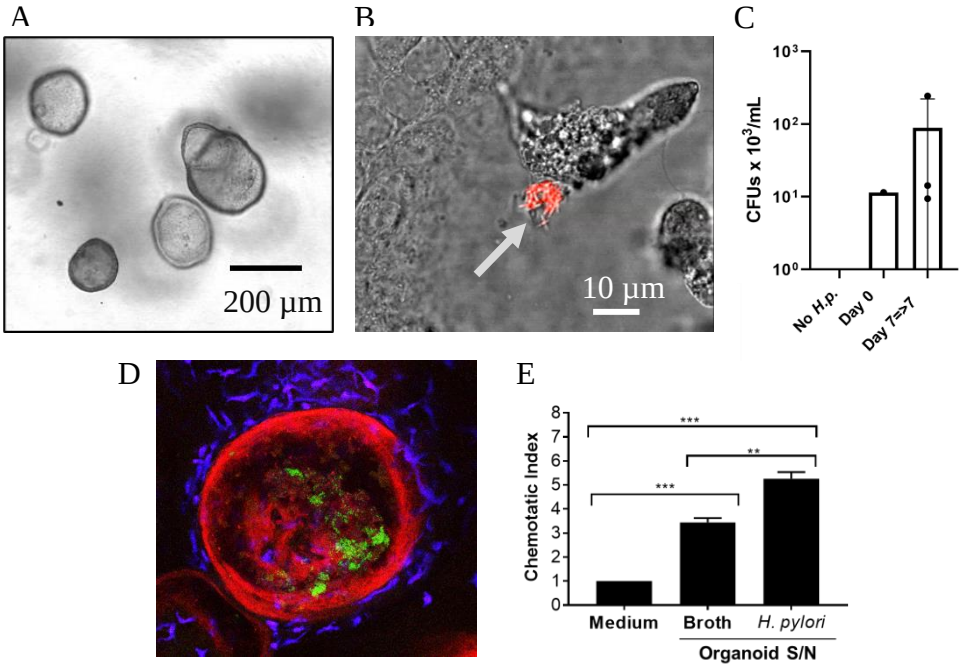

Fig. 1. (**A**) Representative phase contrast image of human gastric organoids. (**B**) *H. pylori* (RFP-expressing strain G7, red) forms biofilm-like structures on the luminal side of the organoid epithelium (arrow). Confocal image. (**C**) *H. pylori* growth in human gastric organoids after 14 d with organoids trypsinized and replated after the first 7 days (Day 7=>7). (**D**) Recruitment of immune cells (dendritic cells, blue) to the basolateral surface of a human gastric organoid (mCherry, red) infected with GFP-*H. pylori* (strain M6, green). (**E**) Human dendritic cells demonstrate increased chemotactic migration towards supernatants (S/N) from organoid cultures infected with *H. pylori*. ** $P \leq 0.01$; *** $P \leq 0.001$.

Biofilms and chronic wounds: An overview of recent Medical Biofilms Lab research

Presenter: **Elinor Pulcini**, Assistant Research Professor[1,2]
Co-author: Garth James[1,2]
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Chronic wounds such as pressure ulcers, venous leg ulcers and diabetic foot ulcers fail to progress through normal expected patterns of healing by remaining in a chronic infected and inflammatory state. Chronic wounds are considered to be biofilms based on their response or lack of response to treatment (i.e. antimicrobial tolerance) and their ability for regrowth in a manner similar to biofilms. The Medical Biofilms Laboratory has conducted numerous chronic wound studies including assessment for the presence of biofilms using microscopy, determination of the microbial community within wounds and effects of treatment on those wounds. This presentation will provide an overview of past and present chronic wound studies conducted by the MBL.

Accumulation of protoporphyrin IX by biofilm bacteria attenuates bovine neutrophil responses

Presenter: **Joey S. Lockhart**, Postdoctoral Fellow
Co-authors: Douglas W. Morck
Affiliation: Biological Sciences, University of Calgary, Calgary, AB, CAN.

Anaerobic gram-negative bacteria such as *Fusobacterium necrophorum* and *Porphyromonas levii* are etiologic agents that cause necrotic infections like periodontal disease, bovine interdigital phlegmon, digital dermatitis, and hepatic abscesses. Both species can form biofilms as a virulence mechanism, and they are commonly isolated together from infection sites. Host immune responses towards bacterial biofilms are frequently ineffective, resulting in persistent infection and chronic inflammation in a variety of biofilm-mediated pathologies in animals and humans alike. Neutrophils are critical first responder cells that are essential in the initiation of inflammation and clearance of bacterial invaders, and a better understanding of their interactions with biofilm bacteria is required for the development of new strategies to combat biofilm-mediated infections.

In this work, we employed a mixed-species model of biofilm growth to investigate *in vitro* neutrophil functional responses towards small molecules released from biofilm bacteria compared to those from planktonic cultures. Neutrophils were isolated from whole blood via hypotonic lysis and bacterial biofilms were generated using previously validated methods for the cultivation of mixed-species biofilms. Spent media from biofilm or overnight planktonic cultures were collected, centrifuged, and filtered to remove viable bacteria. Supernatants were fractionated via <3kDa-cutoff filtration and exposed directly to neutrophils. Neutrophil responses to the soluble factors in the fractionated supernatants were assessed with a fluorescent microplate assay for reactive oxygen species and chemotaxis was measured with a transmigration assay. Neutrophils generated substantial amounts of reactive oxygen species and transmigrated towards planktonic products but did not respond with similar intensity to biofilm factors. *Porphyromonas* species lack several of the necessary enzymes for heme synthesis, and therefore possess mechanisms to acquire a small, heat-stable, heme precursor known as protoporphyrin IX from host sources. *Porphyromonas* species accumulate protoporphyrin IX at the cell surface as a protective mechanism against environmental pressures such as oxidative stress or attack from host immune cells. The findings presented here suggest that the accumulation of protoporphyrin IX in the biofilm mode of growth inhibits bovine neutrophil functional responses. To our knowledge, this is the first study that investigated the direct impact of protoporphyrin IX on bovine neutrophil function and the results may help explain the inability of host immune cells to eradicate biofilm-mediated infections.

Application of PNA-FISH based-methods for bacterial detection and localization in biofilms

Presenter: **Laura Cerqueira**, Junior Researcher
Affiliation: Laboratory for Process Engineering, Environment, Biotechnology and Energy; ALiCE - Associate Laboratory in Chemical Engineering; Chemical Engineering Department; Faculty of Engineering, University of Porto, POR.

Nucleic acid mimics fluorescence *in situ* hybridization (NAM-FISH) is a molecular assay where fluorescently labelled NAMs such as peptide nucleic acids (PNA), are used to penetrate microbial cells and hybridize with specific rRNA sequences [1]. In earlier works, we have reported the design of several PNA probes, targeting specific pathogens in food, clinical and environmental samples (e.g. *Helicobacter pylori*, *Salmonella* spp., *Aspergillus fumigatus*; *Legionella* spp.) [2-6]. One of the advantages of the NAM-FISH is that, in addition to the detection of microorganisms, it also allows to localize individual cells in multispecies and multistrains biofilms, hence allowing the study of their three-dimensional spatial distribution [7]. PNA proved an advance over standard FISH because the neutrally charged, synthetic nature and shorter length of the PNA can contribute to an improved diffusion of the probe through the biofilm and to a better signal-to-noise ratio of the probe due to a stronger affinity of PNA to the target sequences [8,9]. Adapting PNA-FISH to target mRNA and combining it with spectral imaging using high-resolution CLSM, will lead to improved multiplexing ability. This allows to study gene expression in single cells either in suspension or in biofilms, combining spatial and functional information of the cells in their original environment [10]. Here it is intended to show a preliminary model for the use of mRNA PNA-FISH to study the regulatory network of *L. pneumophila*, helping to clarify the organization and functional development of biofilms and understand the roles that spatial and temporal heterogeneity plays in *L. pneumophila* virulence in water systems under different physiochemical conditions, disinfection treatments, and in the presence of diverse microbial consortia.

Special Presentation

Functions of the *Pseudomonas aeruginosa* biofilm matrix proteins

Presenter: **Boo Shan Tseng**, Assistant Professor
Affiliation: School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV, USA.

Approximately 80% of bacteria are capable of forming biofilms. Understanding how the extracellular biofilm matrix helps not only hold the cells together, but also protect the resident cells against external stressors is crucial to expanding our knowledge of this common bacterial lifestyle. While the extracellular DNA and exopolysaccharides in the biofilm matrix have been relatively well studied, much less is known about the matrix proteins. Using *Pseudomonas aeruginosa* as our model biofilm organism, we previously developed a method to identify proteins that are enriched in the extracellular matrix. In this talk, I will describe two such matrix proteins (the non-specific outer membrane porin OprF and serine protease inhibitor ecotin) and their roles in biofilm formation and in protecting the resident cells from the immune response. Overall, better understanding of the biofilm at a molecular level will aid in the development of technologies that will allow for the manipulation of biofilms to benefit humanity.

SESSION 4: Engineered Biofilms, *Matthew Fields, Session Chair*

From bones to sustainable building materials

Presenter: Chelsea Heveran , Assistant Professor
Affiliation: Mechanical Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Cementitious materials are ubiquitous in the built environment but require high energy, time, labor, and material resources to manufacture. Cement manufacturing necessitates high temperature processing and is associated with 5-9% of anthropogenic carbon emissions. The impressive resource needs and limited re-use and recycling of cement and concrete are at odds with a resource-limited future. To address these challenges, it is necessary to re-imagine how building materials are manufactured.

Biomineralization offers a new toolbox for eco-manufacturing more sustainable building materials. Microbial induced calcium carbonate precipitation (MICP) has been used at the Center for Biofilm Engineering for projects ranging from sealing leaking oil and gas wells to coarsening soils. MICP is also useful for binding together aggregate to form load-bearing materials. However, several challenges need to be overcome to efficiently manufacture biomineralized building materials with adequate strength for common load-bearing applications. We look to bone as an inspiration for more efficient manufacturing and improved material properties for biomineralized building materials. Bone is strong, tough, lightweight, and self-assembles at body temperatures. Bone achieves these remarkable properties as a biomineralized scaffold with many levels of structural hierarchy. The location and rates of biomineralization are controlled by properties of the scaffold and are also directed by the activities and products of remodeling bone cells.

We are working towards engineering biomineralized building materials that utilize several strategies inspired by bone. In this talk, we will focus on the results of biomineralizing scaffolds that range from hydrogels to living fungal mycelium. We will also discuss strategies to modify the surface of aggregate to control bacterial attachment and the location of the resulting biomineralization. Finally, we will explore ongoing efforts and next steps in designing bone-inspired sustainable building materials.

Figure 1. Biomineralized scaffolds for engineering sustainable building materials.

Untapped potential of fungal-based biofilms for water treatment and resource recovery

Presenter: **Erika Espinosa-Ortiz**[1,2], Assistant Research Professor
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Fungi are not only the major decomposers in nature, but they are also intimately involved in the biogeochemical processes underpinning metal and mineral transformations in the environment. Despite their unique properties and fundamental roles in the biotransformation of organic and inorganic compounds in nature, fungi have not been widely exploited for bioremediation processes and recovery of resources. In this presentation, I will provide an overview of the characteristics that make fungal systems advantageous for bioremediation, waste treatment processes and biorecovery of high-value products. As an example of an application for wastewater treatment, I will discuss the ability of fungi to transform toxic forms of metalloids, e.g., selenium and tellurium, commonly found in mining-impacted waters, into valuable products such as nanoparticles. I will also address the challenges of developing the next generation of fungal-based systems for wastewater treatment, which are envisioned to exploit the natural association and synergistic interactions that exist between fungi and other microbes in natural environments.

