Montana State University
Center for Biofilm Engineering
Bozeman, Montana

montana biofilm SCIENCE & TECHNOLOGY meeting

JULY 17–19, 2018

presentation
and poster

proceedings

Table of Contents: Presentation Abstracts

SESSION 1: Biofilm Methods

SESSION 2: Bacteriophage and Biofilm

SESSION 3: Strategic Communication and Data Resources

SESSION 4: Medical Biofilms

SESSION 5: Biofilm Physiology

SESSION 6: Biofilm Imaging

SESSION 7: Industrial Biofilms

Table of Contents: Poster Abstracts

Industry Posters (non-CBE)

Center for Biofilm Engineering Posters

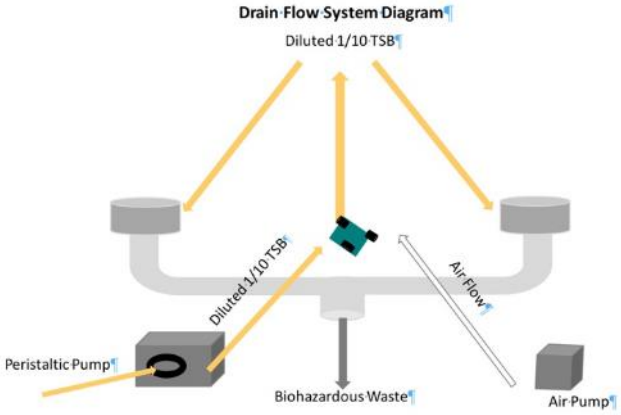
Presentation Abstracts

SESSION 1: Biofilm Methods

Biofilm removal and control issues in food processing: Biofilm reactor and drain models

Presenter: **Charles J. Giambrone**, Vice President, Technical Services, Food Safety Division
Co-Authors: Griffin Jadwin*, Erin Dalmata
Affiliation: Rochester Midland Corporation, Food Safety Division, Corporate Headquarters, Rochester NY, USA. (*Griffin Jadwin is no longer an employee of Rochester Midland Corp.)

A dynamic flow drain system was developed in a laboratory setting to generate mixed strain *L. monocytogenes, S. typhimurium, E. coli* replicate biofilms on surfaces of the drain and polyethylene carrier replicates inside the drains. The ability to test foaming cleaners' remediation capabilities gives information to the sanitarian by providing a general database for a variety of cleaning technologies which can be used in remediation of a bio-contaminated drain. Due to the nature of biofilms and the need for either longer contact time or concentration, we challenged these biofilms against various Rochester Midland foaming cleaning products at high end use concentrations for a five-minute contact time. Control titer samples were compared against exposed carriers to determine overall microbial biofilm reductions of each cleaner. A total of eight RMC formulations were applied in this laboratory study. The data accumulated showed a significant variance (between 1-5 logs) in log reduction between the formulations used to remediate the bacterial biofilm. The most significant reduction came from our chlorinated caustic product, F-364 at 5.21 Logs. The second most efficacious came from our chlorinated soft-metal cleaner, Foam-Safe with 4.48 Logs. The two least effective were our Quaternary blend, F-29 sanitizer, and neutral cleaner, F-204 with only Log 1.67 and Log 1.94 respectively.



Chemical Formulation	Usage Concentration
F-29	1000 ppm QAC
F-204	1/10
FA 6700	5/64
Sterilex Disinfectant/ Activator	1/1/10
Scale off II / Perafoam	1/20
EnviroGuard Sanitizer Perafoam	1000 ppm PAA
Foam Safe	1/10
F-364	1/10

Novel methods to study multispecies biofilms

Presenter: **Nuno Filipe Azevedo**, Assistant Professor
Affiliation: Laboratory of Process Engineering, Environment, Biotechnology and Energy (LEPABE), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

It is now common knowledge that most clinical, environmental and industrial biofilms are constituted by more than one species of microorganisms. These multispecies biofilms generally exhibit a different behavior from the monospecies biofilm models that are studied in the lab, and this can have profound consequences to the conclusions being drawn from a study. For instance, it is well-known that clinical

