# Presentation
# & Poster Proceedings

**JULY 13-15, 2021**

****** All times are Mountain Daylight Time ******

**Virtual Meeting**
**Originating from Bozeman, Montana**

*The Center for Biofilm Engineering and its Industrial Associates gratefully acknowledge Elsevier for sponsoring the 2021 Montana Biofilm Virtual Meeting.*


*Biofilm* focuses on hypothesis- or discovery-driven studies on microbial cells that grow in multicellular communities and demonstrate different gene expression, growth rate, behavior and appearance to those that are in planktonic (free-living) state.

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Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA

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**Matthew Fields**, CBE Director, Professor, Microbiology and Cell Biology, MSU
**Paul Sturman**, CBE Industrial Coordinator

## SESSION 3: Industrial Biofilms



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## Ask the Experts

# SESSION 1: Biofilm Matrix

### The biofilm matrix as a cooperative and competitive trait

*Presenter*: **Hans Steenackers**, Assistant Professor
*Affiliation*: Microbial Communities and Antimicrobials Lab (MICA Lab), University of Leuven, Belgium.

Bacteria commonly form dense biofilms encased in a matrix of extracellular polymeric substances. In this presentation I will highlight the social nature of the biofilm matrix by showing that it can both mediate cooperation between bacteria belonging to the same strain as well as competition between different strains. I will provide evidence that both social characteristics are interesting targets for novel antimicrobial therapy. Based on *Salmonella typhimurium* as a model organism, we showed that the biofilm matrix can function as a cooperative, public trait that is shared between individual cells and that enhances both cell attachment and antimicrobial tolerance. Consistently, interference with biofilm matrix production by 2-aminoimidazole based compounds reduced bacterial attachment and invoked sensitivity to antibiotics and disinfectants. Most interestingly, social evolution theory predicts that inhibiting such public traits selects against resistance. The reason is that a resistant strain will produce the public trait and pay a cost to do so, while susceptible strains will also be able to use the public trait but without paying the cost. To test the theory in practice, we compared the matrix inhibitor to conventional antimicrobials in an evolutionary experiment. While resistance against conventional antimicrobials rapidly evolved, we saw no evolution of resistance to matrix inhibition. We further showed that a resistant strain is outcompeted by a susceptible strain under matrix inhibitor treatment, providing critical support for the evolutionary robustness of public trait inhibitors. We validated the activity of the 2-aminoimidazoles against biofilms of a broad range of other bacterial and fungal pathogens and are currently developing these compounds towards several medical, industrial, and agricultural applications.

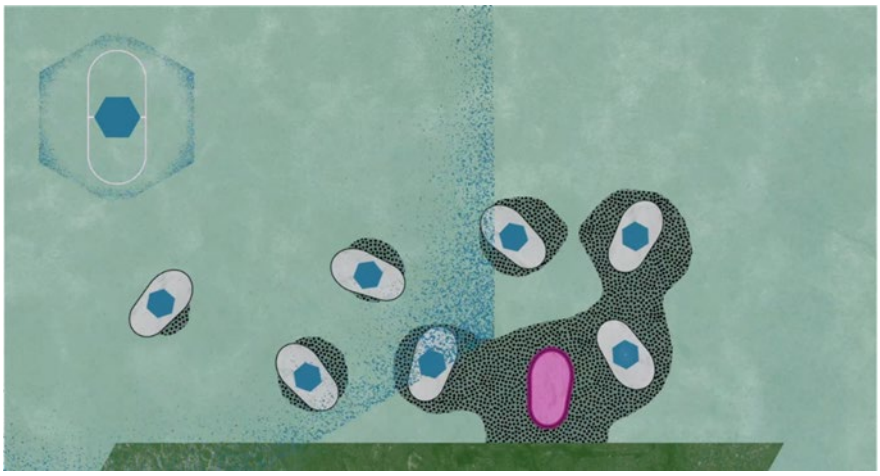


*FIGURE 1. The matrix inhibitor (upper left) interferes with matrix production (grey dots) in an evolutionarily robust manner. Animation video: https://www.micalab.be/news/inhibiting-bacterial-cooperation-is-an-evolutionarily-robust-anti-biofilm-strategy*

Biofilm matrix production can also be involved in the competition between different strains and species. Indeed, we showed that *Salmonella* residing in a mixed-species community can increase the production of its biofilm matrix in order to protect against antimicrobials produced by competitors. We proved this upregulation of biofilm matrix to be part of a broad defensive response regulated by stress response systems that detect the damage caused by competition (=competition sensing). Most worrisome, this defensive response not only provided protection against competitors, but also enhanced the tolerance of *Salmonella* against treatment with clinical antibiotics. We therefore explored whether interfering with competition and the competitive response could weaken *Salmonella* and increase the susceptibility to antimicrobial treatment. As a proof of concept, we showed that reducing the biofilm density with a compound that interferes with

adhesion lowered the competition in the mixed-species community. Consequently, biofilm formation was no longer upregulated and the enhanced tolerance of *Salmonella* in presence of competitors was abolished (unpublished data). Overall, our work highlights the potential of antimicrobial strategies that interfere with competitive traits.

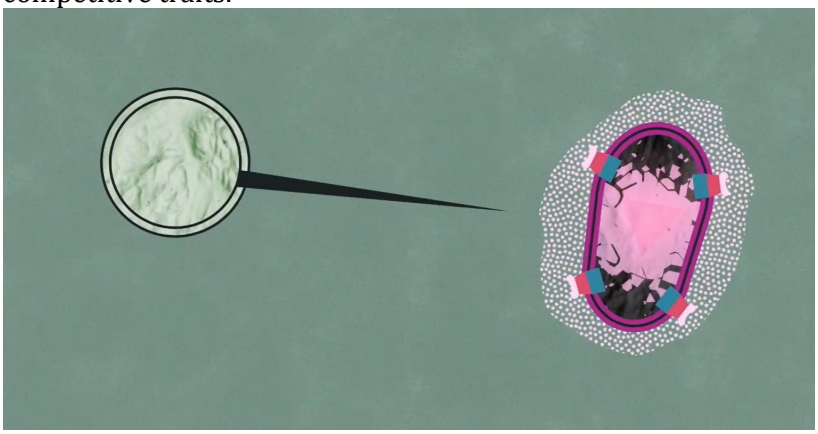


*FIGURE 2. Salmonella enhances its matrix production (white dots) and antimicrobial efflux as a response to harm caused by competitors (toxins injected by T6SS (black needle)). This provides protection against competitors, but also impedes clinical antibiotic therapy. Animation video: https://www.micalab.be/news/bacteria-use-stress-responses-to-detect-and-respond-to-competitors*

### Biofilm mechanics as a surface survival mechanism for them but a drag for us

*Presenter*: **Paul Stoodley**, Professor[1,2]
*Affiliation*: [1]Department of Microbial Infection and Immunity, Department of Orthopaedics, Ohio State University, OH USA.
[2]Department of Mechanical Engineering, University of Southampton, UK.



Microbial biofilms are assemblages of cells usually attached to a surface and held together by a self-produced extracellular polymeric slime (EPS) matrix. Biofilms are ubiquitous in the natural environment and are highly problematic in industry and medicine where they cause corrosion, fouling, contamination and chronic medical and dental infections. A hallmark feature of biofilms is their ability to allow bacterial survival in highly diverse environments despite physical, chemical and biological challenges and stresses. In man-made and natural environments biofilms may be exposed to shear stresses which range from close to zero to many magnitudes with fluctuation frequencies covering a wide range of time scales. Rheological and indentation tests show that the same biofilms can behave as elastic solids, viscoelastic solids, viscoelastic liquids or viscous liquids depending on the nature of the applied mechanical stress. Recent observations of high velocity impacts with air jets and water spays show that they rapidly form interfacial instabilities allowing them to flow over surfaces with velocities of meters per second. This survival mechanism can also explain high pressure drops and drag associated with biofouling in pipelines and on ship hulls. Traditionally rheometers have been used to measure the viscoelastic properties of soft solids and viscous liquids. Since many control strategies require the break-up of biofilm, mechanical testing allows a method of quantifying the effect of such treatments with interpretations based on molecular interactions in the EPS. Rheometer methods have also been recently developed to assess the influence of biofilm on drag and to characterize detachment events as a function of shear. A better understanding of how bacterial biofilms respond to fluid flow provides new opportunities to develop more effective control strategies.