Building synthetic biofilm with 3D hydrogel printing

Presenters: **Isaak Thornton**[1,2], PhD Student, **Kathryn Zimlich**[1,3], PhD Student
Co-authors: Matthew Fields[1,4], Jim Wilking[1,5]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA. [3]Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA. [4]Microbiology & Cell Biology. Montana State University, Bozeman, MT, USA. [5]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Microbial biofilms are spatially structured, and this structure often gives rise to emergent properties not observed in planktonic, free-living populations. Biofilms have vast potential for metabolic diversity in both natural and man-made systems and there is growing interest in harnessing this diversity for applications such as bioremediation. The notion of applied bio-systems follows the ecological principle that increasing diversity can expand utilization of simple (CO_2, CH_4) and complex resources (toxic, recalcitrant chemicals) in an efficient manner. However, processes governing the assembly and evolution of microorganisms in a biofilm, which may generate and sustain diversity (*i.e.*, carrying capacity), are not well understood. To design biofilms for specific applications, more basic research into structure-function relationships of microbial communities is needed.

The collective function of a biofilm is coupled to the structure and composition of the community. For example, a biofilm in contact with a nutrient source develops internal nutrient gradients which depend on factors like thickness, cell density, and microbial composition. These nutrient gradients drive heterogeneities in growth rate, gene expression, and cell phenotype, which, in turn, alter the structure and composition of the

biofilm. Understanding these structure-function relationships requires the ability to control and manipulate the structure and composition of the community; however, biofilms that self-assemble naturally are difficult to manipulate experimentally. To understand these complex biological systems, methods of controlling and manipulating biofilm structure and composition are needed.

3D bioprinting is a rapid fabrication technique that could provide control over the structure and composition of living materials. Bioprinting has been used extensively to structure mammalian cells and tissues with complex geometries and finely tuned chemical, mechanical and biodegradable properties. By contrast, bioprinting microorganisms and biofilms is a growing but underdeveloped field with opportunity for constructing synthetic biofilm that can be experimentally manipulated. Here, we demonstrate 3D printed hydrogels with encapsulated *Pseudomonas fluorescens* growing in the hydrogel matrix. We anticipate this technology will advance into a powerful method for studying structure-function relationships in microbial communities.

Ureolytically induced calcium carbonate composites as natural adhesives

Presenter: **Sobia Anjum**[1,4], PhD student
Co-authors: Kendall Parks[2,4], Kaylin Clark[3,4], Chelsea M. Heveran[3,4], Albert Parker[4] , and Robin Gerlach[2,4]
Affiliation: [1]Civil Engineering, [2]Chemical & Biological Engineering, [3]Mechanical Engineering, [4]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Most of the synthetic adhesives currently in the market are non-renewable petroleum products and during production, use or postproduction emit volatile organic compounds (VOCs). Petroleum dependency of synthetic glues and outdoor emission of VOCs is harmful to the environment. Additionally, there are concerns with indoor accumulation of VOCs and their adverse human health effects for workers and users. There is a demand for sustainable, natural, and natural-synthetic hybrid materials as adhesives driven by a need for lower petroleum product dependency, and less detrimental environmental and human health effects of adhesives. State governments have been lowering the existing thresholds for VOC emissions to try to adhere to Clean Air Act regulations. Human health concerns with VOCs have also brought about regulations by OSHA adding VOCs used in adhesive production to Permissible Exposure Limit (PEL) to protect workers' health.

Natural adhesives provide an alternative to synthetic adhesives if they can be developed as standalone adhesive materials or as natural-synthetic hybrid material without VOCs. The alternative material we are exploring as a bioadhesive is a Ureolytically Induced Calcium carbonate Composite (UICC). The underlying reaction for the formation of UICC is ureolysis, driven by bacteria or the enzyme urease. Urea hydrolysis generates carbonate ions which in the presence of dissolved calcium can form calcium carbonate. The enzyme source (bacteria or extracted enzyme), organics and calcium carbonate aggregate to form the composite adhesive. UICC with and without additives have been previously studied for a broad array of applications utilizing its adhesive capabilities, including soil stabilization, concrete remediation, creating subsurface barriers etc. Applications in field work have also shown that UICP can be carried out with low-cost industry-based materials without losing efficiency of the process.

While a lot of research has been conducted on the stability of UICC consolidated structures, the adhesive strength of UICCs as standalone materials has yet to be tested. In this work we optimized the concentrations of bacterial cells, calcium, and type of organic additives to optimize the shear strength of UICC as an adhesive. Maximum lap shear strength achieved on the glass surfaces was 2MPa. Longer curing times to increase the shear strength of the composite resulted in glass failure and breakage before the composite failed. Comparable shear strength of the composite could be achieved on stainless steel surfaces too. Surface coverage analysis of the composite on glass surfaces after failure showed that the predominant mode of failure was adhesive failure. Durability testing of the composites at various relative humidities (50%, 80%, and fully immersed) and various temperatures (-20°C, 25°C, 100°, and 300°C) showed that the composite retained its shear strength at moderate humidity of 50% and at temperatures ranging from -20°C up to100°C.

These data provide evidence for successful application of the UICC as a suitable bioadhesive for glass and stainless-steel surfaces at a broad range of humidity and temperature conditions.

SESSION 5: Biofilms in Space, *Elizabeth Sandvik, Session Chair*

Potential for biofouling control by nutrient removal in an ISS water system

Presenter: **Elizabeth Sandvik**, Research Engineer
Co-authors: Phil Stewart, Darla Goeres, Paul Sturman
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Water recycling and recovery systems are critical components of crewed space exploration. The longest operational water recovery system in space is the Water Processor Assembly (WPA) on the International Space Station (ISS). While successfully recovering potable water from wastewater steams for over a decade, intermittent biofilm fouling events have reduced system performance, at times requiring component replacement to return the system to operation. One proposed strategy to reduce growth potential in the WPA is selective nutrient removal or reduction of target nutrients entering the water recovery system. To identify growth-limiting nutrients, a nutrient limitation screen was performed using a synthetic wastewater medium based on the water chemistry of the ISS WPA and a five-species multidomain consortia of ISS isolates. Batch assays were performed with individual nutrients (N, P, S, Zn, Ni, Ca, and trace nutrients) absent or provided in excess with follow-up experiments in CDC biofilm reactors. Testing showed growth in these models was phosphate-limited with phosphate removal reducing microbial growth of total cells by 1.7 $\log_{10}$(CFU total cells/mL) in the bulk fluid and 1.5 $\log_{10}$(CFU total cells/cm^2) in biofilm in biofilm reactors. Adding magnesium increased growth of all organisms but particularly increased growth of *Burkholderia contaminans*, *Methylobacterium organophilum*, and the fungal strain *Lecythophora mutabilis*. In addition, the magnesium greatly increased floc formation in the bulk fluid as well as biofilm on surfaces. This study identified phosphate and magnesium as target nutrients for biofilm control on the ISS and increased our knowledge of how the known fluctuations of nutrients and inhibitory metals in the ISS WPA may contribute to intermittent growth-permissive conditions in which biofouling potential increases.

Sensing slime: Microfabricated sensors to detect biofilm for space applications

Presenters: **Matthew McGlennen**[1,2], PhD student, **Haley Ketteler**[1,4], Master's Student
Co-authors: Markus Dieser[1,3], Michael Neubauer[1,2], Erick Johnson[2], Christine Foreman[1,3], Stephan Warnat[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA. [3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA. [4]Electrical and Computer Engineering, Montana State University, Bozeman, MT, USA.

In natural and human-inhabited environments, most microorganisms exist as organized communities attached to surfaces known as biofilms. Biofilm contamination is a major issue in human space exploration such as aboard the International Space Station (ISS) as it can cause failure of water reclamation components and corrosion to surfaces. Currently, biofilm management of the water reclamation system aboard the ISS consists of replacing failed components as part of an annual maintenance schedule. While a feasible approach for spacecraft orbiting Earth, long-duration, manned spaceflight missions will require systems adept at monitoring and controlling biofilm growth autonomously. Microfabricated sensors that leverage electrochemical techniques may be an effective solution for autonomous biofilm detection.

Electrochemical Impedance Spectroscopy (EIS) is a powerful technique for characterizing bulk and interfacial properties in aqueous, solid, and gas systems. The technique is based on applying an oscillating voltage at a single frequency to a device under test (DUT) and measuring the complex electrical current. Varying the frequency and calculating the complex resistance/impedance allows modeling the DUT using electrical equivalent circuits. Changes to the recorded spectra indicate *in situ* biofilm formation and increased microbial

concentrations in the media. In combination with EIS for microbial detection, oxygen, pH, temperature, and conductivity measurement devices may be incorporated onto the same miniaturized sensor footprint. Collectively, these data may help provide important information about biofilm formation during space-exploration missions. Currently, we have developed microfabricated EIS and temperature sensors that are small (~9x26 mm), low-cost, and amenable for real-time monitoring of biofilms.

The focus of this presentation will discuss the design, testing, and application of microfabricated sensors to monitor biofilm formation being developed here at the Center for Biofilm Engineering. The talk will highlight (i) the design of a simulated microgravity reactor under development, (ii) testing sensors in the simulated microgravity biofilm reactor, and (iii) analysis of sensor stability and data validation. Finally, the talk will discuss the future directions of microfabricated sensors, and the ongoing development and applications currently being evaluated.

Off-planet production of high-protein foods using fungal biomats

Presenter: **Ross Carlson**[1]
Co-authors: Laura Camilleri[1], Rich Macur[2], Renata Black[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Nature's Fynd, Bozeman, MT USA.

Production of fresh, nutritious food is essential for long duration, off-planet NASA missions. A novel bioreactor technology which uses fungal biomats as a food source has been developed through a collaboration of Nature's Fynd (formerly known as Sustainable Bioproducts), Montana State University, and BioServe Space Technologies. The technology capitalizes on the unique growth characteristics of an extremophilic, filamentous fungus isolated from a geothermal spring in Yellowstone National Park, named *Fusarium* strain flavolapis (also referred to as "strain MK7"). Strain MK7 is a Generally Recognized as Safe (GRAS) food ingredient and is now available to the US public as breakfast patties and cream cheese. The bioreactor technology is relatively simple, does not require energy during growth other than temperature control, requires little water, and rapidly grows dense, easily harvested, consolidated biomats for food with little to no remaining waste. The bioreactor technology has been demonstrated on Earth to efficiently convert NASA mission-relevant feedstocks, such as inedible plant materials and other wastes, into thick (> 6 mm) high-protein, edible, fungal biomats. The overarching goal of this project is to demonstrate the use of the novel bioreactor technology for growing high-protein food on the International Space Station (ISS). An ISS flight experiment is now underway to evaluate the effects of both micro-gravity and space radiation on bioreactor performance. Samples are expected to be returned to Earth in late July 2022.

Microbial isolation and characterization from two flex lines from the urine processor assembly onboard the International Space Station

Presenter: **Hang Ngoc Nguyen**[1], Microbiologist
Co-authors: G. Marie Sharp[2], Sarah Stahl-Rommel[1], Yo-Ann Velez Justiniano[3], Christian L. Castro[1], Mayra Nelman-Gonzalez[2], Aubrie O'Rourke[4], Michael D. Lee[5], Jill Williamson[6], Chelsea McCool[7], Brian Crucian[8], Miten Jain[9], and Sarah L. Castro-Wallace[8]
Affiliation: [1]JES Tech, Houston, TX, USA. [2]KBR, Houston, TX, USA. [3]Aerodyne Industries LLC, Cape Canaveral, FL, USA. [4]Exploration Research and Technology, NASA Kennedy Space Center, Merritt Island, FL, USA. [5]KBR, Space Biology, Ames Research Center, Moffett Field, CA, USA. [6]NASA George C. Marshall Space Flight Center, Huntsville, AL, USA. [7]Jacobs Space Exploration Group, Marshall Space Flight Center, Huntsville, AL, USA. [8]Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, TX, USA. [9]Bioengineering, Physics, Northeastern University, Boston, MA, USA.