multispecies biofilms may exhibit increased tolerance to antimicrobial agents, and that environmental multispecies biofilms feature an increased ability to degrade compounds. Most of the biofilm forming devices can be easily adapted to grow multispecies biofilm models. Assuming that our choice of the biofilm setup has been accurate, the bottleneck is hence on the ways that we use to examine such a biofilm. One of the most important parameters to evaluate is the prevalence of the different species. Traditionally, this is accomplished by standard plate counts on nutrient-rich or selective agars. However, in a recent study, we have shown that three different methods (plate counting, q-PCR and FISH) provide very different values for the prevalence of each biofilm population (Fig. 1A), even when these methods have been previously optimized and provide similar results on planktonic populations [1]. Another important aspect is the spatial location of the different populations. FISH is a widely-used technique to identify and spatially locate populations within biofilms. Nonetheless, the method generally requires harsh permeabilization steps and is carried out at high temperatures, meaning that it is an end-point analysis. Employing nucleic acid mimics (NAMs), we have showed that hybridization can occur at low temperatures and also in the absence of toxic permeabilization agents [2], results that pave the way for the application of FISH in living microbial cells.
Finally, bioinformatics tools have evolved from the 1-dimensional to the three-dimensional modelling of polymicrobial structures. One of the most promising technologies is agent-based modelling (ABM) [3]. As an example, we have recently developed an ABM model to predict phenotypic switching in *C. albicans* when this microorganism is exposed to AHL molecules secreted from *P. aeruginosa* (Fig. 1B). This technology allows to assess the dynamic behavior of individual cells taking into account the spatial location of cells and molecules.

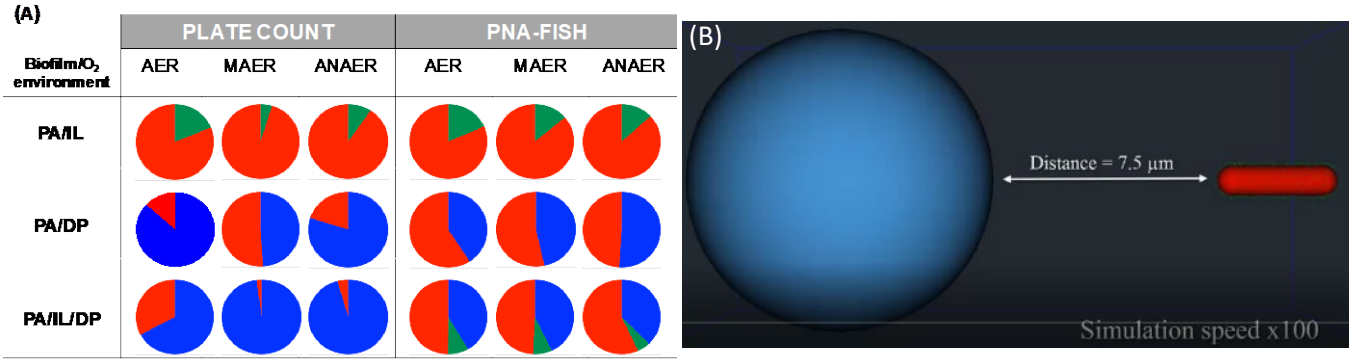


Figure 1 – (A) Different prevalence of species in a three species biofilm model using plate counts and FISH. (B) Agent based modeling of *C. albicans* and *P. aeruginosa* interactions.

References

[1] Lopes, S. et al.: Scientific Reports (in press); [2] Fontenete, S. et al.: Plos One. 2016, 11:e0148353; [3], Perez-Rodriguez G. et al.: Biofouling. 2018, 34(3):335-345.

Advances in 3D hydrogel printing for biofilm engineering

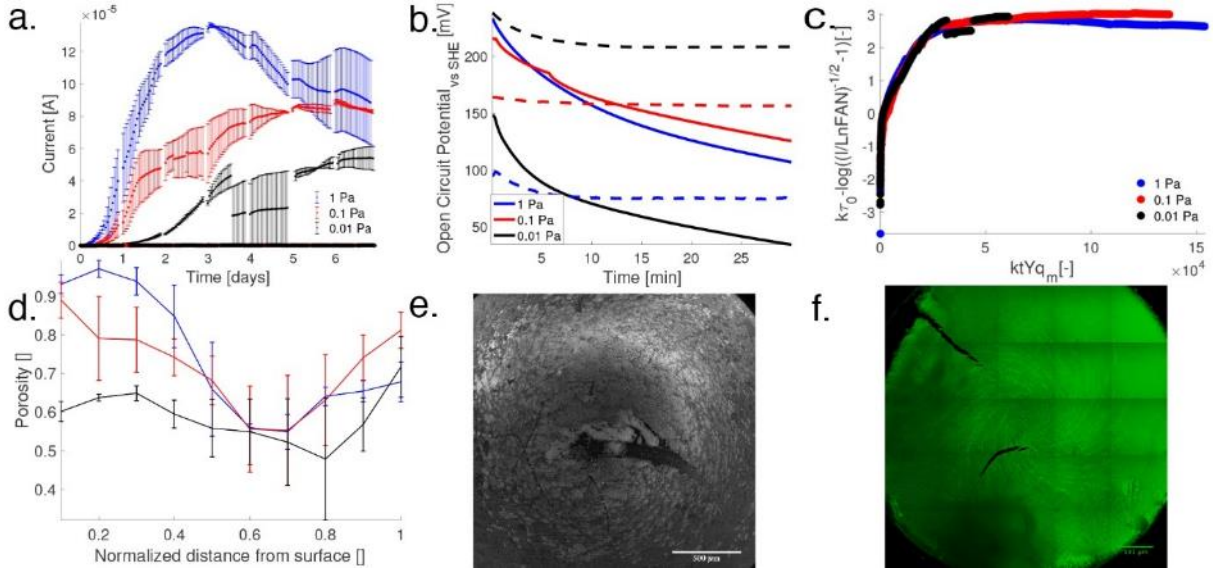
Presenter: **Jim Wilking**, Assistant Professor
Affiliation: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Abstract not available.