### Secreted, large-scale, extracellular membrane systems in microbial biofilms (SLEMS)

*Presenter*: **Matthew Fields**, Director[1], Professor[2]
*Co-authors*: L.C. Franco[#,1,2], A.S. Wu[#,3], M. Joo[3], N.J. Mancuso[3], A. Gorur[3], A. Leung[3], D.M. Jorgens[3], J. Remis[3], J. Correa[3], J. Ivanisevic[4], G.E. Siuzdak[4,$], M.W. Fields[*1,2], and M, Auer[*3]
*Affiliation*: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[3]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
[4]Scripps Center for Metabolomics and Mass Spectrometry, The Scripps Research Institute, La Jolla, CA, USA.

The biofilm matrix is increasingly being realized to contain a variety of intra- and intermatrix interactions that contribute to and control biofilm behavior; however, extracellular membranes have not been previously reported despite the occurrence of membrane vesicles in many types of microorganisms. *Desulfovibrio vulgaris* biofilms exhibited extracellular, elongated structures that in cross section appeared membranous, or as complex geometrical enclosed shapes devoid of cells. Non-osmicated, UAc only stained biofilm sample revealed an unstained thin core structure that upon osmication became black, indicating the thin structure was lipid-based. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers, and metal precipitation occurred predominantly on the extracellular structures. 3D renderings after Serial Block Face Scanning Electron Microscopy (SBF/SEM) demonstrated long lamellar structures associated with metal deposits that extended up to 100 μm, essentially the entire length of imaged biofilm. Due to involvement with metal precipitates, biofilms were grown under electron-acceptor limitation (EAL), and the distribution of extensive membrane structures increased. Quantification of total fatty acids as FAMEs indicated that the EAL-biofilm had 3-fold increased FAME content, and untargeted metabolomics experiments indicate the increased occurrence of twelve long-chain fatty acids that included methyl-hexanoic and methyl-heptanoic acid. EAL-biofilms exposed to Cr(VI) showed increased viability compared to biofilms grown under a balanced condition, and these results suggested a protective role for the membrane structures during metal exposure. To our knowledge, this is the first report of secreted, large-scale, extracellular membrane systems in microbial biofilms (SLEMS), and the described structures have implications for microbial biofilms and the evolution of biological systems.



### Matrix in the context of biofilm 3D printing

*Presenter*: **Reha Abbasi**, Postdoctoral Researcher
*Affiliation*: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Microbial biofilms are multicellular communities of microbial cells that colonize and adhere to surfaces. Biofilms are abundant in nature, critical for global food chains and nutrient cycling, are increasingly used for biotechnological applications, and are implicated in a wide variety of industrial and medical problems such as biofouling, corrosion, and infection. There is a long-standing need to understand structure-function relationships in microbial biofilms, which are important for fundamental and applied purposes. 3D bioprinting is a rapid fabrication technique that provides exquisite control over the structure and composition of multicellular systems. 3D bioprinted microorganisms have been used to produce bioproducts such as cellulose, investigate quorum sensing mediated communication between bacterial aggregates, and study antimicrobial resistance; however, progress in this field is limited by the print materials used to mimic the extracellular matrix. Here, we will present a review of the different natural and synthetic polymers used to simulate the extracellular matrix in biofilm 3D bioprinting applications. We will also discuss alternative possibilities for mimicking the extracellular matrix.

## PANEL DISCUSSION

**Uncovering the hidden potential and known challenges of the biofilm matrix**

*Panelists*: Matthew Fields[1,2], Director, Professor; Sarah Finn[3], Microbiology Manager; Jan Hodges[4], Director of Quality Assurance and Regulatory Affairs; Hans Steenackers[5] Assistant Professor; Paul Stoodley[6,7], Professor
*Moderators:* Darla Goeres[1,] Research Professor; Jim Wilking[1,8], Associate Professor
*Affiliation*: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[3]Kersia, Wexford, Ireland.
[4]Quest Medical, Allen, TX, USA.
[5]Microbial Communities and Antimicrobials Lab (MICA Lab), University of Leuven, Belgium.
[6]Department of Microbial Infection and Immunity, Department of Orthopaedics, Ohio State University, OH USA.
[7]Department of Mechanical Engineering, University of Southampton, UK.
[8]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Most biofilm definitions include a qualifying statement that the biofilm bacteria are encased in an extracellular material of their own making. The matrix makes biofilm uniquely, well, biofilm. This panel will explore what is known about the biofilm matrix, and what gaps exist in our understanding and how this knowledge may be used to implement better control strategies in an industrial and medical context.

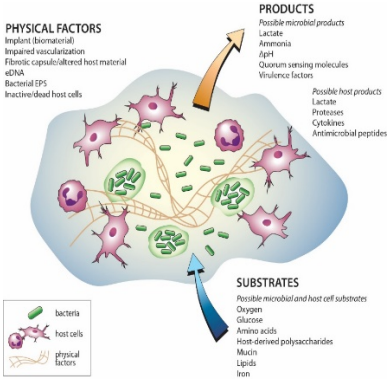
## SESSION 2: Medical Biofilms

**The infectious microenvironment and biofilms**

*Presenter*: **Thomas Bjarnsholt**[1,2], Professor
*Co-authors*: Philip S. Stewart[3], Regents Professor
*Affiliation*: [1]Department of Immunology and Microbiology, Costerton Biofilm Centre; University of Copenhagen, Denmark.
[2]Department of Clinical Microbiology, University of Copenhagen, Denmark.
[3]Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.



Biofilms are increasingly associated with many chronic infections across the health field. The main problem with chronic infections is that biofilms are difficult to treat as bacteria in biofilms are tolerant to antimicrobials and the immune system. In this presentation, I will highlight the challenge that biofilms pose in implant-related infections and wounds, both in relation to treatment but also to the immune system. However, what is a biofilm, do we all know what we talk about in vitro vs in vivo? Also, what is the infectious microenvironment of infections, and why do you need to know about this?



### The wound microbiome

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

The microbiology of wounds has been studied for over 40 years. First with culture-based methods and then with DNA analysis methods. Both methods are evolving but both also have inherent biases. Culture is biased towards bacteria that can be grown under laboratory conditions, although methods for culturing fastidious bacteria continue to improve. DNA analysis can be biased during extraction, PCR amplification, sequencing, and data analysis. Most studies have focused on "chronic" wounds. These wounds are better termed "hard to heal wounds" (HTHW) as new approaches and technologies improve healing. Cutaneous wounds are exposed to a wide range of microorganisms from the skin and environment. However, most wounds heal within weeks. HTHW can last for months to years and usually have underlying comorbidities such as diabetes, venous insufficiency, and immune deficiencies. Overall, new molecular approaches have confirmed culture-based results that *Staphylococcus* and *Pseudomonas* tend to predominate in HTHW, although some wounds harbor diverse microbial communities including anaerobic genera, such as *Anaerococcus* and *Peptoniphilus*. Molecular analyses also revealed that skin-associated genera, such as *Corynebacterium*, that were previously overlooked as contaminants in typical clinical culture, often had high relative abundances in wounds. Specific pathogens have seldom been correlated with HTHW, although a whole metagenome-based study suggested that certain strains of *S. aureus* were associated with poor healing. Recent studies have also suggested that poor wound healing is associated with particular groups of bacteria, population shifts, and community stability. It is unclear whether some bacteria or groups of bacteria could be beneficial. Most studies have been based on the relative abundance of particular genera/species and not the activity and interactions between microorganisms and the wound, knowledge that will be important for further improvements in wound healing.