Urine, humidity condensate, and other sources of non-potable water are processed onboard the International Space Station (ISS) by the Water Recovery System (WRS) to yield potable water. Recycling crew urine has

occurred since 2008 via the Urine Processor Assembly (UPA), which processes urine in batches. As this occasionally requires the urine to sit stagnant for extended periods of time, a pretreatment solution containing a strong acid, phosphoric acid (H_3PO_4), and a strong oxidizing agent, hexavalent chromium (Cr^{6+}), is added to stabilize the stored urine. While this pretreatment solution provides microbial control, areas within the WRS likely harbor microbial communities that have not been previously investigated. As such, the opportunity was pursued to microbially characterize two flex lines from the UPA that had been removed and returned to Earth for refurbishment. The residual water from these lines, as well as flush water, was evaluated. Bacterial culture and culture-independent analysis revealed the presence of *Burkholderia*, *Paraburkholderia* and *Leifsonia*, while fungal culture revealed *Fusarium* and *Lecythophora*. Hybrid de novo genome analysis of the five distinct *Burkholderia* isolates speciated them as *B. contaminans*. Not surprisingly, multiple chromium-resistance and transporter protein gene clusters were present in these genomes and separated them from other *B. contaminans* assemblies in NCBI upon pangenomic analysis. Interestingly, the gene clusters in these isolates were also unique as compared to other *B. contaminans* isolates from the ISS Potable Water Dispenser (PWD). As the PWD is at the opposite end of the WRS from the UPA, these findings indicate that unique populations exist within distinct niches in the WRS. Beyond genomic analysis, Environmental Scanning Electron Microscopy (ESEM) provided a visual display of fungal-bacterial biofilms within the flex lines. This is the first evidence of biofilm formation within flex lines from the UPA onboard the ISS. For all bacteria isolated, biofilm potential was further characterized with the *B. contaminans* isolates demonstrating the most considerable biofilm formation. Moreover, the genomes of the *B. contaminans* revealed secondary metabolite gene clusters associated with quorum sensing and biofilm formation. Collectively, these data identify the UPA flex lines as novel areas of biofilm growth within the WRS. Further investigation of these organisms and their resistance profiles will enable engineering controls directed toward biofilm prevention in future space station water systems.

Preliminary results of the ISS-operated *Pseudomonas aeruginosa* 'Space Biofilms' experiment

Presenters: **Pamela Flores**[1], PhD Candidate; **Luis Zea**[1], Assistant Research Professor
Co-authors: Samantha McBride[2], Kripa Varanasi[2], Daniel Wyn Mueller[3], Jiaqi Luo[3], Frank Muecklich[3]
Affiliation: [1]University of Colorado Boulder, Boulder, CO, USA. [2]Massachusetts Institute of Technology, Cambridge, MA, USA. [3]University of Saarland, Saarbrücken, DE.

Biofilms have been reported in all the Soviet, Russian, and American space stations, as well as the International Space Station. They may have negative impact to mission success in at least two aspects: degradation or impediment of engineering systems' functionality, and increase in the likelihood of crew infections. Nevertheless, biofilms have not been systematically studied in terms of how their growth and gene expression differs in microgravity with respect to Earth. To address this, the Space Biofilms project included a bacterial study performed in the International Space Station (ISS), where *Pseudomonas aeruginosa* was launched in stasis and, once on orbit, was exposed to 1cm^2 coupons of different materials submerged in culture media, and incubated for one to three days. The tested materials were selected due to their relevance to spaceflight components (stainless steel (SS) 316 and passivated SS316) or to nosocomial infections (catheter-grade silicone), and to try to replicate a previous spaceflight study (cellulose membrane). Additionally, and to interrogate potential solutions, two novel material approaches were included: a silicon wafer with a microscale texture impregnated with silicone oil developed at the Massachusetts Institute of Technology (referred to as LIS), and the same silicone coupons but with a repeating channel microtopography 3 μm in periodicity. After their incubation period, the samples were either fixed in 4% paraformaldehyde or preserved in RNAlater for post-flight phenotypic and transcriptomic studies, respectively. In this presentation, we compare and contrast how the biofilms' biomass, thickness, and surface area coverage changed as a function of incubation time, material, and gravitational condition. Furthermore, we present the preliminary results of our transcriptomic analyses, which includes differential gene expression and pathway enrichment analysis to gain mechanistic insight into the observed processes, among others.

POSTER ABSTRACTS

CBE Poster #794

Date: 03/22
Title: **Determination of biosurfactant producing microbes for biotechnological applications**
Authors: **Aspen Burke**[1,2], Markus Dieser[1,2], D. Smith[3], Christine M. Foreman[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA. [3]Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA.
Sponsored by: MONT Empower Scholars Program

Surfactants are chemicals with many valuable industrial and remediation applications, like helping clean up oil spills. They are amphipathic, meaning they contain a hydrophilic (water-soluble) group, and a hydrophobic (water-insoluble) group. This allows surfactants to reduce surface tension, emulsify liquids, and simply acts as a detergent. Unlike synthetic surfactants, biosurfactants are naturally produced by microorganisms, low in toxicity, and more eco-friendly and biodegradable. Much of what is known about biosurfactants is derived from studying model organisms such as *Pseudomonas* and *Bacillus*, however, these organisms are less environmentally relevant. To date, many natural environments, such as those in cold climates, have been largely overlooked in the search for biotechnologically relevant organisms. To fill this gap, cold temperature microorganisms previously gathered by the Foreman Research Group in Antarctica were screened for their biosurfactant producing ability.

CBE Poster #795

Date: 03/22
Title: **Investigation of ice nucleation activity of microplastics**
Authors: **Christy Teska**[1,2], Markus Dieser[1,2], Christine M. Foreman[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Undergraduate Scholars Program (USP)

Microplastics are some of the world's major contaminates today. Recent research has determined that these tiny particles are carried in the atmosphere via rain or snow and transported around the globe. Mineral particles, dust, black carbon, or biological material (e.g., microbes, pollen, fungi) in the atmosphere can act as ice nucleators and thus, affect precipitation patterns. Whether microplastics have an effect on precipitation remains unknown. We hypothesize that these particles can initiate ice nucleation (i.e., the freezing process) as has been shown with ice nucleation of biological samples. The purpose of this research is to observe ice nucleation activity of microplastics found in snow and rain.

CBE Poster #796

Date: 07/2022
Title: **Phycosome activity during the diel cycle**
Authors: **Isaac Miller**[1,2], Huyen Bui[2], Robin Gerlach[2,3], Matthew Fields[1,2]
Affiliation: [1]Microbiology & Cell Biology, Montana State University, Bozeman, MT, USA. [2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Phycosomes, or algae-associated microbiomes, can impact the growth, productivity and stability of algal cultures. While the influence of the phycosome on culture productivity is understudied, recent efforts have characterized phycosome communities based on taxonomy (*e.g.* 16S rRNA gene sequencing) and potential

metabolic capacity (*e.g.* metagenomic sequencing). Correlating single or multiple taxa with positive or negative outcomes for algae cultures is challenging in cultures where the phycosome is diverse and conditions are dynamic. Expanding the repertoire of molecular tools and refining experimental approaches are both essential in answering challenging questions related to the role of the phycosome in culture health, physiology and stability. Here, we use time-resolved sampling coupled with activity-based probing to gain a better understanding of how dynamics in the phycosome community correlate with algal physiology and culture productivity.

Chlorella sorokiniana SLA-04 is a highly productive, alkali-tolerant microalgae isolated from the alkaline Soap Lake (Washington, USA). In this study we used bioorthogonal non-canonical amino acid tagging (BONCAT) to determine the translationally active fraction of the SLA-04 phycosome. We assessed SLA-04 phycosome composition and activity during the light and dark periods of the diel cycle under high alkalinity conditions (HA, initial pH of 10.3, starting total alkalinity of 150 mM), and low alkalinity conditions (LA, initial pH of 8.7, and no added alkalinity). We separated phycosome communities based on level of physical attachment to algal cells to determine if spatial distribution of activity is even in the phycosome. We monitored algal physiology to correlate activity in the phycosome with culture productivity. HA and LA cultivation resulted in similar chlorophyll and cell numbers but HA cultures had significantly higher lipid content. HA cultures, which experienced smaller daily variations in pH than LA cultures, had more active phycosomes. During the dark growth period, phycosomes in HA cultures showed more significant activity than in the light period. This research (1.) outlines a new method for monitoring phycosome activity and function and (2.) provides foundational knowledge about diel cycling and resulting shifts in phycosome activity as it relates to algal physiology.

CBE Poster #797

Date: 07/2022

Title: **Influence of antimicrobial coatings on biofilm accumulation of multidomain ISS wastewater isolates**

Author: **Madelyn Mettler**[1,2]

Co-authors: Brent Peyton[1,2]

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

Biofilm formation in the ISS water system has resulted in system failure, requiring the replacement of parts and reevaluation of the wastewater renewal process. One approach to reducing biofilm growth is the application of antimicrobial coatings to the surfaces of the wastewater treatment system, specifically the wastewater tank. This, combined with approaches like nutrient depletion and the use of human-friendly biocides could greatly reduce biofilm accumulation in the ISS and other future craft. This project seeks to determine the biocidal effect of several antimicrobial coatings against five microbes commonly isolated from the ISS water system. The consortium consists of four bacteria: *Ralstonia insidiosa, Burkholderia contaminans, Methylobacterium organophilum*, and *Cupriavidus metallidurans*, as well as a fungus, *Lecythophora mutabilis*. Biofilms are grown in a normal gravity system (CDC biofilm reactor) with a medium that mimics the chemical composition and nutrient availability of the ISS wastewater. Antimicrobial coatings are evaluated on two materials found in the ISS water system: Inconel, a nickel-chromium alloy, and Teflon. This project is unique as it employs a five species biofilm that crosses the bacteria and eukarya domains, rather than single-species biofilms traditionally used in research. Additionally, though the experiments take place at normal gravity, the organisms, medium and materials used closely match those found *in situ* on the ISS. Biofilm prevention/reduction is evaluated based on viable cells in the biofilm via colony forming units on selective agar plates in the coated system compared to an uncoated control. Confocal and scanning electron microscopy provides information regarding the spatial arrangement of each species within the biofilm. Preliminary data from several coatings will be presented.