Shear stress mediates metabolism and growth in electroactive biofilms

Presenter: **A-Andrew D. Jones III**[1,2], Future Faculty Fellow
Co-Authors: Cullen R. Buie[2] Associate Professor
Affiliation: [1]Northeastern University, Boston, MA,
[2]Massachusetts Institute of Technology Cambridge, MA, USA.

Electroactive bacteria like *Geobacter sulfurreducens*, and *Shewanella onedensis*, can produce electrical current as part of their respiration and this has been exploited in bioelectrochemical systems. These bacteria grow to greater thickness and stay more active than soluble respiring bacteria because their electron acceptor is always accessible. In engineered environments, corrosion resistant metals uptake the oxidation current from the bacteria producing power. While beneficial for engineering applications, this induces pH stress on the bacteria unlike the naturally occurring process where an electrochemically reduced metal combines with protons released during metabolic activity. To reduce bacterial death, some bioelectrochemical systems use forced convection to enhance mass transport of both nutrients and byproducts. Convective transport through the biofilm is negligible, as should be expected with micron size pores in biofilms. However, in previous work it has been difficult to separate bulk mass transport from momentum transport (i.e. fluidic shear stress). In this study, we use a rotating disc electrode to emulate a practical flow system while decoupling mass transport from shear stress. This is the first study to isolate the metabolic and structural changes in the electrochemically active biofilm due to shear stress (a). We find that increased shear stress reduces the biofilm development time, not predicted by initial voltage (b), and while increasing the metabolic rate, at the cost of sustained growth and metabolic activity (a,c). Furthermore, we find that biofilm health is negatively affected by higher shear stress over long-term growth due to biofilm memory of the fluid flow conditions present during the initial phases of biofilm development (d-f). These results not only provide guidelines for improving performance of bioelectrochemical systems, but also provide potential explanations of general biofilm behavior. For example, one may operate a reactor at high shear to decrease time to reach maximum metabolic rates before decreasing shear for steady state operation. Furthermore, the aspect of biofilm memory discovered may help explain the presence of channels within biofilms observed in other studies.



The versatility of shockwaves in destroying biofilms

Presenter: **Iulian Cioanta**[1], Vice President of Research and Development
Co-Authors: Garth James[2] and Paul Sturman[2]
Affiliation: [1]SANUWAVE Health, Inc., Suwanee, GA;
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The Pulse Acoustic Cellular Expression (PACE®) Technology was recently approved by FDA for the treatment of diabetic foot ulcers (DFUs). The chronicity of DFUs is generated in many cases by the presence of biofilms in the wound bed. Based on this assumption, SANUWAVE Health, Inc. started the collaboration with the Center for Biofilm Engineering, to assess the effect of PACE technology on biofilms. Thus the PACE® technology was evaluated for *in vitro* effects on medical, marine, and monuments biofilms. The species of inoculation organisms used for testing were Gram-negative bacteria (*Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus*), and marine microorganisms. Biofilms were grown on polycarbonate or Danby marble coupons in a CDC Biofilm Reactor using methods similar to ASTM E2562-17. Coupons were exposed to 500 to 8,000 shockwave pulses, at 4 shocks/second and at the highest energy setting (E6) for the PACE® System (SANUWAVE Health, Inc.). The amount of remaining biofilm on the coupons was assessed using plate counts as well as confocal scanning laser microscopy (CSLM) with LIVE/DEAD® staining. Based on the lower 95% confidence intervals of the Michaelis-Menten regressions, for *S. aureus* medical biofilms, a 2 log reduction could be achieved with 779 pulses and a maximum log reduction of at least 3.2 could be achieved with 6528 pulses. For *P. aeruginosa* medical biofilms, a 2.0 log reduction could be achieved with 626 pulses and a maximum log reduction of at least 2.8 could be achieved with 4889 pulses. For *P. aeruginosa* monuments biofilms (grown on marble), a 3.5 log reduction could be achieved with 500 pulses and a 4.3 log reduction was achieved with 3000 pulses, which was the largest amount of pulses used in the study, to prevent any possible detrimental effects on the integrity of the marble surface. For marine microorganisms a 4.0 log reduction was achieved with 1000 pulses and a log reduction of 4.9 with 8000 pulses. For both medical and non-medical biofilms, the CSLM imaging results indicated that this reduction was due to biofilm removal. It should be noted that the PACE® System used in this testing was a commercially available system for stimulating regeneration of human tissue and was not optimized for removing or killing biofilms on hard surfaces. Optimization for specific applications may lead to an even better performance against biofilms. It is also important to note that the shock wave application was completed in a non-flowing/static liquid environment, which means that the biofilm removal was strictly the result of the shock waves and was not facilitated in any way by a liquid flow/stream. Based on these research results, shock wave technology may be suitable for biofilm eradication for a broad-range of medical and industrial applications.