### Factors that influence microbial ingress into luer activated valves for intravascular administration sets

*Presenter*: **Elinor Pulcini**, Assistant Research Professor
*Affiliation*: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The use of luer activated valves, also known as needle-less or needle-free connectors, was mandated for use in the United States in the early 1990s in response to the need to reduce needle stick injuries and the subsequent transfer of bloodborne pathogens in health care workers (HCW). These connectors provide a means of access in intravascular administration sets in order to give medications, provide nutrition therapy, provide chemotherapy, and for use in hemodialysis. The mandated use of these connectors resulted in a reduction of needle-stick injuries in HCW. Unfortunately, however, there has been a rise in infection rates since the 1990s. Central line-associated bloodstream infections (CLABSI) are associated with as many as 28,000 intensive care unit (ICU) patient deaths per year and an estimated cost burden of $46,000 per case. While not responsible for all CLABSI cases, bacterial contamination of connectors, resulting in patient sepsis, are implicated in a large number of these cases. Improper/inadequate disinfection of connectors, connector design and long-term use of connectors can contribute to microbial ingress.

### Biofilms as hot spots for gene transfer

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

Are biofilms really hot spots of gene transfer promoting the spread of antimicrobial-resistant microorganisms? This presentation reviews the literature on gene transfer and mutation in biofilms including how biofilms can influence key processes such as horizontal gene transfer by conjugation or transformation, emergence of antibiotic resistance via mutation, and vertical transmission of new strains. Classic microscopy investigations using bacteria tagged with fluorescent proteins visually confirm that horizontal gene transfer

(HGT) occurs in biofilms. The extent of this transfer depends dramatically on how the biofilm is formed. If a donor and recipient strain are pre-mixed, the frequency of HGT is high. If a biofilm is formed first by a recipient strain and then inoculated with a donor, HGT is typically limited to the zone in which the two strains physically touch each other and is less extensive. In other words, mixing and contact of the respective organisms are critical drivers of HGT. Experiments with mixed species biofilm, such as a model dental plaque community, confirm that genetic determinants of antibiotic resistance can move between different species in a biofilm. Experiments with isolated DNA, which can also transform biofilm cells, point to the possibility that eDNA in the biofilm matrix might function as a genetic reservoir that could be tapped to create cells with new capabilities or resistance profiles. Antibiotic resistance mutants arise spontaneously in biofilms just as they do in other cultures, a reality that is made clinically relevant by the accumulation of mutants in the lungs of individuals with cystic fibrosis. Vertical transmission is likely impacted by heterogeneous growth rates within biofilms. Biofilms are often characterized by zones of slow growth or dormancy which are expected to reduce the speed at which resistant subpopulations expand. Vertical transmission of antibiotic-resistant can also be expected to be throttled within a biofilm by the common antimicrobial tolerance manifested in the biofilm mode of growth. There is not a simple answer to the question of whether biofilms can be considered hot spots, but it is clear that gene transfer, mutation, and vertical transmission all readily occur in biofilms.

## SESSION 3: Industrial Biofilms

### Beer draught line challenge: Biofilm vs. chemistry

*Presenter*: **Darla Goeres**, Research Professor of Regulatory Science
Co-authors: Kelli Buckingham-Meyer, Lindsey Miller
*Affiliation*: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

In the US, there has been a resurgence of craft brewing. The Brewers Association website (www.brewersassociation.org/statistics/craft-brewer-defined/) defines a craft brewer as small (less than 6 million barrels of beer per year) and independent (less than 25% of craft brewery is owned or controlled by a beverage alcohol industry member that is not itself a craft brewer). Beer quality becomes compromised when draught lines become contaminated with undesired bacteria or yeast that produce off-flavors, aromas, and haze. Although beer is generally not a welcoming environment for unwanted microbes because of the alcohol content, craft beer contains high concentrations of carbon desirable to many bacteria and yeast, especially the honey or fruit beers. In a bar with a long draw system, the kegs are stored in a cooler separate from the beer dispense tap. The keg is connected to a tap via multiple meters of tubing, which provides a large surface area to which the bacteria can attach. This tubing can stay in place for multiple years. If there is an unfortunate contamination event that enables bacteria to enter the line, the bacteria will find locations within the system to hide and form biofilm, FIGURE 1. Once the biofilm forms, the challenge is that the bacteria and yeast within it become more tolerant to disinfection. The extracellular matrix, which can consist of beer stones, proteins, or lipids, may contain some of the compounds contributing to the off flavors. Additionally, the physical properties of the biofilm matrix often make it difficult to remove from the system once established. A study was done to understand the impact repeated exposure to cleaning chemistries has on biofilm growth, kill/removal, and subsequent regrowth in beer draught lines. Vinyl hose was conditioned to simulate 1, 2, and 5 years of use. A mixed species biofilm was then grown in the conditioned hoses, and the viable cell density assessed. The biofilm was then treated with caustic and the reduction of cells quantified. Finally, the hose was refilled with fresh media and the remaining biofilm allowed to regrow. The data collected demonstrates a clear trend between simulated age of the hose and biofilm accumulation on the surface. Interestingly, treating the biofilm with the recommend caustic solution effectively killed and/or removed the biofilm from the surface, regardless of the starting cell density, demonstrating how effective the caustic is at controlling biofilm accumulation in a beer draught line. The biofilm in the hose that had the longest

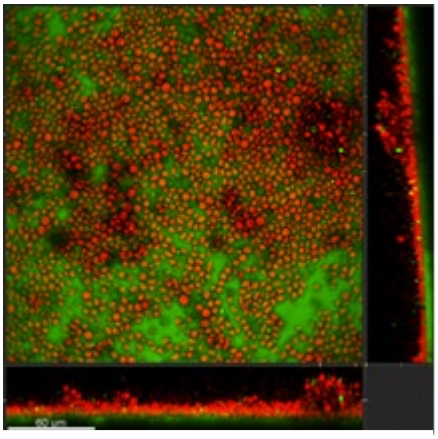


*FIGURE 1. Example of biofilm from a contaminated beer line. L.A. Miller*

exposure to the cleaning chemistry was able to recover more quickly, though, suggesting that the treatment left viable cells that were protected in the biofilm. This means that eventually, even the strong chemistry used in this study would cease to be as effective. We suggest tailoring the cleaning to key system parameters, such as age of the system, type of beer served, and volume of product sold. In the near term, though, the guidance provided in the "Draught Beer Quality Manual" will keep the lines clean, but distributers need to understand that the system components may need replacing more often.

### Impacts of an antibacterial coating on the growth of ISS isolates for single and mixed domain biofilms

*Presenter*: **Madelyn Mettler**[1,2], PhD student
*Co-authors*: Ceth Parker[3], Postdoctoral researcher
Brent M. Peyton[1,2], Professor
Kasthuri Venkateswaran[3], Senior Research Scientist
*Affiliation*: [1]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA.

Many studies of biofilm growth and prevention in medical and industrial systems on Earth have been completed. However, biofilm growth extends to human habitable systems such as the International Space Station (ISS) and crew vehicles used in space travel. Studies evaluating biofilms in these environments have been more limited. Biofilms found on the ISS pose increased threats to astronaut health and equipment integrity compared to those on Earth due to the difficulty of medical care, equipment replacement, and regular cleaning in space. A better understanding of biofilm treatment and/or prevention is necessary for prolonged space travel to places such as Mars or for a flight base on the Moon. Additionally, many laboratory biofilm studies have been completed using only a single microbial species. However, in industrial, medical, and environmental systems it is more likely that biofilms are far more diverse. Natural biofilms consist of multiple microbial species, often belonging to different domains of life. There have been limited studies evaluating multispecies and multidomain biofilms even though they are more relevant models to naturally occurring biofilms. This project aims to evaluate the effect of material type and a novel antimicrobial coating on biofilm growth of microbial isolates from the ISS. The materials evaluated are all found on the ISS and include uncoated controls and antimicrobial-coated stainless steel, Teflon, Inconel (nickel-chromium alloy), and titanium. Biofilm growth studies were completed using CDC biofilm reactors in normal gravity. Experiments included reactors run with a single microbial species and reactors run with two species of different domains. Data using the CDC reactor to evaluate single species and mixed *Pseudomonas aeruginosa* (bacteria) and *Rhodotorula mucilaginosa* (yeast) biofilms will be presented.