CBE Poster #798

Date: 07/ 2022

Title: **Pharmaceutical effects on aerobic granular sludge morphology and treatment capacity**

Authors: **Kylie Bodle**[1,2], Madeline Pernat[1,2], Catherine Kirkland[1,2], Heidi Smith[1]

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Sponsored by: Montana IDeA Network of Biomedical Research Excellence (INBRE), National Institutes of Health

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

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

CBE Poster #799

Date: 06/2022

Title: **Analyzing the performance of aerobic granular sludge to reduce PFAS and conventional nutrients from wastewater in sequencing batch reactors**

Authors: **Tasnim Sultana Ritu**[1], Catherine M Kirkland[1,2]

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Sponsored by: Montana Water Center, USGS 104b Water Resources Research Program

The project assesses the performance of the aerobic granular sludge (AGS) to remove Poly-perfluoroalkyl substances (PFAS) and conventional nutrients like carbon, nitrogen, and phosphorus from synthetic wastewater in sequencing batch reactor (SBR). AGS are a novel microbial community which may be effective in reducing the PFAS from wastewater via sorption. PFAS are a class of man-made chemicals used as surfactants, fire retardants and coating materials. The project will be conducted with two specific PFAS which are perfluorooctanoic acid (PFOA), perfluoro octane sulfonate acid (PFOS). PFAS compounds are very persistent in the environment and can lead to adverse health outcomes in humans. PFAS can migrate from consumer products and enter the influent of wastewater treatment facilities (WWTF). PFAS compounds are poorly removed by conventional wastewater treatment methods making effluent from WWTF a significant source of PFAS in the environment. Other objectives of this project are to monitor how PFAS influence the treatment of conventional wastewater constituents and the granule's structure and morphology. Two SBRs were started with floccular sludge from seed granules. Steady state performance in both reactors must be achieved before dosing of PFAS into one reactor. The second SBR is a control. Both reactors have been operating for around 328 days. The size distribution of the granules shows 19% granules have diameters larger than 2mm, 57% granules in the range of 2-0.5mm, and 24% granules smaller than 0.5 mm. The concentration of total suspended solids and volatile suspended solids in the SBRs ranges from 27-28 mg/ml and 20-21 mg/ml, respectively. The sludge volume index is 14-15 ml/g indicative of robust and dense granules. Some standard laboratory analytical methods for nitrogen, phosphorus and organic carbon are used to monitor the removal efficiencies of the granules. The granules contribute to the removal of 99.78%

ammonia, 75.66% phosphate, 55.59% nitrite, 59.38% nitrate and 91.07% organic carbon. Solid phase extraction (SPE) and liquid chromatography with mass spectrometry (UPLC with ESI Q-TOF-MS) have been used to assess the removal of PFOA and PFOS both from liquid and sludge phases. Prior to dosing PFOS and PFOA to the experimental SBR, methods have been developed for the extraction and mass spectrometry analysis for liquid and sludge samples contaminated with PFAS. The lowest limit of PFOA and PFOS detected in mass spectrometry is 5 ug/L in the extracted sample which corresponds to concentrations in the reactor in the ng/L range. The recovery of PFOA and PFOS from liquid sample is 85-95% and from sludge sample is 70-85%. Partitioning of PFAS between the sludge and aqueous phases will be tracked to assess how effective biofilm-based treatment may be in removing these harmful organics from wastewater.

CBE Poster #800

Date: 07/2022

Title: **Highlights of a recent publication on biofilm harvesting & disaggregation**

Authors: **Kelli Buckingham-Meyer**, Lindsey Miller, Diane Walker, Albert Parker, Paul Sturman, Darla Goeres

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

Biofilm growth methods are made up of four steps: growth of biofilm in an appropriate model, treatment of the biofilm, harvesting the biofilm, and analysis. Here, biofilm harvesting refers to removal of biofilm from a surface and disaggregation of the biofilm clumps into a homogeneous cell suspension. Out of these four steps, harvesting generally receives the least attention in the literature, yet inadequate harvesting results in biased data. This poster presents the results of a literature review to determine the most commonly used techniques to harvest and disaggregate biofilm, types of surfaces where the techniques were applied and efficiency of the chosen techniques, the advantages and disadvantages of the most common methods, checks for verifying the effectiveness of the techniques and the minimum information required when reporting on harvesting techniques.

CBE Poster #801

Date: 07/2022

Title: **Structured hydrogel microcapsules for understanding cell physiology in a chronic wound biofilm community**

Authors: **Shawna Pratt**[1,2], Connie Chang[1,2]

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Chronic wounds, or wounds with extended and abnormal healing pathologies, are frequently colonized by polymicrobial communities. The unique traits of these diverse communities, including enhanced pathogenicity, suggest that interspecies interactions in chronic wounds play a significant role in wound healing outcomes. The heterogeneous physical environment of chronic wounds further complicated these interactions. Although impactful, in-vitro methods to study community interactions within varied physical environments are underdeveloped. We investigated the impacts of multispecies interactions between *Staphylococcus aureus* and *Pseudomonas aeruginosa* using a catalog of microenvironments fabricated from hydrogels using single- and multiple-emulsion drop-based microfluidics techniques. This work presents novel methods for studying microbial interactions with single-cell resolution and constitutes the first application of all-aqueous core-shell microparticles to the study of prokaryotes and, more specifically, multispecies microbial communities. With this method, we investigate the ultimate and transient population dynamics and the cell and colony growth physiology in various microparticle core structures. This novel approach provides a unique tool and additional perspective as we seek to understand the impacts of multispecies interactions and their role in chronic wound infections.

CBE Poster #802

Date: 07/2022

Title: **Using synthetic ecology to decode complex consortial interactions**

Authors: **Martina Du**[1,2], Jeremy Chacón[3], William Harcombe[3], Ross Carlson[1,2]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA. [3]Ecology, Evolution, and Behavior, University of Minnesota, St. Paul, MN, USA.

Sponsored by: Montana INBRE, Montana State University USP, National Institutes of Health

From chronic wounds to nitrogen cycling in soils, metabolite cross-feeding is essential for ensuring the stability, resilience, and productivity of microbial communities. Most naturally occurring microorganisms live in complex consortia where metabolites are readily exchanged. Replicating these multispecies interactions with defined, engineered cocultures can help dissect the basis for microbial food webs providing a rational basis for controlling both beneficial and harmful consortia. Here, synthetic ecology was used to study the cellular economics of amino acid and organic acid exchange between obligate cross-feeding *Escherichia coli* strains. The coculture model was comprised of two strains, an arginine secreting strain that cannot catabolize the sugar lactose, and an arginine auxotroph which can catabolize lactose and secrete organic acid byproducts. The interactions created a robust and stable system in which the consortium composition shifted in response to stressors and resource availability.

The relative value of the exchanged metabolites changed with environment as quantified by changes in the abundance of the two strains. The consortium, when grown as planktonic batch culture, resulted in a reproducible 98:2 ratio of organic acid producer: arginine producer. However, when the consortium was grown as a biofilm, the consortium population shifted to a 50:50 ratio of the strains. The biofilms were further analyzed using O_2 microelectrode measurements and qPCR analysis of laser microdissected biofilm samples. The experimental results were integrated into a computational model and system parameters interrogated to identify possible physicochemical parameters controlling consortium behavior. Differences in O_2 availability were identified as a likely driver of the different consortium results. Steady state chemostat experiments were designed to confirm these predictions. Synthetic consortia with engineered interactions can decode complex natural systems by reducing the number of unknowns.

CBE Poster #803

Date: 06/2022

Title: **Investigating material properties of subsurface rock formations modified by engineered mineral precipitation**

Authors: **Kayla Bedey**[1,2], Olayinka Durojayea[2], Laura Dobeck[3], Dustin Crandall[4], Jonathan Mooree, Alfred B. Cunningham[2], Adrienne J. Phillips[2], Catherine M. Kirkland[1,2]

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Sponsored by: US Energy (DOE), EPSCoR

Fractures in shale serve multiple purposes, for example, in the recovery of resources in hydraulic fracturing or as potential harmful leakage passages through caprocks that may contribute undesired fluids to the atmosphere or functional groundwater aquifers. A proposed method to seal or influence fracture properties is Ureolysis Induced Carbonate Precipitation (UICP), a bio-mineralization technology driven by the enzymatic hydrolysis of urea, resulting in the formation of calcium carbonate. This study uses the microbe, *Sporosarcina pasteurii*, as the source of the urease enzyme that catalyzes the chemical reaction. The resulting calcium carbonate can bridge the gaps in fractured shale formations and reduce fluid flow through fractures. However, there is little information on how this process simultaneously affects the material property of the resulting biomineralized shale. This study represents the first step toward determining the influence of UICP treatment

on shale material and mechanical strength properties. This study combines micro-X-ray computed tomography (CT) and low field nuclear magnetic resonance (NMR) relaxometry techniques to characterize the changing material properties of a 2.54 cm (1 in) diameter, 5.08 cm (2 in) long fractured shale core taken from an outcropping of Marcellus shale before and after UICP treatment. The fractured untreated shale core was placed in a reactor operated at 60°C and ambient pressure and injected with fluids containing *Sporosarcina pasteurii* and other calcium mineralizing media prepared in a subsurface-like artificial ground water. Injection through the core continued until fluid could no longer be injected, with an apparent permeability reduction (2-3 days). CT scanning, reconstruction, and image analysis techniques have been conducted on the pretreated and biomineralized shale core. The results show a reduction in the fracture volume, and consequently a reduction in permeability, after UICP treatment. Permeability and porosity changes have also been assessed with NMR, and the resulting fracture volume changes were compared with μ-CT methods. After fractures are successfully sealed, the biomineralized shale cores will be subject to mechanical tension testing to determine the strength of UICP as a fracture sealant. This project aims to further develop optimal laboratory injection strategies to seal fractures with UICP by subjecting variety of shale formations to actual subsurface conditions in the lab and then closely characterize the material and mechanical property changes of the biomineralized shale so that engineered or natural rock fractures can be better understood.

CBE Poster #804

Date: 05/2022

Title: **Durability of microbially produced calcium carbonate adhesives**

Authors: **Kaylin Clark**[1,3], Sobia Anjum[1,4], Ethan Viles[1,3], Robin Gerlach[1,2]

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Volatile organic compounds (VOCs) are major indoor air pollutants released by common adhesives. Bio-based adhesives can reduce pollutant concentrations to create healthier living and working environments. One promising bio-based adhesive is a Microbially Induced Calcium Carbonate Precipitation (MICP) composite. MICP is induced by ureolysis, which generates carbonate ions, which can then bind with calcium ions to produce calcium carbonate. Soy protein isolate (SPI) is added to produce an MICP-SPI adhesive – previous work at the CBE has shown that this composite can be an effective bio-adhesive for glass and stainless-steel surfaces. The durability of this adhesive was tested at different temperatures and relative humidities (RH): -20˚C, 100˚C, 300˚C, 50% (RH), 80% (RH), and fully immersed in water at room temperature (23 ± 2 ˚C) for 7 days. Samples exposed to each condition were compared to control samples, which were tested after curing the adhesive for 48 hours at ambient, uncontrolled room conditions, prior to receiving any controlled temperature or humidity exposure. The single lap shear strength of the adhesive increased by about 41% after exposure to -20˚C, with most of the glass slides themselves experiencing failure before the adhesive failed. As temperature increased, shear strength decreased, with an observed 26% decrease from the control when exposed to 100˚C; at 300˚C most of the adhesive joints failed without any external applied force. The shear strength of the adhesive increased relative to the control by approximately 16% after exposure to 50% RH for 7 days, and decreased by about 90% at 80% RH; the lap joints fell apart quickly even during gentle handling full immersion in water for 7 days. This work will contribute to a future life cycle assessment which will aid in understanding the environmental impact, durability, and recyclability of these MICP bio-adhesives.