SESSION 2: Bacteriophage and Biofilm

A bacteriophage integrase regulates virulence factor production by *Pseudomonas aeruginosa*

Presenter: **Patrick R. Secor**, Assistant Professor
Co-authors: Lia A. Michaels, Caleb Schwartzkopf, Zie Xie, Autumn J. Robinson, Jake Cohen, Devin Hunt
Affiliation: Division of Biological Sciences, University of Montana, Missoula, MT, USA.

Filamentous Pf bacteriophage (phage) are Inoviruses that infect *Pseudomonas aeruginosa,* an important opportunistic pathogen. The majority of laboratory and clinical *P. aeruginosa* isolates already harbor Pf prophage integrated into their chromosomes[1]. When *P. aeruginosa* forms a biofilm, some of the most highly expressed genes belong to Pf phage[2]. Unlike many phage, however, Inoviruses like Pf do not

typically lyse and kill their bacterial hosts—rather, they are continuously extruded[3] and accumulate in the biofilm matrix[4]. In previous work, we discovered that when filamentous Pf phage accumulate in the biofilm matrix, they spontaneously align and assemble a highly ordered liquid crystalline network [4]. Relative to a non-crystalline matrix, bacteria within a liquid-crystalline biofilm matrix are better able to survive desiccation and antibiotic treatment[4]. We also found that filamentous Pf phage increase mucus viscosity causing *P. aeruginosa* to became trapped at infection sites, establishing a non-invasive infection phenotype[5]. Our present work focuses on how Pf phage modulate the virulence potential of *P. aeruginosa*. We discovered a novel role for the Pf phage integrase IntP in regulating *P. aeruginosa* virulence factors. IntP catalyzes the integration and excision of the Pf genome into a specific location in the *P. aeruginosa* chromosome. When *intP* was deleted, *P. aeruginosa* produced less of the green pigment pyocyanin, an important quorum-regulated virulence factor[6]. This finding may explain why deleting the Pf prophage (and *intP* along with it) from the *P. aeruginosa* chromosome reduces virulence *in vivo*[7]. We also find that overexpressing IntP markedly enhances pyocyanin production. Together, these results indicate that IntP can control pyocyanin production. How does a phage integrase regulate a small molecule virulence factor? As a first step to answering this question, we deactivated the integrase activity of IntP by introducing a point mutation to the catalytic tyrosine residue ($IntP^{Y308F}$). Unlike wild-type IntP, $IntP^{Y308F}$ did not promote Pf prophage excision from the chromosome or affect pyocyanin production. IntP may affect pyocyanin production through two general mechanisms. First, IntP-induced prophage excision may promote Pf phage replication and, thus, the expression of Pf genes that may enhance pyocyanin production. However, neither IntP nor $IntP^{Y308F}$ induced the production of infectious phage particles. Second, IntP-induced prophage excision may activate phage suppression mechanisms. For example, H-NS DNA binding proteins silence phage DNA[8] and regulate virulence factor production[9]. Experiments to test these possibilities are currently underway. Because of their contribution to phenotypes associated with biofilm formation and infection pathogenesis, Pf phage may be novel therapeutic targets. Indeed, we recently received R01 funding to develop an anti-Pf phage vaccine to treat and prevent *P. aeruginosa* infections. Understanding how Pf phage regulate virulence factors may offer additional therapeutic targets to combat *P. aeruginosa* infections.

References

[1]Knezevic et al. Prevalence of Pf1-like (pro)phage genetic elements among *P. aeruginosa* isolates. *Virology* 483, 64-71, (2015). [2]Whiteley et al. Gene expression in *P. aeruginosa* biofilms. *Nature* 413, 860-864, (2001). [3]Rakonjac et al. Filamentous Bacteriophage: Biology, Phage Display and Nanotechnology Applications. *Curr Issues Mol Biol* 13, 51-76 (2011). [4]Secor, P. R. et al. Filamentous Bacteriophage Promote Biofilm Assembly and Function. *Cell Host Microbe* 18, 549-559, (2015). [5]Secor, P. R. et al. Filamentous Bacteriophage Produced by *P. aeruginosa* Alters the Inflammatory Response and Promotes Noninvasive Infection *In vivo*. *Infect Immun* 85, (2017). [6]Dietrich et al. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *P. aeruginosa*. *Mol Microbiol* 61, 1308-1321, (2006). [7]Rice et al. The biofilm life cycle and virulence of *P. aeruginosa* are dependent on a filamentous prophage. *Isme J* 3, 271-282, (2009). [8]Castang & Dove. Basis for the essentiality of H-NS family members in *P. aeruginosa*. *J Bacteriol* 194, 5101-5109, (2012). [9]Diggle, et al. MvaT and the regulation of N-acylhomoserine lactone production and virulence gene expression. *J Bacteriol* 184, 2576-2586 (2002).