### Fungal biofilms: The good, the bad, and the unknown

*Presenter*: **Erika J. Espinosa-Ortiz**, Assistant Research Professor
*Affiliation*: Department of Chemical and Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Whereas most biofilm research has focused on bacterial biofilms, little is known about biofilms formed by fungi. Diverse fungi are capable of colonizing surfaces and develop biofilms, either as single species or in association with other fungi or other microbes including algae and bacteria. Fungi have been detected within biofilms associated with rocks, acid mine drainage, pipe walls, buildings and historic monuments, medical implants, and water systems. This presentation will focus on describing the relevance of fungal biofilms, unveiling some of the aspects of fungal biofilm formation, and presenting the 'good' (desirable/beneficial) and the "bad" (undesirable/harmful) aspects of fungal biofilms in the medical, environmental, and industrial fields.

*(This abstract continues on the next page)*

This presentation will also provide an overview of bioreactor systems and characterization tools developed and used at the CBE for studying fungal biofilms. Finally, some of the current projects on the potential use of fungal biofilms in different engineering applications (*e.g.*, bioremediation, biomanufacturing) will be discussed.

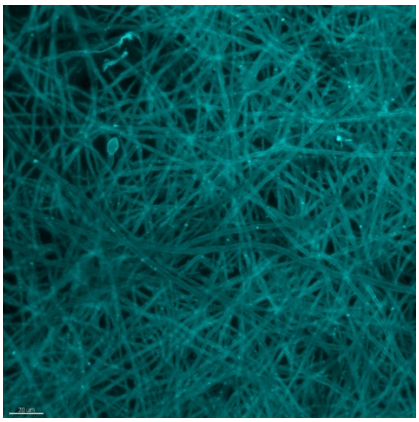


*FIGURE 1. Fungal biofilm.*

**Sensing slime: Microfabricated sensors to detect biofilm**

*Presenter*: **Matthew McGlennen**[1,2], PhD Candidate
*Co-authors*: Markus Dieser[1], Michael Neubauer[1,2] Christine Foreman[2,3] and Stephan Warnat[1,2]
*ffiliation*: [1]Department of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.



In natural and engineered environments, the vast majority of microorganisms exist as organized communities known as biofilms. Biofilms are complex communities composed of microorganisms and organic matter that attach to surfaces. Biofilm contamination is a significant factor in the degradation of industrial fluids, causing biofouling and corrosion of equipment, the imperilment of product quality, and posing occupational safety risks. Current methods to determine biofilm contamination in industrial environments are expensive, have long lead times (hours to days), and require qualified personnel to perform the analysis. Therefore, a proper mechanism to detect biofilm promptly would be beneficial to control industrial processes. However, industrial sites often do not have the resources for wet chemical biofilm analysis. Microfabricated sensors that leverage electrochemical techniques such as electrochemical impedance spectroscopy (EIS) may be an effective alternative for analyzing biofilm growth in these environments. 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. We have developed microfabricated EIS sensors that are small (~ 9 x 26 mm), low-cost, and amendable to use in a wide variety of environments, providing exciting opportunities real-time monitoring of biofilm in industrial settings. This presentation will discuss the design, development, and testing of microfabricated sensors to monitor biofilm formation being developed at the Center for Biofilm Engineering. The talk will highlight several examples of where the sensors are being implemented, namely *(i)* in the manufacturing industry where metalworking fluids (MWFs) are applied, *(ii)* in water-recirculation systems aboard the international space station (ISS), and (*iii*) in icy environments containing microorganisms. Finally, the talk will discuss the future direction of microfabricated sensors and how further improvements can be made.

# CBE *Virtual* Open House

## POSTERS

### CBE Poster #782

*Date*: July 2021
*Title*: **Confocal microscopy techniques for identification of pharmaceutical impacts on aerobic granular sludge**
*Authors*: **Kylie Bodle**[1,2], Heidi Smith[2], Catherine Kirkland[1,2]
*Affiliation*: [1]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
*Sponsored by*: Montana IDeA Network of Biomedical Research Excellence (INBRE), National Institutes of Health

Aerobic granular sludge 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. However, many knowledge gaps remain regarding biofilm structure within granules; in particular, structural differences between lab-grown and environmentally grown granules are largely unexplored. To that end, selective EPS staining, as well as fluorescent *in situ* hybridization (FISH), were performed on three different granule samples: those grown in lab-scale sequencing batch reactors and those from full-scale treatment facilities in Utrecht, NL and Rockford, IL. Methods developed during this research will be used to evaluate the impacts of common pharmaceutical compounds on granular sludge structures, as granular sludge shows great promise as a pharmaceutical treatment biotechnology.

### CBE Poster #783



*Date*: July 2021
*Title*: **Examining carbon flux through alkali-tolerant alga under light stress**
*Authors*: **Charles J. Holcomb**[1,2], Adrienne D. Arnold[1,3], Ross P. Carlson [1,2,4], Robin Gerlach[1,2,4]
*Affiliation*: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA
[3]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[4]Thermal Biology Institue, Montana State University, Bozeman, MT, USA.
*Sponsored by*: DOE Award # DE-EE0008247 / 000

Algae have been used for the production of biofuels for decades. A strain of alga, *Chlorella sorokiniana* str. SLA-04, was recently isolated from Soap Lake, WA that can grow over a range of pH 7-11. This strain is very efficient at using the dissolved inorganic carbon that is more bioavailable in the lake due to the alkaline conditions. The alkali-tolerance of the strain can reduce the costs of biofuel production by reducing the need for $CO_2$ sparging and the need for an algal pond to be in close proximity of a $CO_2$ source (ex, coal plants). This study uses a combination of *in silico* predictions and experiments to analyze the changes in biomass composition in response to light availability. Low light predictions suggest that the majority of carbon will be stored in the form of starch, while high light favors the production of lipids. These predictions have been used to design ongoing experiments to confirm the behavior of SLA-04 under light stress conditions. It is additionally hypothesized that the extra carbon available to the cultures under alkaliphilic conditions will increase SLA-04's tolerance to high light stress, a potential benefit for industrial production of biofuels.

## CBE Poster #784

*Date*: 07/2021
*Title*: ***In silico* analysis of value-added chemical production from methane**
*Authors*: **Adrienne D. Arnold**[1,2], Ross P Carlson[1,3]
*Affiliation*: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA
*Sponsored by*: Molecular Biosciences Program, Montana State University; NSF Award #1736255 (BuG ReMeDEE); DOE Award # DE-EE0008247 / 000

Methanotrophs are organisms that can use methane as their sole carbon and energy source. They play a crucial role in carbon cycling in the environment and are also of economic interest, as they can consume methane, a cheap carbon source that is often flared from industrial sites. In this study, metabolic modeling was used to investigate the production of value-added byproducts from methane by a type II methanotroph. These byproducts range from chemical feedstocks like methanol and formaldehyde to reduced carbon compounds like acetate and ethanol. Even the biomass itself can be utilized as a protein source. In our predictions, we manipulate cultivation conditions like nitrogen source and $O_2$ availability to increase production of these byproducts. We also examine the tradeoffs between consumption of methane and production of nitrous oxide, a potent greenhouse gas that methanotrophs are known to release under some cultivation conditions. These *in silico* predictions provide information that can be used for industrial design and to understand methanotroph interactions in the environment.