CBE Poster #805

Date: 07/ 2022

Title: **Characterizing aerobic granular sludge MRI contrast though artificial biofilm models**

Authors: **Matthew R. Willett**[1,2], Kylie Bodle[1,3], Joseph D. Seymour[1], Catherine M. Kirkland[1,3]

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Aerobic granular sludge (AGS) are compact, spherical biofilm aggregates used in wastewater treatment to simultaneously remove carbon, nitrogen, and phosphate. Previous studies have demonstrated that magnetic resonance imaging (MRI) can provide important insights into the internal structure of these complex, heterogenous granules. Of particular interest are water-like voids and solid-like dark regions which appear within the granule, which still have not been fully explained. This study explores how major components of the granule—proteins, polysaccharides, and cells—contribute to the contrast in T_1/T_2-weighted MRI by analyzing artificial biofilm models with well-defined properties. Preliminary findings have showed that certain gel-forming polysaccharides are a major source of T_2 contrast, while other polysaccharides show minimal contrast. Additionally, proteins, the most abundant granule component, lower the T_2 contrast, while bacterial cells enhance it.

CBE Poster #806

Date: 07/2022

Title: **Wide phylogenetic and functional diversity of McrA-encoding archaea in Yellowstone hot springs revealed by metagenomics and mesocosm experiments**

Authors: **Mackenzie Lynes**[1,2,3], Viola Krukenberg[1,2,3], Zackary Jay,[1,2], Anthony Kohtz[1,2,3], Rachel Spietz[1], Christine Gobrogge[4], Roland Hatzenpichler[1,2,3,5]

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Sponsored by: National Science Foundation

Yellowstone National Park (YNP) contains the world's highest concentration and largest diversity of geothermal features. While many studies have addressed the microbiology of geothermal environments, only a few have focused on methanogenic archaea in YNP. Based on the results of a geochemical (40 parameters) and molecular (methyl-coenzyme M reductase subunit A, *mcrA* amplicon sequencing) survey of 100 geothermal features across four thermal areas in YNP, we have identified the Lower Culex Basin (LCB) as an area of high interest. Metagenome sequencing of sediment samples from three hot springs within the LCB resulted in the recovery of 12 metagenome assembled genomes (MAGs) encoding *mcrA* and other key genes essential to Mcr-mediated metabolism. Four MAGs were found to be affiliated with classical methanogens within the *Euryarchaeota* phylum and eight with other lineages implicated in methane or alkane metabolism (*Verstraetearchaeota, Hadesarchaeota*, and *Archaeoglobus*). The recovered MAGs were comparatively small (0.73-1.78 Mbp) and mostly complete (87.5-100%) with low contamination (0-4.5%), with the exception of a *Hadesarchaeota*-affiliated MAG (72% complete). The twelve *mcrA*-encoding MAGs recovered reflect the potential for methanogenic pathway diversity in YNP geothermal environments and suggests more novel lineages may be recovered by future investigations. Mesocosm experiments were performed to assess the potential for hydrogenotrophic, aceticlastic, and hydrogen-dependent methylotrophic methanogenesis pathways under close to *in situ* conditions, conditions meant to stimulate methanogenic activity, and conditions inhibiting bacterial metabolism via treatment with antibiotics. Methane headspace measurements

in combination with 16S rRNA gene sequencing identified an active and diverse methanogenic community unique to the three hot springs. These results demonstrate the potential to study the activities of uncultured methanogens with a goal to isolate them from their natural environment. Next, we plan to incubate organisms enriched from this community with ^{13}C-labeled substrates and track their uptake under methanogenic conditions into FISH-identified cells using Raman microspectroscopy (SIP-FISH-Raman). Novel methanogens will be targeted in order to provide experimental evidence of their activities.

CBE Poster #807

Date: 07/2022

Title: **Bio-trapping of ureolytic microorganisms on sand surfaces to enhance microbially induced calcium carbonate precipitation**

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Microbially induced calcium carbonate precipitation (MICP) has been widely studied for its potential to improve sustainability in the construction industry and geotechnical applications. The presence of urease-producing bacteria is essential for catalyzing the conversion of urea into ammonium and carbonate ions to promote $CaCO_3$ precipitation. The effectiveness of the MICP process highly depends on the spatial uniformity of $CaCO_3$ precipitation and the concentration of bacteria. In this study, the functionalization of sand surfaces with amine groups was adopted for the first time to trap urease-producing bacteria on the sand surface from the planktonic phase to enhance MICP. Bioactive sand surfaces were prepared using 1% (3-Aminopropyl) triethoxysilane, APTES, to promote adhesion of ureolytic microorganism *Sporosarcina pasteuri* on sand surfaces. This approach enables concentration of urease producing bacteria on the sand surface, which can then precipitate a full monolayer of $CaCO_3$ acting as a binder to adjacent sand particles. The ureolytic activity, microbial growth, and viability after cell immobilization on APTES treated sand were measured using colorimetric Jung assay, Optical Density (OD600), and Confocal laser scanning microscopy (CLSM) of live/dead stained cells, respectively, and compared to untreated sand. The results demonstrated that the bio-trapping of microorganisms on APTES-treated sand surfaces reduced the growth rate of microorganisms and compromised microbial viability. Although the growth of the microorganisms as measured with optical density was slowed with the APTES treatment, ureolytic activity was maintained, and urea was completely hydrolyzed although at a slower rate. Bio-trapping of ureolytic microorganisms on sand particles can promote uniform and effective biocementation of sand.

CBE Poster #808

Date: 07/2022

Title: **Using fungal mycelium as a scaffold for biomineralized building materials**

Authors: **Ethan Viles**[2], Erika Espinosa-Ortiz[1,4], Arda Akyel[1,4], Robin Gerlach[1,4], Adie Phillips [1,3], Chelsea Heveran[1,2]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA. [3]Civil Engineering, Montana State University, Bozeman, MT, USA. [4]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Introduction: Traditional concrete manufacturing requires large amounts of energy due to the high temperatures used. This traditional method is estimated to contribute to about 8% of global CO_2 emissions. Microbial biomineralization happens at lower temperatures and may be used as a concrete alternative that would lower CO_2 emissions. Bacterial microbial induced calcium carbonate precipitation (bMICP) is used to

create low weight bearing structures and seal cracks. Previous work shows that scaffolded mineralized materials are more efficient, but these structures lacked environmental durability due to their gelatin scaffolds. Here we draw inspiration from biomineralized tissues, such as bone, as they form from a robust mineralized scaffold. Fungal mycelium has the potential to serve as a robust scaffold for biomineralization. To understand how biomineralization efficiency and biomineral characteristics differ between self-mineralized fungal scaffolds and bacterial-mineralized fungal scaffolds, *Neurospora crassa* was mineralized using 4 different techniques. A fungal MICP condition using malt media (fMICP-malt) method, another fMICP method using a medium where urea is the only nitrogen source (fMICP-urea), an abiotically induced calcium carbonate induced precipitation (AICP), and a bacterial-MICP (bMICP) where the ureolytic bacteria, *Sporosarcina pasteurii*, mineralizes the mycelium scaffold.

Methods: For fMICP scaffolds, *N. crassa* was cultured for 10 days in a medium containing calcium and urea. For AICP and bMICP scaffolds, *N. crassa* was cultured for 5 days in calcium-free medium then autoclaved. Scaffolds for AICP were then washed and placed into a solution containing calcium and bicarbonate. The pH was increased, and scaffolds were incubated (22C, 30min). For bMICP scaffolds, *S. pasteurii* was cultured on the fungal scaffolds in calcium free medium for 24 hours. After 24 hours, the scaffolds were placed in growth media containing urea and calcium for another 24 hours. Dissolved calcium and dissolved urea were measured throughout all mineralization techniques, and calcium content of the scaffolds were determined using a calcium digest. Electron microscopy and X-ray diffraction analyses were used to investigate the morphology and elemental composition of scaffolds.

Results: Electron microscopy illustrated different morphologies between all four different mineralization techniques. The two fMICP conditions both formed small minerals, but fMICP-urea had hyphae coated with mineral while fMICP-malt had little individualized hyphae and a coating of mineral on the outside of a mat of mycelium. AICP produced minerals that also coated the outside of the mycelium, but these were much larger than for fMICP-malt. bMICP produced the largest minerals of the four conditions. These minerals were clearly incorporated within the mycelium, not just on the outside of the mycelium. XRD showed that all scaffolds had two calcium carbonate polymorphs present, calcite and vaterite. bMICP was the only scaffold to have anything other than calcite and vaterite found by XRD which was calcium phosphate. fMICP samples showed more ureolytic activity and a faster decrease in dissolved calcium than fMICP-malt samples. AICP samples showed an instantaneous drop of dissolved calcium with the pH increase. BMICP showed the most ureolytic activity and the fastest decrease in dissolved calcium than any other MICP method.

Discussion: It is demonstrated that bMICP of fungal scaffolds offers the most efficient way to mineralize fungal scaffolds as ureolytic activity and dissolved calcium decrease is the fastest compared to other MICP scaffolds. The precipitation of calcium carbonate within the mycelium itself, and not just on the outside of the scaffold from bMICP scaffolds may provide stronger structures than other scaffolds. Future work will include nanoindentation analysis and scaling up these techniques to analyze the strength and stiffness of these scaffolds.

CBE Poster #809

Date: 06/2022
Title: **Optimizing total nitrogen removal in a two-stage vertical flow treatment wetland**
Presenter: **Kristen Brush**, Christopher Allen, Ellen Lauchnor, Otto Stein
Affiliation: Civil Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Since initial start-up in 2013, Bridger Bowl's pilot scale, two-stage, vertical flow treatment wetland has proven to be an effective system for removing ammonia and chemical oxygen demand (COD) from high strength wastewater. With increasingly strict discharge permit regulations, the focus is now to optimize operations for total nitrogen removal without compromising ammonia and COD removal. The first stage of the wetland is designed to remove COD initially by aerobic processes in an unsaturated layer, and then by anoxic processes in an underlying saturated layer. The COD consumed in the saturated zone fuels total nitrogen removal via

denitrification of the nitrate entering through the recycle stream. The second stage of the wetland is fully aerobic and acts as a nitrification reactor, converting >95% of influent ammonia to nitrate. In what is believed to be a carbon-limited system, it is hypothesized that increasing the quantity of COD within the saturated zone will enhance denitrification, and therefore total nitrogen removal. Operational parameters that influence COD concentration in the first stage are the saturation level, the recycle ratio, and the dose volume and dose frequency of the septic tank influent. During operations for the 2021 – 2022 ski season, the septic dose volume will be increased while maintaining the same daily influent and recycle flowrates. Results from this operational change will indicate if larger, less frequent influent doses improve total nitrogen removal when compared to smaller, more frequent influent doses. Expanding operational flexibility of the two-stage vertical flow wetland design will allow for broader industry application and increase the design's ability to meet the moving target of water discharge regulations.

CBE Poster #810

Date: 06/2022

Title: **The influence of biomineralized contaminated waste plastics in reinforced cement mortar**

Authors: **Kylee Rux**[1,2], Seth Kane[1,3], Michael Espinal[1,3], Cecily Ryan[1,3], Adrienne Phillips[1,2], Chelsea Heveran[1,3]

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The demand for cement infrastructure and plastics is continuously increasing, and the production of these materials generates greenhouse gas emissions. An additional problem is the low recycling rate of common plastics — with only 9% of plastics ever produced being recycled. Instead, these waste plastics are accumulating in landfills and the environment. One contributing factor to low plastic recycling rates is that contamination from food waste necessitates treatment steps that may increase the cost and reduce the quality of recycled plastic. Researchers have made headway against these challenges by using waste plastic as reinforcement in cementitious materials. However, increased plastic leads to a decrease in compressive strength. To combat these losses, it may be possible to use microbially induced calcium carbonate precipitation (MICP) to coat waste plastic in calcium carbonate and improve the properties of plastic-reinforced cementitious materials. The objective of our research was to understand how much clean and oil-contaminated waste plastic can replace cement in mortar and whether MICP coating of waste plastic enhances the strength of plastic-reinforced mortar. The performance of plastic-reinforced mortar cylinders was investigated using compressive strength tests at a 5%, 10%, and 20% volume replacement for cement. Results indicate that incorporating untreated clean plastics (HDPE, PVC, LDPE1, LDPE2) into the mortar matrix at a 20% replacement produces compressive strengths sufficient for several applications such as foundation walls, garages, and sidewalks. MICP-treatment on clean waste plastic did not significantly improve the compressive strength of the specimens. This biomineralization technique on oil-coated waste plastics (LDPE1, LDPE2, HDPE) rescued the strength relative to cylinders containing untreated oil-coated plastics by 28.28%, on average. However, washing the same oil-coated plastics with only water resulted in compressive strengths similar to that of MICP-treated, contaminated plastics. Ultimately, incorporating greater volumes of waste plastics into mortar at optimized replacement ratios could improve the sustainability of cementitious composites by the dual mechanisms of reduced cement production and repurposing plastic waste.