A potential biocontrol strategy for healthcare facilities using a surfactant-supplemented bacteriophage cocktail for the control of carbapenemase-producing *Klebsiella pneumoniae* biofilms

Presenter: **Ariel J. Santiago**, ORISE Research Fellow
Co-Authors: Mustafa Mazher and Rodney M. Donlan
Affiliations: Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA.

In clinical settings, biofilms are often associated with acute and chronic infections. However, they can also serve as environmental reservoirs, harboring antibiotic resistant organisms, ultimately facilitating their spread. Carbapenemase-producing *Klebsiella pneumoniae* (KPC+) are members of a group of antibiotic resistant organisms known as carbapenem-resistant Enterobacteriaceae (CRE), which are currently a major public health concern. The spread of these organisms within health care facilities has been documented to be, in some instances, a result of contaminated surfaces associated with hospital handwashing sinks as well as corresponding premise plumbing (e.g. sink p-traps). The focus of this work is to investigate the effectiveness of a bacteriophage (phage) cocktail, and its supplementation with a non-ionic polyoxyethylene-polyoxypropylene block copolymer surfactant (Pluronic P103), as a potential biocontrol strategy against these organisms.

Methods

Phages used in this study were isolated from municipal wastewaters and screened for lytic activity towards *K. pneumoniae* 1016 KPC+ (pKPC_UVA010). Four phages were identified (SNP_1-3; RLS_1), three of which exhibited depolymerase activity. *K. pneumoniae* biofilms were cultivated in 96-well microtiter plates for 48 h at 37°C, followed by a phage cocktail treatment (2 h). Reduction in biofilm biomass was quantified using the crystal violet (CV) assay. To determine the biocidal effects of our phage cocktail, *K. pneumoniae* biofilms were cultivated on stainless steel coupons in 12-well plates for 48 h at 37°C, treated with a phage cocktail (2 h), and quantified by viable plate counts of planktonic- and biofilm-associated cells. A series of similar experiments were conducted to determine if addition of the surfactant Pluronic P103, would enhance the lytic activity of the phage cocktail or penetration into the established biofilm. This was done by determining phage titers of the phage cocktail for both the planktonic- and biofilm-phases.

Results

The biomass of phage-treated *K. pneumoniae* biofilms (48 h) was reduced by nearly 35%, relative to untreated controls ($p<0.01$), as determined by the CV assay. Phage-cocktail treatment resulted in an approximate 2.5 log reduction in biofilm-associated cell viability, relative to untreated controls, as indicated by viable plate counts. Addition of P103 (0.5 mg/L) did not appear to enhance the biocidal effects of the phage cocktail; however, titers of biofilm-associated phage in the surfactant-supplemented phage cocktail indicated a slight, albeit significant ($p<0.05$), increase in phages present in this phase, suggesting that penetration into the biofilm may be enhanced.

Conclusion

The combination of biocidal and dispersive properties of these phages make them useful for potential environmental applications targeting CRE in healthcare facilities**.** Furthermore, this work serves as a proof of concept in the use of bacteriophage as a viable treatment option in reducing the bioburden of these pathogens.

Life in the human gut microbiome

Presenters: **Seth Walk**, Assistant Professor
Affiliation: Department of Microbiology & Immunology, Montana State University, Bozeman, MT, USA.

The human gastrointestinal tract is a microbially dominated ecosystem, and recent research has identified important interactions that help support individual members of the microbiome. It has been known for some time that cells of the human epithelium, called goblet cells, secrete a thick layer of mucus in the distal gut and that at least some microorganisms preferentially colonize this glycoprotein-rich matrix, thus establishing a diverse biofilm. Compared to studies that catalog the presence/absence and relative abundance of different bacteria in the gut during disease states, remarkably little is known about life within the biofilm of the gut. Research in the Walk Lab is beginning to address this important microbial lifestyle because we believe life in the human gut requires a biofilm. A brief background as well as results from ongoing research projects will be presented as evidence in support of this hypothesis.

SESSION 3: Strategic Communication and Data Resources

BRaID: Biofilm data fusion

Presenters: **David Millman**[1], Assistant Professor, and **Thiru Ramaraj**, Bioinformatics Research Scientist
Affiliation: [1]Gianforte School of Computer Science, Montana State University, Bozeman, MT, USA. [2]National Center for Genome Resources, Santa Fe, NM, USA.