## CBE Poster #785

*Date:* July 2021
*Title:* **Using nuclear magnetic resonance to characterize EPS of aerobic granular sludge with the aid of contrast agents**
*Authors:* **Matthew Willett**[1,2], Kylie Bodle[1,2], Joseph D. Seymour[2,3], Catherine M. Kirkland[1,2]
*Affiliation:* [1]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.



Aerobic granular sludge (AGS) consists of compact, spherical biofilm aggregates used in wastewater treatment to simultaneously remove carbon, nitrogen, and phosphate. Previous studies have demonstrated that ultra-high field nuclear magnetic resonance (NMR) imaging can provide important insights into the internal structures of these complex, heterogenous granules. However, these studies have not elucidated how proteins or polysaccharides, the main constituents of the extracellular polymeric substances (EPS), influence the NMR relaxation behavior observed. This study explores AGS structure using alginate beads with added model proteins and polysaccharides to characterize their relaxation behavior, and ultimately uncover details of the composition and structure of the EPS in an actual granule. To help achieve this, various contrast agents—including superparamagnetic iron oxide nanoparticles (SPIONs) and magnetotactic bacteria—are being employed to provide greater contrast enhancement between the different regions inside the model granules.

## CBE Poster #786

*Date*: July 2021
*Title*: **Impacts of pharmaceuticals on aerobic granules vs. planktonic cultures**
*Authors*: **Madeline Pernat**, Kylie Bodle, Catherine M. Kirkland
*Affiliation*: Department of Civil Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
*Sponsored by*: Montana IDeA Network of Biomedical Research Excellence (INBRE)

Aerobic granular sludge (AGS) is a novel wastewater treatment technology with several advantages over the conventional activated sludge process, including increased biomass retention, the ability to withstand toxic shock loads, and accelerated treatment rates. Aerobic granules are spherical biofilm aggregates consisting of diverse bacterial populations bound together by extracellular polymeric substances (EPS) in the absence of a

carrier material. The EPS matrix is a complex hydrogel structure whose molecular makeup is largely unknown but is the reason why biofilms are more resistant and resilient than planktonic cells. Aside from providing structural stability, EPS acts as a buffer between the microbial community and the surrounding bulk fluid, making granules less vulnerable to negative changes in their environment, such as the presence of certain pollutants. Due to their complex EPS structure and microbial diversity, aerobic granules may offer a promising solution to the growing concern over the presence of pharmaceuticals and personal care products (PPCPs) in wastewater. Aerobic granules are expected to better treat these compounds than conventional activated sludge systems since bacterial flocs used in conventional systems do not contain EPS and are therefore more susceptible to the negative impacts of PPCPs. The overarching purpose of this research is to explore the extent to which AGS can remove PPCPs from wastewater and how PPCPs influence the removal of the typical wastewater constituents: carbon, nitrogen, and phosphorus. In this study, enriched planktonic cultures of ammonia- and nitrite-oxidizing bacteria grown from crushed aerobic granules will be exposed to PPCP-laden media. The microbial density and nitrogen removal efficiency of these cultures will be measured in both long- and short-term exposure experiments. Comparisons will later be drawn between these planktonic cultures and aerobic granules cultivated in a laboratory reactor dosed with the same PPCPs. These results will demonstrate the extent to which the EPS matrix aids in the protection from and treatment of PPCPs.

## CBE Poster #787

*Date*: 6/21

*Title*: **Removal of PFAS from synthetic wastewater using aerobic granular sludge in sequencing batch reactor**

*Authors*: **Tasnim Sultana Ritu**, Catherine M Kirkland

*Affiliation*: Department of Civil Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

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



Poly-perfluoroalkyl substances (PFAS) are a class of man-made chemicals used as surfactants, fire retardants and coating materials that include perfluorooctanoic acid (PFOA), perfluoro octane sulfonate acid (PFOS) among other chemicals. 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) where they are poorly removed by conventional wastewater treatment methods making effluent from WWTF a major source of PFAS in the environment. Aerobic Granular Sludge (AGS) is novel microbial community that can be used for treatment of wastewater. The extracellular polymeric substance (EPS) structure in AGS may facilitate removal of PFOA/PFOS via sorption. AGS will be used to remove PFAS, since AGS is both cost effective and energy efficient compared to conventional activated sludge. It also has excellent settleability, high biomass retention and tolerance to toxicity. Removal mechanisms such as sorption and biodegradation may contribute to PFAS removal efficiency. Liquid chromatography with mass spectrometry (LC-MS/MS) will be used to assess the extent to which PFOA and PFOS partition to the sludge phase using a mass balance approach. Microscopy will be used to monitor morphological and structural changes. Other nutrients in wastewater such as carbon, nitrogen, phosphorus will be measured to see how PFAS influence the conventional treatments of wastewater. It is expected that EPS in granules will improve the sorption of PFAS relative to activated sludge and reduce the PFAS load from wastewater.

## CBE Poster #788

*Date*: 07/21

*Title*: **Investigation of complete groundwater denitrification utilizing an environmentally relevant bacterial co-culture**

*Authors*: **Uve Strautmanis**[1,2], Heidi Smith[1,2], Sara Altenburg[2], Matthew Fields[1,2]

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

Primary motivations for studying the subsurface are to expand the knowledge of Earth's microbial diversity and the subsurface microorganisms under low nutrient conditions that significantly impact C, S, N, P and mineral cycles. One such biogeochemical cycle of importance to groundwater systems is microbial denitrification, the reduction of nitrate ($NO_3^-$) from organic and inorganic sources back to atmospheric nitrogen ($N_2$). However, little is known about the extent of microbially-mediated denitrification in groundwater environments. The key to harnessing microbial potential is to find the optimal set of parameters that promotes enhanced rates of denitrification. In anaerobic environments, oxygen is not readily available for respiration, therefore microbes must use alternative electron acceptors such as $NO_3^-$ to respire, reducing $NO_3^-$ to $N_2$. To investigate the environmental parameters that influence denitrification this work uses a co-culture of *Rhodanobacter sp. R12* and *Acidovorax sp. 3H11* that when grown together, can complete full biotic denitrification. Batch experiments mimicking field conditions were run using the *Rhodanobacter sp. R12* and *Acidovorax sp. 3H11* co-culture under varying pH values, dissolved oxygen concentrations, carbon sources, and amino acids. Samples were analyzed for growth performance, nitrate reduction, and single cell analysis including the integration of stable isotope probing with Raman Microspectroscopy and the identification of individual microbial cells and fluorescent in-situ hybridization (FISH). This will quantitatively track the abundance of individual organisms across treatments. Higher rates of denitrification are expected to occur when the organisms are grown together and in anaerobic conditions at a pH of 7.

## CBE Poster #789



*Date*: July 2021

*Title*: **Investigation of Raman spectroscopic signatures with multivariate statistics: an approach for cataloguing microbial biosignatures**

*Authors*: Mitch W. Messmer[1,2], Markus Dieser[1,2], Heidi J. Smith[1,3], Albert E. Parker[1,4], Christine M. Foreman[1,2,]

*Affiliation*: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[4]Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA.

*Sponsored by*: National Aeronautics and Space Administration and the National Science Foundation

Spectroscopic analyses are an integral tool for the exploration of extraterrestrial systems. Spectrometry instruments have been used in NASA's previous missions to Mars to measure geological conditions and are equipped on the *Perseverance* rover to search directly for signs of microbial life. Vibrational Raman spectroscopy can be used for *in-situ* detection of organic compounds that are foundational to biology. As these signals can be difficult to detect amongst heterogeneous environmental samples, techniques are needed to discern trends in spectral data for distinguishing between abiotic factors and biological material with potentially unknown compositions. In this project, Raman spectra were measured for bacterial species isolated from the Greenland and Antarctic Ice Sheets. Unsupervised K-means clustering, followed by targeted, supervised techniques were investigated as a potential method for effectively analyzing spectral data. K-means clustering was successful at differentiating isolates based on the spectral features associated with carotenoids. The cluster means also identified spectral features corresponding to other cellular compounds, which served as target regions for subsequent supervised analysis. Supervised PERMANOVA showed statistically significant difference when comparing pigmented and non-pigmented isolates with respect to the target regions associated with common cellular compounds. The use of unsupervised K-means analysis in conjunction with targeted, supervised analyses provides a generalized analytical approach that can be used to

effectively identify trends in spectral data without assuming prior knowledge of the sample, making it beneficial for application in searching for signs of life on extraterrestrial environments.