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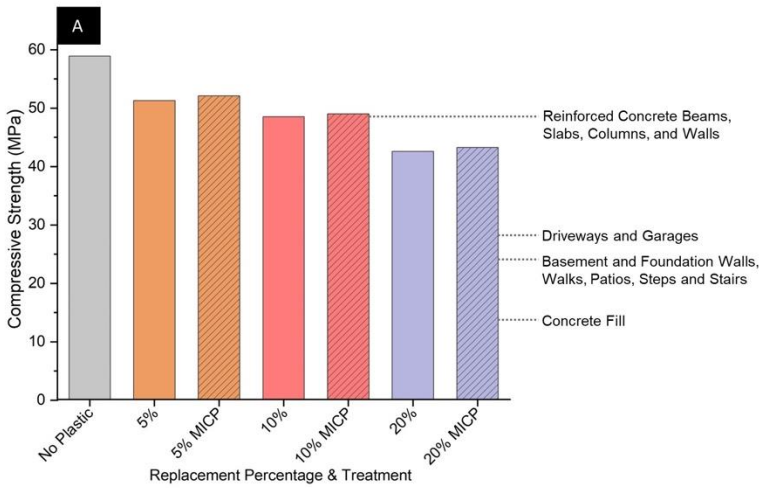

Figure A. Mortar containing clean waste plastic at a 20% replacement had adequate strength for several applications (strengths shown are averages across HDPE, PVC, LDPE1, LDPE2 at the given replacement percentage). The MICP treatment did not have a significant effect on the compressive strength.

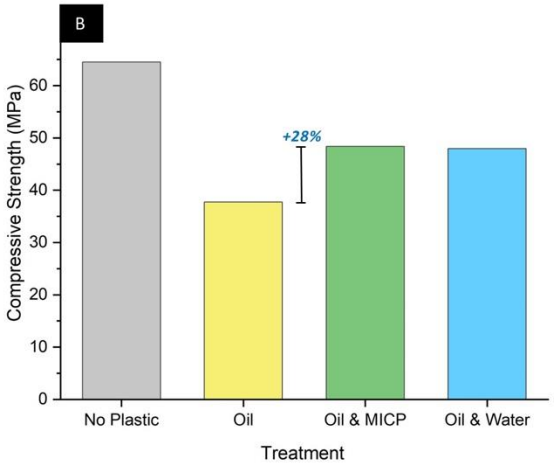

Figure B. Washing or MICP-treatment of oil-coated waste plastics rescued the strength relative to mortar containing untreated oil-coated plastics. Both treatment techniques resulted in similar strengths at a 5% replacement (strengths shown are averages of HDPE, LDPE1, LDPE2).

CBE Poster #811

Date: 06/2022

Title: **2-amino-5-nitrothiazoles and 2-aminoimidazoles; warheads for the eradication of biofilm-forming bacteria**

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

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[2]Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA.

Bacteria that have the propensity to form biofilms have presented a considerable challenge for the medical field in the treatment of chronic infections. Structure activity relationship (SAR) studies were conducted on novel 2-amino-5-nitrothiazole (ANT) derived antimicrobial agents via Minimum Inhibitory Concentration (MIC) assays and Minimum Biofilm Eradication Concentration (MBEC) assays and the conclusions of that study will be presented. In addition, N-trimethylsilyl-2-amino-5-nitrothiazole (N-TMS-ANT) was synthesized for the direct synthesis of ANT derived antimicrobial agents. The utility of this novel reagent will also be presented. Finally, the potential use of poly-substituted 2-aminoimidazoles as effective adjuvants will be presented.

CBE Poster #812

Date: 07/2022

Title: **Microbial nitrate-dependent iron oxidation in a coal waste rock bioreactor**

Authors: **Hannah Koepnick**[1,2], Brent Peyton[2], Ellen Lauchnor[1,3]

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Sponsored by: Teck Resources

The microbial process of anoxic nitrate-dependent iron oxidation (NDFO) could enhance removal of metal and metalloid contaminants in mining wastewater. Microorganisms performing NDFO produce iron (oxy)hydroxides by coupling Fe(II) oxidation with nitrate reduction. During this process, metal(loid) contaminants may be removed by precipitation, by co-precipitation, and by adsorption to the iron (oxy)hydroxides. Additionally, some contaminants, such as selenium, may be reduced by the same denitrifying

microbes that perform NDFO. This study investigated the potential of microorganisms from a bioreactor treating coal mining waste to perform NDFO. Fill material and monitoring well water were collected from an active *in situ* bioreactor treating coal mining waste and were used to construct anoxic batch reactors. Reactors were amended with aqueous Fe(II) and/or methanol to determine the feasibility of NDFO and assess competition of NDFO with heterotrophic denitrification using the carbon amendment. The reactors were incubated at 10˚C for approximately 3 months, with substrates re-amended when they became depleted. In reactors amended only with Fe(II), iron oxidation and nitrate reduction occurred concurrently, indicating that the microbial community performs NDFO. In reactors amended with both Fe(II) and methanol, some iron oxidation occurred, but the microbes preferentially used methanol for heterotrophic denitrification. Future work will analyze the microbial community involved in NDFO, and will investigate the impact of NDFO on removal of selenium and nickel, two contaminants of concern in the *in situ* bioreactor.

CBE Poster #813

Date: 07/2022

Title: **Effect of engineered grain boundaries & surface finish on microbiologically influenced corrosion (MIC) of copper 101**

Authors: **Amit Acharjee**[1,4], Yagmur Keskin[2,4], Brent Peyton[2,4], Roberta Amendola[1,4], Matthew Fields[3,4]

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

The annual cost of damage due to corrosion is estimated to be around 2.5 trillion USD globally. Microbially influenced corrosion (MIC) is found to be responsible for approximately 20% of annual corrosion cost and 50% of pipeline infrastructure failures and, in most cases, sulfate reducing bacteria (SRB), which exist in a broad range of anaerobic environmental conditions (e.g. shallow marine and freshwater sediments, soil and deep subsurface environments), were considered responsible for the corrosion damage. Most MIC research mainly focuses on the effect of microorganisms on the metallic substrate, while very little concern is given to the influence on the corrosion process of the intrinsic and extrinsic substrate properties like microstructure and roughness. This work focuses on the incorporation of low energy engineered grain boundaries, namely twin boundaries, into the microstructure of copper achieved through different thermal treatments and evaluates the effects of different surface morphologies achieved through metallographic grinding or polishing. The correlation between these surface properties and MIC is investigated with the sulfate-reducing bacterium, *Desulfovibrio alaskensis* G20. 3D optical profilometry, Electron Backscatter Diffraction (EBSD) Analysis, Field Emission Scanning Electron Microscopy (FE-SEM), Energy Dispersive X-Ray (EDX) and Auger nanoprobe analyses are used to investigate corrosion morphology and product development. Characterization is conducted before and after exposure to both biotic and abiotic environments. Results from this work represents a novel and inexpensive approach to reduce MIC by taking advantage of material surface properties which is easy to control during the manufacturing process.

CBE Poster #814

Date: 07/2022

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

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

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

Understanding the fate and transport of environmentally relevant bacteria in groundwater is critical to the prediction of fate and transport of microbial biomass and activity as well as the enhancement of potential bioremediation in subsurface porous environments. In this study, we examined the transport and adsorptive capacity of *Stenotrophomonas* EB106-03-01-XG87 isolated from the contaminated Bear Creek Aquifer in Oak Ridge, Tennessee that contains high levels of nitrate and heavy metals. A limited understanding of the transport processes of relevant bacteria at this site remains a large hurdle in estimating the distribution and dispersal of microbial biomass, and *Stenotrophomonas* ASVs observed from field samples are equally distributed as groundwater or sediment-associated populations. Therefore, *Stenotrophomonas* EB106-03-01-XG87 represents a base case bacterial population for insight into fate and transport in a simulated porous media. In this study, *Stenotrophomonas* EB106-03-01-XG87 was inoculated into packed bed reactors which mimicked key environmental conditions from the aquifer. Namely, the PBR was composed of sand particles that represented particle size distributions observed at the field site and two flow rates (low and high) that simulated the upper and lower bounds of groundwater flow *in situ* (triplicate reactors). All reactors were operated anoxically for a period of 10 days with an artificial groundwater medium. The low flow case was approximately 4.9mL/hr. and the high flow case was approximately 11.8mL/hr. These flow rates represent the upper and lower bounds of the pore velocities observed at the site. Over the course of the experiment, effluent samples were taken periodically, and cell counts were performed using a DAPI/BONCAT counter stain to determine total cell counts as well as activity levels over time. The timing of these samples was based upon breakthrough curves which utilized Br- as a non-reactive tracer at each flow rate. At the final time point, the reactors were disassembled, and a sand core was removed from the reactor. Cells were removed from the core material in quartiles (e.g., bottom 25% of core was lower quartile) to determine the cell distribution within the porous media. We observed that the partitioning *Stenotrophomonas* is highly dependent on flow rate. Under the high flow condition 10-30% of the total cells were permanently associated with the sand particles. However, under the low flow condition approximately 50-60% of the total cells permanently adsorbed to the sand particles. Additionally, growth was significantly higher under the high flow condition, likely a result of higher nutrient loading with the same amount of oxygen introduced. Interestingly, under all conditions, the time to peak effluent matched the corresponding Br- breakthrough curve. Overall, 65-80% of total planktonic cells were active over the course of the experiment in the high flow case, and 80-85% were active in the low flow case. Toward the end of the experiment, the activity level dropped to approximately 10%, and this level is similar to the activity levels in the sand associated particles. Current work includes batch sorption experiments with varying concentrations of live and dead *Stenotrophomonas* cells.