The "Biofilm Resource and Information Database (BRaID)" is a collaboration between the Center for Biofilm Engineering (CBE), Gianforte School of Computing at Montana State University, Bozeman and the National Center for Genome Resources (NCGR), Santa Fe, NM. The BRaID project is developing a public web portal and database dedicated to storage, analysis, and communication of diverse forms of biofilms-specific data, including nucleotide sequences, images, video, chemistry (water, surface, and extracellular polymer matrix), remediation history, clinical outcomes, and geospatial data. BRaID will complement existing databases by developing a data representation paradigm that solves the problems posed by this data challenge and will implement new metrics and algorithms for the formal description and analysis of biofilms data. It aims to provide the premier resource that offers users the ability to ask complex questions (including those currently impossible to answer), permitting quantitative comparisons among biofilms, supporting remediation efforts, and enabling predictions about outcomes. This talk will focus on what has been accomplished in the initial phase of the project.

Fostering a common vision to mobilize innovation across government, industry, and academic partnerships

Presenter: **Jayne Morrow**, Senior Science Policy Advisor
Affiliation: National Institute of Standards and Technology, Gaithersburg, MD, USA.

In the increasingly data intensive, complex, and dynamic world in which we live, many of the most important challenges facing society require the science and technology community to work differently. Increasingly, the most innovative research is often conducted at the interface or intersection of two fields of science and many of great modern-day discoveries have leveraged the power of effective collaboration. The fundamentals of teams that thrive, collaboration models, and evolving work models, as well as insights from open data access and integration will be discussed. Several examples of strategic

initiatives that have led to fruitful innovation will be discussed with the goal of identifying common methods and tactics that may be beneficial to bring to the biofilm research community. Several of these methods include - creating a space for diversity of thought to flourish, boosting the diversity and the innovation potential of teams, messaging the work and fostering engagement through the process of identifying the science and technology gaps. Models, tools, examples of successful roadmaps and some common best practices for implementation that truly foster community collaboration across disciplines, sectors and stakeholders will be presented in order to opportunities to mobilize the biofilm research community to achieve more by working together.

The elements introduced during this talk will be translated into the poster session where the group will work to uncover opportunities to work together under a common vision for science and technology investment in the next wave of biofilm research.

SESSION 4: Medical Biofilms

Clinical efficacy of biofilm disruption technology developed with testing at the CBE

Presenter: **Alicia Tetlak** RM(NRCM), Microbiologist/Lab Manager
Affiliation: Next Science, LLC, Jacksonville, FL, USA.

Innovation of in-vitro biofilm test methods that can be predictive of in-vivo or naturally forming biofilms is essential for the development of anti-biofilm products. With the assistance and expertise of the Center for Biofilm Engineering, Next Science has commercialized several products that focus on the removal of biofilms and the destruction of the microorganisms within them. Clinical trial and post-market surveillance data of these products has substantiated the in-vitro results seen during product development. These data demonstrate efficacy of these products in treating biofilm-implicated diseases – chronic wounds and prosthetic joint infections and underscores the importance of the continued collaboration between industry and the academic community.

Diabetic wound care in our veteran population

Presenter: **MaryCloud Ammons Anderson**, Research Scientist, Idaho Veterans Research and Education Foundation
Affiliation: Boise VA Medical Center, Boise, ID, USA.

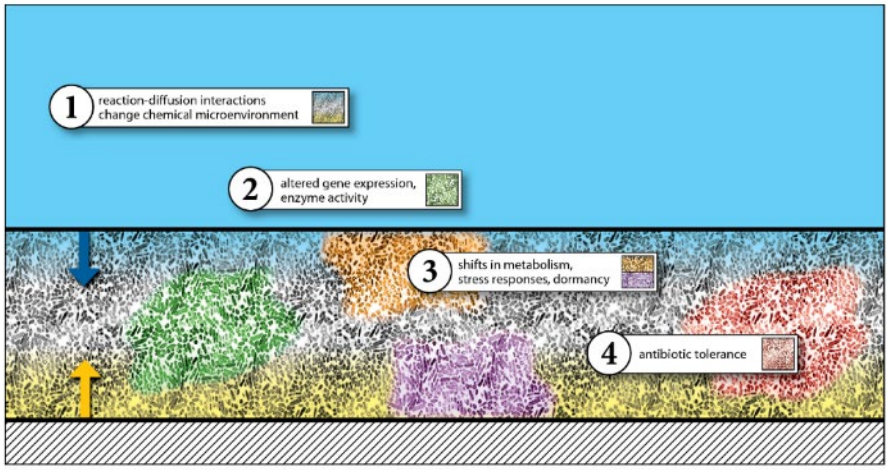
Top priority healthcare initiatives increasingly focus on the issue of non-healing in wound care and the resultant socioeconomic burden. In the general population, the convergence of an exponential increase in the prevalence of Type II Diabetes and a lack of innovation in preventative care has resulted in an estimated $237 billion in annualized direct healthcare costs, including upwards of $58 billion associated with limb amputation due to non-healing wounds. With an estimated 30 million diabetics in the United States currently and projections of upwards of 350 million diabetics by 2050, according to the American Diabetes Association, the issue of non-healing wounds will only amplify in the foreseeable future. Non-healing wounds in diabetics is of particular concern to the Veterans Health Administration (VHA), where one in three patients is either pre-diabetic or diabetic (versus one in ten patients in the general population). Currently, about 9 million veterans are enrolled in the VHA, with around a quarter of the diabetic patient population at risk of developing a non-healing ulcer. The impact on the veteran population includes significant increase in risk to short-term mortality, with a five-year survival rate of around 30% post-diagnosis of a non-healing ulcer, and severe economic impact to the VHA system, with an estimated $46,000 per year per person in cases of continual foot ulcer care. The unsustainable costs to the VHA system and the need for quality-of-life improvement for veteran diabetic patients has resulted in the VA prioritizing innovations in care directed at preventing amputations due to non-