## CBE Poster #790

*Date*: June 2021
*Title*: **Understanding microbial Interactions in fungal-bacterial biofilms: implications for selenium remediation**
*Authors*: **Sandra Kohl**, Gretchen Gutenburger, Erika Espinosa-Ortiz
*Affiliation*: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
*Sponsored by*: Montana CREWs

Traditionally, the use of single cultures or communities of either fungi and bacteria are used in bioremediation applications, such as remediation of coal mine tailings, chemical waste, and wastewater effluents. However, current findings suggest that mixed-domain systems, e.g. fungal-bacterial cultures, can be more robust compared to their monocultures and can potentially be more efficient at removing pollutants from different environmental matrices. Despite their promising use, there is still a lack of information regarding the potential use of mixed-domain systems for bioremediation. This study aims to establish fungal-bacterial biofilms using relevant pollutant-degrading microbes, including the bacterial species *Pseudomonas putida* and the fungal species *Phanerochaete chrysosporium,* both known to be capable to degrade a wide range of pollutants. Single-species and multi-domain biofilms were grown in a Drip Flow Reactor; different cultivation factors (e.g., pH, sequence of inoculation) were tested to assess their effect on the establishment and growth of the biofilms. The obtained biofilms will be used for the bioremediation of selenium in acid mine drainage.

## CBE Poster #791

*Date*: 07/2021
*Title*: **Observations of carbon and nitrogen removal in treatment wetlands**
*Authors*: **Nina Denny**, Ellen Lauchnor, Chris Allen, Otto Stein, Paul Karcher
*Affiliation*: Department of Civil Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
*Sponsored by*: MSU Undergraduate Scholars Program



Treatment Wetlands (TW) require little mechanical and electrical input yet still treat water as effectively as conventional systems, but TW implementation has been limited by the lack of knowledge of their treatment capacity and design standards and recommendations. This project aims to increase further TW installation by understanding the complex biogeochemical processes and kinetics that drive water treatment in TW. The basic mechanisms of TW are similar to conventional systems because they both rely on microbes to perform processes such as nitrification, denitrification and decomposition of organic matter. These processes can be tracked through Chemical Oxygen Demand (COD) and nitrate levels. Samples of the treated water were taken one hour, three days, seven days, and fourteen days after feeding but data consistently showed that essentially all of the COD and nitrate treatment was happening in the first three days. With this information, a more detailed time analysis was performed on the COD treatment group. Samples were collected at hours zero, one, five, twenty-four, forty-eight and seventy-two. Within the first hour of feeding, 54%-71% of COD was removed in every plant. This is likely an indication that COD sorption significantly contributes to removal in the TW rather than purely chemical degradation. Treatment does not vary much between plant species, providing evidence for the consistency and reliability of TW. This research is ongoing and the current focus is on pushing the limits of sorption and evaluating different carbon sources and high doses of COD to allow for calculation of removal rates.

## LAB DEMOS

### Growing a hard water/biofilm matrix in silicone tubing

*Standardized Biofilm Methods Laboratory*

This video demonstrates a method for growing a biofilm that incorporates hard water deposits. This test method is relevant for determining the efficacy of biocides used in many industrial water systems where hard water deposits pose an additional challenge. A *Pseudomonas aeruginosa* biofilm is grown in silicone tubing for 24 hours at room temperature in a recirculating system. At 24 hours, a solution of calcium chloride and magnesium chloride is fed simultaneously into the tubing with a solution of sodium bicarbonate. The video will show how the formed deposits become encased in the biofilm matrix. The biofilm is then allowed to grow for an additional 24 hours before it is ready for a biocide application. This test method results in a mean biofilm log density equal to 7.6 +/- 0.28 $Log_{10}(CFU/cm^2)$ for n=9 experiments. The tubing surface may also be sampled for total cells or imaged using confocal microscopy.

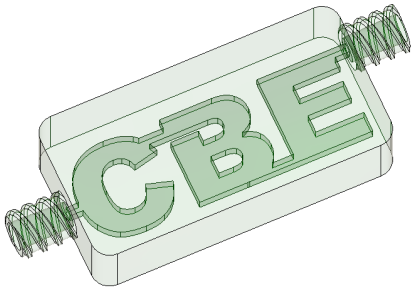
### Colony drip flow reactor

*Medical Biofilms Laboratory*

The colony-drip flow reactor is a hybrid biofilm model that combines the properties of the colony biofilm model and the drip flow reactor model. The colony-drip flow reactor is used in the MBL to mimic the growth of a wound biofilm and to test the efficacy of various wound treatments. This lab demonstration will show how this reactor is both assembled and sampled.

### 3D printing with materials and microbes

*Isaak Thornton, Reha Abbasi*




3D printing can have many uses and applications. Light based, stereolithography printing (SLA), utilizes a laser to selectively polymerize a photoactive resin. This creates a plastic structure with high resolution and smooth walls. The same technology can also be used to print with microbes. Here we demonstrate light based printing of both an acellular millifluidic device as well as a biocompatible hydrogel device with a complex channel printed into the interior. Channel perfusion of both devices is demonstrated using colored dyes. These 3D printing technologies can both be utilized for research in the area of biofilm engineering.



### Simple method for imaging bacteria-immune cell interactions on a surface using confocal microscopy

*Brian A. Pettygrove*

In this demonstration, we present a simple system to quantitatively image interactions between surface adherent bacteria and immune cells over time. This technique can be used to study the growth and structure of early biofilm, the discovery, phagocytosis, and killing of bacteria by immune cells, and the behavior of immune cells on a surface. In brief, bacteria expressing a fluorescent protein are deposited on a glass coverslip, allowed to adhere, and non-adherent cells are subsequently rinsed off. Human serum is then added to opsonize bacteria and condition the surface. Immune cells such as neutrophils are stained with a fluorescent dye for easy visualization and then added to the surface. An inverted confocal laser scanning microscope equipped with an incubation chamber is then used to rapidly image the bacteria-immune cell interactions on the surface for several hours. Analysis of the collected images allows for quantification of bacterial killing and immune cell behavior on the surface. To date, we have used this system to image neutrophil-*S. aureus* interactions but it could easily be adapted to study other cell types such as macrophages or dendritic cells. The current system utilizes an inverted confocal microscope, however a new scheme is proposed to facilitate imaging on opaque surfaces using an upright microscope to broaden the system's application and accessibility.

**A BONCAT-FACS demonstration: Activity-based characterization of microbial phycosome communities associated with the alkali-tolerant green alga *Chlorella sorokiniana* str. SLA-04**

*Isaac Miller*

The alkali-tolerant green alga *Chlorella sorokiniana* str. SLA-04 is of interest to the Algal Research Group at the CBE as it has an elevated capacity to produce biofuel. Briefly, high pH and high alkalinity conditions are advantageous for delivering inorganic carbon to algal cells for carbon fixation during photosynthesis. Higher levels of dissolved inorganic carbon have been linked to higher biofuel production in these cultures. It is hypothesized that high pH, high alkalinity conditions may also benefit algal culture productivity by providing a competitive advantage for the algae over pests and other potential microbial "invaders" in the system. We have been conducting temporal studies to investigate the stability and robustness of xenic cultures, yet little is known about the activity levels of microbes. This demonstration outlines an approach that we are developing to understand microbial activity in the phycosome, or the algae-associated microbiome, under industry-relevant conditions. Bioorthogonal noncanonical amino acid tagging (BONCAT) is a method that can be used to profile activity in microbial populations by tracking the incorporation of synthetic amino acids into new proteins. Fluorescence-activated cell sorting (FACS) is a cytometric method for separating cells based on the presence of a fluorescent signal. When paired together, BONCAT-FACS is a useful tool for identifying the active fraction of complex microbial communities. We have employed BONCAT-FACS to characterize the activity in the phycosome of SLA-04 cultures under low and high alkalinity conditions, light and dark growth cycles, and in closely-attached and planktonic microbial populations. This demonstration illustrates the workflow and preliminary findings.