CBE Poster #815

Date: 07/2022

Title: **Effect of copper and copper surfaces (101) on the growth and initial biofilm formation of *Desulfovibrio alaskensis* g20**

Authors: **Yagmur Keskin**[2,4], Amit Acharjee[1,4], Brent Peyton[2,4], Roberta Amendola[1,4], Matthew Fields[3,4]

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

The U.S. spends nearly $4 billion annually to address biocorrosion challenges posed by sulfate-reducing bacteria. Copper is a unique material that is widely used in water distribution, and previous work has shown unique biological responses to copper in different microorganisms, including sulfate-reducing bacteria. Copper toxicity effects on the growth of planktonic *Desulfovibrio alaskensis* G20, was investigated under electron-donor limited, electron-acceptor-limited, and balanced growth conditions. Planktonic growth was most impacted at the EAL condition, and this condition can simulate water systems in which the ratio of substrate to sulfate levels can be high. Based on these results, the electron-acceptor limited condition was used to further test surface property effects on initial biofilm attachment. The polycrystalline copper (101) was heat annealed at 475C and cut into coupons, and three surface types were tested: no polish, rough polish

(P-400 Grit Silicon Carbide Paper), and fine polish (P-1200 Fine Grit Silicon Carbide Paper and 3 nanometer grinder fiber optic polisher). The inoculated and abiotic control coupons were incubated for 7 days under anoxic, batch conditions. The dissolution of copper was measured from the abiotic controls, and surface roughness was directly correlated to rate of copper dissolution. The untreated surface had the highest rate of copper dissolution (1.13 um/h) and the rough and fine polished surfaces had slower rates (1.06 and 0.07 um/h, respectively) in 48 hours; however, the final maximum level of soluble copper was similar for the three tested surfaces (0.50 um/h) in 120 hours. These observations coincided with observed growth effects on planktonic and initial biofilm cells, in which the fine polished surfaces allowed faster growth of planktonic cells than unpolished surfaces. Likewise, in the unpolished and rough polished surfaces, planktonic growth was slower but initial biofilm formation was increased. The increased interactions at the copper surface for the unpolished and rough polished surfaces corresponded to increased biofilm, cuprous sulfide precipitates, and initial pitting. However, the untreated surface (no polish) appeared to resist surface interactions compared to the rough polish surface, and this result may be due to the presence of an oxidized passive layer. Further work continues to achieve steady-state biofilm growth on different copper surfaces to better understand material property effects on biofilm formation and growth.

CBE Poster #816

Date: 06/2022

Title: **Characterization of the material properties of biomineralized shale using Nuclear Magnetic Resonance (NMR) methods**

Authors: **Olayinka Durojaye**[1,2], Kayla Bedey[1,2], Laura Dobeck[3], Dustin Crandall[4], Jonathan Moore[5], Johnny Rutqvist[6], Alfred Cunningham[1,2], Adrienne Phillips[1,2], Catherine Kirkland[1,2]

Affiliation: [1]Civil Engineering, Montana State University, Bozeman, MT, USA. [2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [3]Energy Research Institute, Montana State University, Bozeman, MT, USA. [4]Leidos Research Support Team, Morgantown, WV, USA. [5]National Energy Technology Laboratory, Morgantown, WV, USA. [6]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Sponsored by: EPSCoR, US DOE and Benjamin Fellowship

Nuclear Magnetic Resonance (NMR), has shown promising applications in studying material properties such as pore size distribution, porosity, and permeability of porous materials, including nanoporous rocks like shales. The US EIA 2018 estimated that shale, generated about 70% of total U.S. dry gas production and 60% of total U.S. oil production. With these significant economic prospects, shale is a lucrative energy source. However, unwanted fractures could develop during hydraulic fracturing of subsurface rocks like shale. Also, in the case of an abandoned gas well, if inherent fractures are not correctly sealed, there could be harmful leakage pass ways through caprocks that may contribute undesired fluids to the atmosphere (aggravating global warming) or functional groundwater aquifers.

Applying a biomineralization technology known as ureolysis-induced calcium carbonate precipitation (UICP) driven by the enzymatic hydrolysis of urease produced by microbes results in the precipitation of calcium carbonate, a biomineral that has been proven to seal tiny fractures in rocks in previous studies. Still, because of shale's microscopic pores and material constituents, there is little information on how this process affects the material property of the resulting biomineralized shale.

This study used a low field 2MHz NMR instrument to characterize the material properties of a 1inch diameter, 5inch long fractured shale core drilled from an outcropping of Marcellus shale before and after biomineralization. The fractured untreated shale sample was placed in a reactor operated at 60°C to simulate typical subsurface conditions and ambient pressure. Two injection strategies were employed, to inject fluids containing *Sporosarcina pasteurii* as a urease source and other calcium mineralizing media.

NMR relaxometry measurements of one-dimensional (T2) and multi-dimensional (T1-T2) relaxation times were obtained from the samples. The multi-dimensional relaxometry detected changes in the various hydrogen populations and their phases, such as viscid hydrocarbon, bound, and immovable water within the

samples, as reflected in the NMR T1-T2 maps. The one-dimensional relaxometry showed the T2 relaxation times analogous to pore size distribution and changes in permeability. The porosity of the shale samples was also obtained by comparing the T2 signal level of each sample with the signal level from a standard water sample. These findings provide vital information, especially in the energy sector where UICP is employed to seal fractures developed during hydraulic fracturing and well repair. Future work would involve further testing of other biomineralized shale samples.

University of California at Irvine

Date: 07/2022
Title: **Cicadas beat infection! Insect inspired nanomaterials to prevent biofilm formation**
Authors: **Kara Fan, Albert Y.**
Affiliation: University of California at Irvine, CA, USA.

Statement of the Problem: More than 1.2 million deaths were associated with antibiotic-resistant infections in 2019. According to the U.S. Centers for Disease Control and Prevention (CDC), 1 out of 25 patients who go to the hospital will develop a hospital-acquired infection. 80% of these infections are due to biofilm formation on implants and frequently touched surfaces. Recently, it was discovered that dragonflies and cicadas have nanopillars on the surface of their wings that are antibacterial and antifouling. However, the mechanisms by which the nanostructures kill bacteria have yet to be determined. Soft antibacterial nanostructures have also not been fabricated. In this study, antibacterial nanopillars were fabricated out of stiff (PMMA) and soft (chitosan) materials to slow down biofilm formation on a variety of different surfaces. The antibacterial mechanisms of the nanopillars were also examined. The nanopillars were fabricated via drop cast lithography in which the solution was first poured into nanohole molds with a periodicity of 500 nm, then crosslinked.

Perfectus Biomed Group 1

Date: 06/2022
Title: **Expression of *Pseudomonas aeruginosa* biofilm forming genes in a CDC biofilm reactor**
Authors: **Jessica Sanders**, C. Ball, Samantha J. Westgate
Affiliation: Perfectus Biomed Group

The Centers for Disease Control (CDC) reactor model allows for biofilm establishment on solid state coupons for commercial product testing. This study aimed to identify transcription profiles of eight key genes involved in early biofilm production and maintenance up to 72 hours. A *Pseudomonas aeruginosa* inoculum (10^6 $CFUmL^{-1}$) was grown aerobically at 37^0C in a CDC reactor for 72 hours. Stainless steel coupons were removed at 2, 6, 18, 24, 48 and 72 hours, sonicated to recover bacteria, and mRNA was extracted for measurement of gene transcription using qPCR. DNA was also extracted to quantify bacterial load. Biofilm samples were compared to planktonic samples taken from the same reactors to establish difference in transcription fold change.

No genes from biofilm samples showed a significant transcription change at 2 hours post-incubation, however three genes (*rsaL*, *pslA* and *pcrV*) were significantly upregulated, and one significantly downregulated (*gyrA*) at 6 hours. All investigated genes were significantly upregulated by 18 hours, with four (*pelA*, *rsaL*, *pcrV* and *gyrA*) peaking at 18 hours, three (*pslA*, *psqC* and *cbrA*) peaking at 24 hours, and the remaining gene (*acpP*) peaked at 48 hours. There was no significant difference between coupon bacterial loads at 24, 48 and 72 hours, however all coupon quantifications were significantly lower than planktonic samples. This study demonstrated expression of eight genes important to biofilm forming pathways and establish early transcription profiles under optimal conditions. This model can be used for identifying mode of action for commercial products which disrupt or prevent biofilm formation.

Perfectus Biomed Group 2

Date: 06/2022
Title: ***Pseudomonas aeruginosa* adhesion gene expression following wound dressing application**
Authors: **Jessica Sanders**, C. Ball, Samantha J. Westgate
Affiliation: Perfectus Biomed Group

The mode of action for wound dressings can differ depending on their application. One method focuses on the adherence of bacteria, which acts to reduce bacterial load by removing bacteria from the infection site. This study aimed to quantify adhesion gene transcription following application of a commercial wound dressing, when compared to a standard gauze. A *Pseudomonas aeruginosa* inoculum (10^6 $CFUmL^{-1}$) was prepared and 100µL applied to the surface of a commercial wound dressing, standard gauze and a negative control (plastic film). After 24 hours static incubation at 37°C, 1mL of planktonic bacteria were taken from each sample and the remaining was removed by washing. Adhered bacteria were recovered in 5mL of sterile PBS by sonication, and mRNA was extracted for measurement of gene transcription using qPCR. Adhered samples were compared to planktonic samples to establish difference in transcription fold change.

No significant difference was detected in bacterial quantification between recovered samples following 24 hours incubation. Transcription of *pelA* was significantly up-regulated (586.10 fold increase) for the commercial wound dressing when compared to the standard gauze (45.43 fold increase). Similarly, transcription of *rsaL* was significantly up-regulated (230.72 fold increase) for the commercial wound dressing when compared to the standard gauze (11.75 fold increase). This study establishes methodology for detection and quantification of bacterial adherence to a wound dressing. Despite similar bacterial load, transcription data differentiated between test items, with a significantly higher expression of both adhesion-related genes identified from the commercial wound dressing recovery when compared to the standard gauze.

LAB DEMOS

Operation and associated methods of the Industrial Surfaces Biofilm Reactor (ISBR) and the CDC Biofilm Reactor

Presenters: **Jack Koepke, Kylee Schafer, Nate Salsburg, Lindsey Miller**; Standardized Biofilm Methods Laboratory, 301 Barnard Hall

Stop by the SBML to observe two biofilm reactors in action. The ISBR is designed to mimic cooling towers; we'll demonstrate how to assemble and operate an ISBR and the method used to grow a mixed species biofilm that contains Legionella. The CDC biofilm reactor is a versatile system that is used for multiple applications and EPA guidelines. One benefit of the CDC reactor is that the fluid dynamics have been modeled. We'll demo the modeling research while showing the CDC reactor operating according to the ASTM standard test method.

Operation of a sequencing batch reactor (SBR) to cultivate aerobic granular sludge

Presenter: **Catherine Kirkland**, Bioprocessing Laboratory, 302 Barnard Hall

Aerobic granular sludge (AGS) are spherical biofilms which self-assemble in a SBR while treating wastewater. The Feed–Reaction–Draw phases of the cycle and the hydrodynamic shear induced by aeration stimulate granulation and simultaneous conversion of organics, nitrogen, and phosphorus. This demo will describe and show the operational methods to grow granular biofilms in laboratory reactors.

A demonstration of 3D biofilm printing

Presenter: **Isaak Thornton**, Soft Materials Laboratory, 312 Barnard Hall

3D printing has rapidly advanced our ability to build complex structures at a sub-millimeter scale. By utilizing biocompatible printing materials, we can begin building living structures with exquisite control over form and composition.

Virus assays used in the Medical Biofilms Laboratory

Presenter: **Kelly Kirker**, Medical Biofilms Laboratory, 316 Barnard Hall

The recent outbreak of the COVID-19 pandemic, caused by the SARS-CoV-2 virus, underscores the need for testing capabilities relevant to novel virus threats. Determining the survival time of specific viruses on surfaces and the most effective disinfectants are important for helping control the spread of viruses. The MBL has gained experience using two different methods of detecting viral titers in solution.

Chemical characterization of biofilms using Surface Enhanced Raman Scattering (SERS)

Presenter: **Bruce Boles, Heidi Smith**; Bioimaging Facility, 334 Barnard Hall

We'll explore the application and benefits of using SERS, with a live biofilm sample, followed by a real-time demonstration on the steps to perform Raman Spectroscopy using SERS.

MSU Simulated Microgravity Reactor

Presenter: **Haley Ketteler**, Microbial Ecology *Laboratory, 334* Barnard Hall

Discover how the MSU Simulated Microgravity Reactor works, its uses, and some of the fascinating theories behind its design.