healing wounds. Key to developing novel prognostic, diagnostic, and therapeutic tools has been the establishment of non-profit, VA medical center-affiliated, research foundations such as the Idaho Veterans Research and Education Foundation (IVREF), housed on the Boise VA Medical Center (Boise VAMC) station. With a focus on host-pathogen interactions between innate immune cells and wound-colonizing microbiomes, the Ammons Lab utilizes a metabolic-interactome platform to better understand the contributing factors to non-healing and, in the long-term, to develop evidence-based protocols that can be directly translated into the wound care clinic. Improved quality of life assessment integration into these evidence-based protocols remains an essential component of developing wound care algorithms that best serve those who have served for us.

How bacteria in biofilms withstand antibiotics

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

Transcriptomic, metabolomic, physiological, and computational modeling approaches were integrated to gain insight into the mechanisms of antibiotic tolerance in an *in vitro* biofilm system. *Pseudomonas aeruginosa* biofilms were grown in drip-flow reactors on a medium composed to mimic the exudate from a chronic wound. After three days, the biofilm was 114 μm thick and contained 9.45 $\log_{10}$ cfu cm^{-2}. These biofilms exhibited tolerance to subsequent treatment with ciprofloxacin. The biofilm specific growth rate was estimated via elemental balances to be approximately 0.35 h^{-1} or one-third of the planktonic maximum specific growth rate. Global analysis of gene expression indicated decreased anabolic activity in biofilms compared to planktonic cells. A focused transcriptomic analysis revealed the induction of multiple stress responses in biofilm cells including those associated with growth arrest, zinc limitation, hypoxia, and acyl-homoserine lactone quorum sensing. Metabolic pathways for phenazine biosynthesis and denitrification were activated in biofilms. A customized reaction-diffusion model was solved to characterize the distribution of oxygen inside the biofilm. It predicted that steep oxygen concentration gradients form when these biofilms are thicker than about 40 μm. Mutants deficient in Psl polysaccharide synthesis, stringent response, stationary phase response, and membrane stress response exhibited increased ciprofloxacin susceptibility in biofilms. These results supported a generalized conceptual model of biofilm antimicrobial tolerance with the following mechanistic steps: 1) establishment of concentration gradients in metabolic substrates and products through reaction-diffusion interactions, 2) active biological responses to these changes in the local chemical microenvironment through shifts in gene expression or alterations of enzyme activity, 3) entry of biofilm cells into a spectrum of states involving alternative metabolisms, stress responses, slow growth,



cessation of growth, or dormancy, and 4) reduced susceptibility of microbial cells to antimicrobial challenges in some of these physiological states.

Microplate hydrogel assay for antibiofilm testing

Presenter: **Kenneth Scott Phillips**, Biofilms Research Group Leader, Center for Devices & Radiological Health
Co-Author: Yi Wang, ORISE Fellow
Affiliation: US Food & Drug Administration, Silver Spring, MD, USA.

Recent work with porcine explant biofilms has shown that they are robust against antimicrobial interventions, and may be more difficult to eradicate than biofilms on abiotic surfaces[1-4]. However, biotic substrates are more challenging to work with and it is difficult to obtain reproducible results between batches. To overcome these challenges, we used ultrasoft high water content hydrogels in place of explant tissue. Previous studies of these ultrasoft materials by our group have shown that they have robust bacterial adhesion and biofilm formation. In this work, we show that biofilms formed on these materials in a high-throughput microplate format are not easily removed by rinsing and provide reproducible test substrates for antimicrobial/antibiofilm assessment. To better understand how this format could be used for real-world testing, we grew green fluorescent protein (GFP) producing *S. aureus* and *P. aeruginosa* biofilms and tested them against several antibiotics — ampicillin, gentamicin, and rifampicin. We monitored the fluorescence over a seven-day period after introduction of antibiotics. Results showed that the amount of antibiotic needed to eradicate biofilm on these substrates was much greater than the MIC in most cases, and varied depending on the antibiotic and organism.