## ASK THE EXPERTS

### Ross Carlson

*System biology & NASA's manned missions to Mars*

Ross is a professor of chemical and biological engineering and has affiliations with the Center for Biofilm Engineering, Thermal Biology Institute, and the Department of Microbiology and Cell Biology. His research group studies biofilms relevant to medical, environmental, and bioprocess fields. For example, his group is studying the systems biology of chronic wound consortia, microalgal growth and nutrient cycling with bacterial partners, and synthetic consortia engineering for enhanced biocatalytic platforms. One current project has the group working on fungal biomat production for NASA food applications for potential ISS, lunar, and Martian missions (in collaboration with Nature's Fynd).

### Erika J. Espinosa-Ortiz

*Fungal Biofilms*

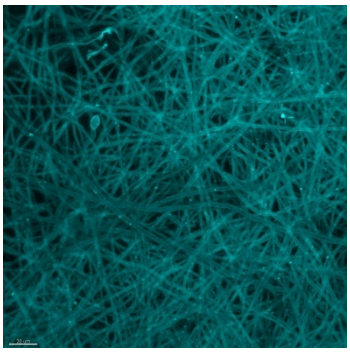


*FIGURE 1. Fungal biofilm*

Diverse fungi have demonstrated the ability to colonize surfaces and develop biofilms, either as single species or in association with other fungi or other microbes including algae and bacteria. Fungi have been detected within biofilms present in different industrial, agricultural, medical, and natural environments. My research focuses on the study of fungal systems and their potential use in different engineering applications. Some of the current projects that I am involved with include the application of fungal-based systems for: (i) water treatment, (ii) biomanufacturing of engineered mycelium composite construction materials, and (iii) bioconversion of lignocellulose waste materials into added-value products. Further, my research also addresses the challenge 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



### Matthew Fields

*Molecular Ecology and Physiology*

Matthew Fields is a professor in the Department of Microbiology & Cell Biology and also serves as director of the Center for Biofilm Engineering at Montana State University. His laboratory uses molecular ecology and physiology to study microbial communities associated with different environments. Laboratory work includes physiology, ecology, and genomics of different organisms relevant to bioremediation, material interactions, groundwater, and biofuels. Ultimately, a driving question is to understand the relationships between structure and function at different scales of biology and the associated ecological and physiological responses. An improved understanding of structure/function relationships will allow predictive modeling and design for a variety of natural and engineered systems. Research projects are currently funded through the Department of Energy, National Science Foundation, the Department of Defense, and private industry.

### Darla Goeres

*Standardized Methods for Biofilm Testing*

The SBML is dedicated to the creation, establishment, and transfer of quantitative biofilm methods for the benefit of academia, government, and industry. Our goal is to design, build, and test laboratory reactors that incorporate the relevant engineering specifications of field systems to recreate a growth environment for predicting the efficacy of biofilm control strategies. In 2020, the CBE capitalized on the SBML's methods development expertise and expanded our role to include regulatory science. Goeres has led the SBML since 2000, and during that time has facilitated the development and approval of five standard test methods with #6 in the works. She has researched biofilms in a range of environments including recreational water, beer draught lines, oil fields, and just about every hard surface in the built environment and industry where biofilm is cause for concern. Stop into her chat room if you are one of the folks who enjoys regulatory science, are curious about what method would be best for your particular biofilm challenge, or would just like to chat about how the CBE has rocked the last 30 years as the first, oldest, and best biofilm center.

**Garth James**

*Medical Biofilms*

The Medical Biofilms Laboratory, led by Dr. Garth James, does research in support of NIH and DOD funded grants in areas such as chronic wounds and tolerance of biofilms to antimicrobial agents as well collaborating with small business on SBIR and STTR funded grants. In addition, the MBL performs custom testing projects for CBE industrial associates and other companies. These projects have included in-vitro evaluation of microbial attachment and biofilm formation on a wide variety of medical devices, including venous access catheters (PIV, PICC, CVC) and needle-free connectors, urinary catheters, and various implantable medical devices (pacemakers, neurostimulators, cochlear implants, surgical mesh, orthopedic implants and spinal fixation devices, dental implants). The MBL has also evaluated antimicrobial lock solutions for both venous access and urinary catheters as well as surgical and wound lavages, antimicrobial wound dressings, toothpastes and mouth washes, and endodontic irrigants. In addition to in-vitro work, the MBL performs ex-vivo analysis of tissues and explanted devices in support of preclinical animal studies and human clinical trials. MBL personnel have expertise in general and molecular microbiology, cell biology, immunology, and virology techniques, as well as scanning confocal laser microscopy, scanning electron microscopy, tissue cryoembedding and sectioning, and fluorescent in-situ hybridization. MBL personnel have extensive experience in designing and adapting in-vitro model systems to perform microbial attachment and biofilm growth experiments under conditions to mimic in-vivo conditions.

**Catherine Kirkland**

*Wastewater Treatment with Aerobic Granular Sludge*

Wastewater treatment is in the midst of a biotechnological revolution in many parts of the developed world, even as approximately half of the world's wastewater continues to be discharged untreated into the environment. Aerobic granular sludge (AGS) is one of the new competing biofilm-based technologies with potential to transform and expand wastewater treatment around the world. AGS consists of spherical biofilm aggregates of diverse microbial communities several millimeters in diameter. The size and structure of the granules promote metabolic diversity and simultaneous biochemical conversions of soluble wastewater constituents like carbon, nitrogen and phosphorus due to oxygen and substrate gradients within the biofilm. The mass of each granule facilitates rapid settling and biomass retention. These innovations reduce the footprint of the treatment plant as well as operations and maintenance costs compared to conventional activated sludge systems. My research group is exploring how the complex matrix of extracellular polymeric substances (EPS) making up the granule structure interacts with recalcitrant organics found in wastewater like pharmaceuticals and per-polyfluoroalkyl substances (PFAS), and how the presence of such compounds influences bioconversion of conventional wastewater constituents. High performance liquid chromatography (HPLC) coupled with quantitative time-of-flight mass spectrometry (QTOF-MS) allows us to assess and quantify the presence of the target compounds in the aqueous phase and adsorbed to the granular biomass. Fluorescence confocal scanning laser microscopy (CLSM) and nuclear magnetic resonance (NMR) imaging is used to compare the morphology and structure of granules exposed to the target compounds versus control granules. Soon, we will include targeted high throughput sequencing (metabarcoding) of the 16S ribosomal RNA genes and transcripts to identify and monitor active members of the microbial community in the AGS over the duration of the study. We aim to identify the primary mechanisms by which the biofilm matrix of the AGS contributes to removal of recalcitrant organics in municipal wastewater.



**Ellen Lauchnor**

*Water Treatment Processes*

Ellen Lauchnor is an associate professor in the Department of Civil Engineering at MSU. Her research group investigates microbially driven processes that contribute to water quality improvement within treatment processes and remediation of contaminated environments. Specifically, her research investigates microbial processes that drive removal of metal contaminants in the environment. Her laboratory and collaborators also investigate microbial contributions to carbon and nitrogen dynamics in engineered wetlands for wastewater treatment.