Using a modified CDC reactor to grow SRB biofilm on copper surfaces

Presenter: **Yagmur Keskin**, Physiology & Ecology Laboratory, 336 Barnard Hall

Heterogenous SRB biofilm leads to localized corrosion on metal surfaces. Initial interaction of SRB biofilm with polycrystalline copper surfaces can give us an idea about bio-corrosion rates and the mechanism of bio-corrosion.

Montana Biofilm Science & Technology Meeting and Workshop

MONTANA BIOFILM MEETING

July 12-14, 2022

MONTANA STATE UNIVERSITY

Hilton Garden Inn Bozeman

Draft AGENDA

7/5/2022 4:24 PM

Monday July 11

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

Tuesday July 12

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

Meeting: Larkspur Ballroom

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

SESSION 1: Defining a Biofilm

8:10-8:15
Session Introduction
Darla Goeres, Research Professor of Regulatory Science, CBE

8:15-8:40
Defining biofilms as biological films: Keeping the cart behind the horse
Matthew Fields

8:40-9:10
Industrial perspective on biofilm biology: Formation, maintenance, and mitigation
Chris Jones, Director, R&D, Sharklet Technologies

9:10-9:35
Important factors for defining medical biofilms
Garth James, PI, Medical Biofilms Laboratory, CBE; Associate Research Professor, Chemical & Biological Engineering, MSU

9:35-10:05 Break

10:05-10:30
The importance of clearly defining biofilm in laboratory standard test methods
Darla Goeres

10:30-11:00
Why evolution in biofilms is different, and some remarkable consequences
Vaughn Cooper, Professor, Microbiology & Molecular Genetics, Computational & Systems Biology, University of Pittsburgh

11:00-11:45
Panel Discussion
Vaughn Cooper
Matthew Fields
Garth James
Chris Jones
Moderator: Darla Goeres

11:45-12:00
Poster Pitches
CBE Researchers

12:00-1:00 Lunch

SESSION 2: CBE Imaging and Analysis Capabilities

1:00-1:15
CBE Imaging capabilities
Heidi Smith, Manager, Bio-Imaging Facility, CBE; Assistant Research Professor, Microbiol. & Cell Biology, MSU

1:15-1:30
CBE Analytical capabilities
Kristen Brileya, Technical Operations Manager, CBE

1:30-1:55
Statistical considerations in image analysis
Al Parker, Biostatistician, CBE; Associate Research Professor, Mathematical Sciences, MSU

1:55-2:10
Physical and chemical imaging of biological samples at ICAL
Sara Zacher, Lab Manager, Imaging and Chemical Analysis Laboratory, MSU

2:10-2:35
Correlative microscopy links identity, morphology, biochemistry, and activity of uncultured microbes
Anthony Kohtz, PhD Student, Chemistry & Biochemistry, MSU, CBE

CBE Open House: Poster session and lab demonstrations

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

Wednesday July 13

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

Meeting: Larkspur Ballroom

SESSION 3: Medical Biofilms

8:00-8:10
Session Introduction
Garth James

8:10-8:40
Is there a universal biofilm defense?
Phil Stewart, Regents Professor, Chemical & Biological Engineering, MSU, CBE

Table of Contents • Agenda

8:40-9:10
Using organoid models to analyze gastrointestinal bacterial infections
Diane Bimczok, Associate Professor, Microbiology & Cell Biology, MSU, CBE

9:10-9:40
Biofilms and chronic wounds: An overview of recent Medical Biofilms Lab research
Elinor Pulcini, Assistant Research Professor, Chemical & Biological Eng., MSU, CBE

9:40-10:10 Break

10:10-10:40
Accumulation of protoporphyrin IX by biofilm bacteria attenuates bovine neutrophil functional responses
Joey Lockhart, Postdoctoral Researcher, Biological Sciences, University of Calgary
*Young Investigator

10:40-11:10
Application of PNA-FISH based-methods for bacterial detection and localization in biofilms
Laura Cerqueira, Junior Researcher, Chemical Engineering, University of Porto
*Young Investigator

Special Presentation

11:10-11:55
Functions of the *Pseudomonas aeruginosa* matrix
Boo-Shan Tseng, Assistant Professor, School of Life Sciences, University of Nevada Las Vegas

12:00-1:00 Lunch

SESSION 4: Engineered Biofilms

1:00-1:05
Session Introduction
Matthew Fields

1:05-1:35
From bones to sustainable building material
Chelsea Heveran, Assistant Professor, Mechanical & Industrial Eng., MSU, CBE

1:35-2:05
Untapped potential of fungal-based biofilms for wastewater treatment and recovery of added-value products
Erika Espinosa-Ortiz, Assistant Research Professor, Chemical & Biological Eng., CBE, MSU

2:05-2:35
Building synthetic biofilm with 3D hydrogel printing
Isaak Thornton, PhD Student, Mechanical & Industrial Engineering, MSU, CBE
Kathryn Zimlich, PhD Student, Microbiology & Cell Biology, MSU, CBE

2:35-3:05
Ureolytically-induced calcium carbonate composites as bioadhesives
Sobia Anjum, PhD Student, Chemical & Biological Eng., MSU, CBE

Strategic Planning Meeting for CBE Members

3:15-5:00
Hilton Garden Inn

6:00 BBQ Dinner
Big Yellow Barn, Bozeman

Thursday July 14

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

Meeting: Larkspur Ballroom

SESSION 5: Biofilms in Space

8:00-8:45
Session Introduction

Potential for biofouling control by nutrient removal in an ISS water system
Liz Sandvik, Research Engineer, CBE

8:45-9:15
Sensing slime: Microfabricated sensors to detect biofilm for space applications
Haley Ketteler, Masters Student, Electrical & Computer Eng., MSU, CBE
Matthew McGlennen, PhD Student, Mechanical & Industrial Eng., MSU, CBE

9:15-9:45
Off-planet production of high protein foods using fungal biomats
Ross Carlson, Professor, Chemical & Biological Eng., MSU, CBE

9:45-10:15 Break

10:15-10:45
Microbial isolation and characterization of two flex lines from the urine processor assembly onboard the International Space Station
Hang Nguyen, Microbiologist, JES Tech, NASA

10:45-11:15
Preliminary results of the ISS-operated *Pseudomonas aeruginosa* 'Space Biofilms' experiment
Pamela Flores, PhD Student, Molecular Cellular & Developmental Biology, University of Colorado, Boulder
Luis Zea, Assistant Research Professor, Aerospace Engineering Sciences, University of Colorado, Boulder

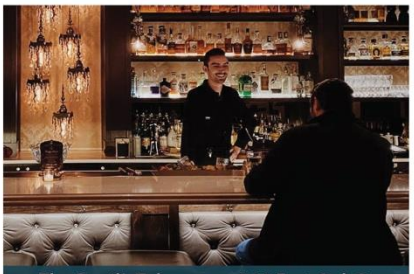
11:15 Meeting Wrap up

CBE gratefully acknowledges *Biofilm Journal* for their sponsorship of this meeting.

Things to Do (and Eat!) around Bozeman!


Nova Cafe • 312 E Main St


Urban Kitchen • 5 West Mendenhall St


The Devil's Toboggan • 113 East Oak St

Breakfast (Downtown)

Nova Cafe is so good. If there's a line, don't worry ... it will be worth the wait.

Western Cafe offers no-frills hearty food prepared in the spirit of a diner and served with authentic western charm. Plus, the waitress just might call you "Hon."

Cateye Cafe loosely enforces a semi-serious list of dos and don'ts. But the food is terrific and there's a $0.50 discount if you wear cateye glasses.

Dinner (Downtown)

Urban Kitchen offers ourmet-ish comfort food that never disappoints and never sits heavy. Plus, craft cocktails.

Sweet Chili Asian Bistro has been named Bozeman's Best Asian Restaurant every year since 2010. Hard to argue.

Copper is located downstairs from Sweet Chili, Copper offers good food, reasonable prices, a terrific atmospher, and a comfortable bar.

Socializing

The Devil's Toboggan is located in the Cannery District (5-minute ride from the Hilton or downtown), and offers fun craft cocktails with a speakeasy feel.

Armory Rooftop Bar offers panoramic views, good food, friendly atmosphere.

The Filling Station is an dive bar offering cold beer, live music, pool, and video poker. Ten-minute ride from the Hilton and downtown.

There are dining treasures throughout this region of the Treasure State. Sir Scott's Oasis in Manhattan, Montana, is a locally owned, no-frills steakhouse that's worth the 25-minute drive west from downtown Bozeman. Montana's Rib and Chop House in Livingston, Montana, is a 30-minute drive east that takes you alongside a historic railroad. Plus, you'll be in beautiful Livingston. If it were me, I'd enjoy a pre-dinner cocktail at the historic Murray Hotel and take a 5-minute walk to the Chop House. Notice that stiff breeze? It's near omnipresent in Livingston. Now imagine that breeze when it's -20F!

Gallatin History Museum

Location: Downtown
Cost: $7.50
Website: gallatinhistorymuseum.org Learn about the rich, wild, and wealthy history of the region.

Montana Grizzly Encounter

Distance from Downtown Bozeman: 15-minute drive
Cost: $8 per adult
Website: grizzlyencounter.org

Montana Grizzly Encounter is a nonprofit rescue and education sanctuarythat features grizzly bears.

Museum of the Rockies at MSU

Location: MSU Campus
Cost: $14.50 for 2-day pass
Website: museumoftherockies.org Dinosaurs, a planetarium, and world-class traveling exhibits.

Discover Montana's Great Outdoors!

Hike 'The M'

Distance: 3 miles RT
Time: 2 hrs (including drive-time)

See that big white M on the southern face of the Bridger Mountains? You can hike to it. Round trip will take about 60 to 75 minutes. The panoramic view is beautiful. Pro tip: Two trails will take you to the M. The most direct (and strenuous) trail is to the right.

Bear Spray Cannisters Available for Loan • Limited Supply

Hike Palisade Falls

Distance: 1 mile RT
Time: 2.5 hrs (including drive-time)

Nestled in the Gallatin Forest near the Hyalite Reservoir is Palisade Falls. As a bonus, the drive through Hyalite Canyon is gorgeous. The half-mile hiking trail is paved, making it one of the few hikes that is truly handicapped accessible. The payoff is a beautiful 80-foot-tall waterfall.

Hike Lava Lake

Distance: 6 miles RT
Time: 4.5 hrs (including drive-time)

You'll enjoy views of the stunning Spanish Peaks and other mountains as you hike to beautiful Lava Lake. It's a heavily traveled trail that folks in these parts love. It's a rocky path, so hiking shoes are preferable to sneakers as you ascend 1,600 feet to the majestic lake.

Guided Fly Fishing (float or wade)

Time Commitment: 6-8 hrs (not including drive-time)
Cost: $650 total for 2 people (includes food & non-alcoholic beverages, waders, boots, flies, rods and reels, and terminal tackle) People travel to Bozeman from all around the world because of the world-class fly-fishing this beautiful region offers. Even first-time anglers will enjoy fishing any one of the rivers that run through it. Not sure you could even cast a line? No sweat. The guides from the award-winning Fins & Feathers will have you fishing in no time.

Bring a Swimsuit and Visit Chico Hot Springs & Day Spa

Time: 1-hour drive time each way.
Cost: $10/adult to swim. Spa prices are *à* la carte.
Website: https://www.chicohotsprings.com
Driving beautiful through beautiful Paradise Valley is a treat unto itself. Then, to take a swim in the hotsprings, is divine. The bar has terrific food, the hotel – established in 1900 – offers fine dining and a terrific wine list.