**Luke McKay**
*DNA/RNA Sequence Analyses*
Sequencing technologies can be used to examine microbial community structure and function through analyses of DNA and RNA. Genes, transcripts, single genomes, and community genomes (metagenomes) are rich with information on potential microbial processes, their evolutionary history, and their impact on diverse environments. At the CBE, we investigate genomic and transcriptomic sequences from a wide variety of medical, industrial, and environmental biofilms. Dr. Luke McKay specializes in sequence analysis with projects ranging from sulfur cycling at hydrothermal vents to natural gas formation in subsurface coal seams."

**Al Parker**
*Biostatistics*
Al Parker is an associate research professor in MSU's Department of Mathematical Sciences and CBE's biostatistician working primarily with CBE's Standardized Biofilm Methods team. His current projects include methods development for the EPA and inter-laboratory study design and analysis for ASTM International. Al's research focus includes modeling complex biological systems (biofilms in the environment and in the lab, neural coding, vision systems), Bayesian inverse problems, iterative sampling from high dimensional densities, bifurcation theory with symmetries, and annealing/clustering schemes. Al will be available during the open house to discuss how to deal with zeros, in vitro and clinical trials, 2D and 3D image analysis, quality control of industrial processes, and multivariate analysis of ecological communities. Be sure to stop by and chat.

**Paul Sturman**
*Partnering with the CBE*
Paul is the industrial coordinator for the CBE. He manages a robust industrial partnership program wherein over 25 companies support research and technology transfer efforts. Paul works closely with these member companies to assist adoption of biofilm-related technologies. His research focus is on investigations of biofilms in industrial water systems, environmental biofilms, and the development of standardized methods for biofilm analysis.



**Philip S. Stewart**
*Biofilm Control*
Dr. Stewart is a senior expert in biofilm science and technology. He has led the Biofilm Control lab at CBE since 1992. His research addresses fundamental mechanisms that protect microbes in biofilms from disinfectants, antibiotics, and the innate immune system as well as practical strategies for managing

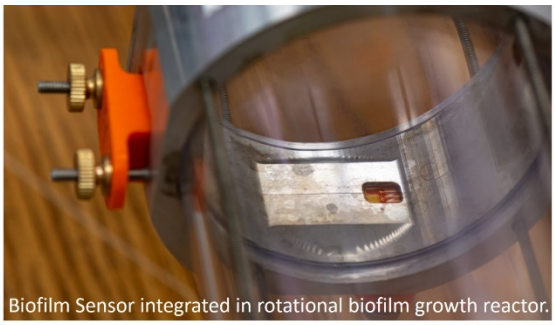
**Diane Walker**
*Food Industry Biofilms*
When the CBE was originally established, the majority of the focus was on biofilms in industrial processes, like the oil and gas industry. Over time, the field expanded to include health-related and medical biofilms, the built-environment, along with other environmental settings. An opportunity exists for the CBE to further expand biofilm research in other arenas like the food industry, as it stands to reason that where there is moisture and nutrients (*i.e.*, food!), biofilms are likely to be found.

- What are areas in the food industry where there is potential for biofilm growth?
- What technologies exist and what is trending for the future?

We are seeking your input! Share your insights about biofilms in the food industry and ask me how we can help!

**Stephan Warnat**

*Biofilm Sensing*


Biofilm Sensor integrated in rotational biofilm growth reactor.

Stephan Warnat, Assistant Professor of Mechanical Engineering, Montana State University. Stephan Warnat is an early Career Researcher and an expert in system integration of miniaturized sensors and actuators for academic and industrial applications. Warnat worked at the internationally noted Fraunhofer Institute for Silicon Technology, Germany, and developed new sensor integration concepts for innovative systems in collaborations with academic and industrial partners. His interdisciplinary education in electrical engineering, materials science, and mechanical engineering fosters a mission driven development, testing, and integration of new sensing mechanisms. Current research questions address how microfabricated sensors can measure reliably biofilm growth and water quality parameter through an in-situ sensor integration in harsh environmental and industrial applications. Currently available systems lack the capability to autonomously measure changes in bio-geochemical processes that can be utilized to study dynamic processes for extended times. The current projects are related to application such as biofilm growth under microgravity conditions and bio-geochemical parameter detection in soil and ice.


7/8/2021 6:15 PM

# Draft AGENDA

****All times are Mountain Daylight Time (MDT)**

## Tuesday July 13

**9:15–9:25**
**Opening Remarks**
Matthew Fields
CBE Director
Professor, Microbiology & Immunology, MSU
Paul Sturman, CBE Industrial Coordinator

### SESSION 1: Biofilm Matrix

**9:25–9:30**
**Session Introduction**
Matthew Fields

**9:30–10:10**
**The biofilm matrix as a cooperative and competitive trait**
Hans Steenackers, Assistant Professor, Microbial & Molecular Systems, KU Leuven, Belgium

**10:10–10:50**
**Biofilm mechanics as a surface survival mechanism for them but a drag for us**
Paul Stoodley, Professor, Microbial Infection & Immunity; Director, Campus Microscopy & Imaging Facility, The Ohio State University

**10:50–11:20 Break**

**11:20–12:00**
**Secreted, large-scale, extracellular membrane systems in microbial biofilms**
Matthew Fields

**12:00–12:40**
**Matrix in the context of biofilm 3D printing**
Reha Abbasi, Postdoctoral Researcher, CBE

**12:40–1:00 Break**

### PANEL DISCUSSION

**1:00–2:00**
**Uncovering the hidden potential and known challenges of the biofilm matrix**
Matthew Fields

Sarah Finn, Senior Microbiology Manager, Kersia

Jan Hodges, Director, QA & Regulatory Affairs, Quest Medical

Hans Steenackers

Paul Stoodley

Moderator:
Darla Goeres, Research Professor of Regulatory Science, CBE

Co-Moderator:
Jim Wilking, Associate Professor, Chemical & Biological Engineering, MSU, CBE

## Wednesday July 14

**9:15–9:25**
**Opening Remarks**
Matthew Fields
Paul Sturman

### SESSION 2: Medical Biofilms

**9:25–9:30**
**Session Introduction**
Garth James, PI, Medical Biofilms Laboratory, CBE; Assoc. Research Professor, Chem. & Bio. Eng., MSU

**9:30–10:10**
**The infectious microenvironment and biofilms**
Thomas Bjarnsholt, Professor, International Health, Immunology & Microbiology, University of Copenhagen, Denmark

**10:10–10:50**
**The wound microbiome**
Garth James

**10:50–11:20 Break**

**11:20–12:00**
**Factors that influence microbial ingress into luer activated valves for intravascular administration sets**
Elinor Pulcini, Assistant Research Professor, Chemical & Biological Engineering, MSU, CBE

### SPECIAL PRESENTATION

**12:00–12:40**
**Biofilms as hot spots for gene transfer**
Phil Stewart, Regents Professor, Chemical & Biological Eng., MSU, CBE

**12:40–1:00 Break**

**CBE *virtual* Open House**
- **Posters**
- **Lab Demos**
- **Ask the Experts**

**1:00–3:00**

# Thursday July 15

**9:15–9:25**
**Opening Remarks**
Matthew Fields
Paul Sturman

## SESSION 3: Industrial Biofilms

**9:25–9:30**
**Session Introduction**
Darla Goeres

**9:30–10:10**
**Beer draught line challenge: Biofilm vs. chemistry**
Darla Goeres

**10:10–10:50**
**Impacts of an antibacterial coating on the growth of ISS isolates for single and mixed domain biofilms**
Madelyn Mettler, PhD Student, Chemical & Biological Eng., MSU, CBE

**10:50–11:20 Break**

**11:20–12:00**
**Fungal biofilms: The good, the bad, and the unknown**
Erika Espinosa-Ortiz, Asst. Research Professor
Chemical & Biological Eng., MSU, CBE

**12:00–12:40**
**Sensing slime: Microfabricated sensors to detect biofilm**
Matt McGlennen, PhD Student
Mechanical & Industrial Eng., MSU, CBE

**12:40–1:00 Break**

**Strategic Planning Meeting**
**1:00–2:30**
CBE Industrial Associates