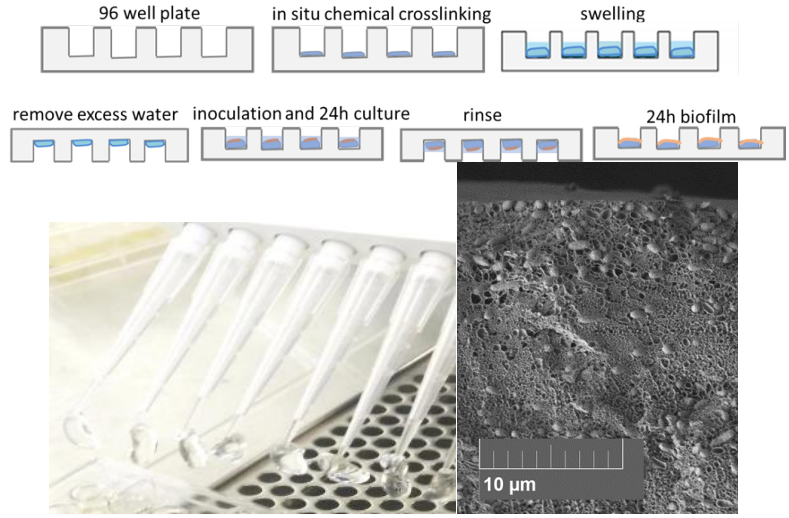


Figure 1. Left: Image of pre-formed hydrogel from microplate wells. Center: Schematic showing experimental procedure. Right: Cryo-SEM image showing *P. aeruginosa* community in microplate hydrogels.

References:

[1]Phillips, P; Yang, Q; Sampson, E; Progulske-Fox, A.; Antonelli, P; Shouquang, J; Schultz, G. Development of a novel ex vivo porcine skin explant model for the assessment of mature bacterial biofilms. *Wound Repair Regen* 2013.
[2]http://ifyber.com/services/antimicrobial/
[3]Wang, Y; Leng, V.; Patel, V.; Phillips, K.S. Injections through skin colonized with Staphylococcus aureus biofilm introduce contamination despite standard antimicrobial preparation procedures. *Nature Scientific Reports* 2017. 7:45070.

[4]Wang, Y.; Tan, X.; Xi, C.; Phillips, K.S. Removal of Staphylococcus aureus from skin using a combination antibiofilm approach. *NPJ Biofilms and Microbiomes* 2018**.** In press.

Biofilm: The clinical dilemma

Presenter: **Eric L. Johnson**, MD, Medical Director
Affiliation: Bozeman Health Wound and Hyperbaric Medicine, Bozeman, MT, USA.

This presentation will focus on the "clinical" aspects of biofilm in patient care. A "bench to patient care" approach will be reviewed and the ongoing challenges that a busy practicing clinician considers in assisting his/her patient to heal.

Characterization of Mycobacterium chimaera biofilms on medical device materials

Presenter: **Archana Siddam**, Commissioner's Fellow
Co-Authors: Shari Zaslow, Yi Wang, Kenneth Scott Phillips, Mathew Silverman, Patrick Regan, Jayaleka Amarasinghe
Affiliation: Winchester Engineering and Analytical Center, US Food and Drug Administration, Winchester, MA, USA.

Mycobacterium chimaera is a slow-growing nontuberculous mycobacterial (NTM) species that is widely distributed in the environment, including soil and water. It has been recently identified as the causative agent of the ongoing global outbreak of invasive infections among patients who had undergone cardiothoracic surgeries. Initial epidemiological studies identified aerosolized *M. chimaera* as the potential source isolated from contaminated heater cooler devices (HCDs), which is used to regulate a patient's body temperature during cardiothoracic surgery. The observed *M. chimaera* resistance to various antibiotics and disinfectants are hypothesized to be due to its biofilm forming properties. Failure of complete eradication of *M. chimaera* from HCDs even after following manufacturer's decontamination guidelines, suggest an urgent need to develop efficient strategies for disinfecting these HCDs. Since the *M. chimaera* contamination reemerges after subjecting HCDs to a strict disinfection protocol, we hypothesize that *M. chimaera* cells are potentially growing in a biofilm enclosed in a matrix of extracellular polymeric substance (EPS). The study aims to first understand biofilm forming properties of *M. chimaera* through the characterization of these biofilms on various medical device materials and then to develop an effective HCD decontamination protocol. We have demonstrated that under laboratory conditions *M. chimaera* forms robust biofilms on various medical device materials including polystyrene, stainless steel and Titanium within a week of incubation, with increased biofilm mass accumulation over the period of six weeks. This study provides one of the very first insights into the early stages of biofilm formation as well as later stage of biofilm accumulation by *M. chimaera* on medical device surfaces. Findings from this study are anticipated to aid in the successful eradication of biofilm formation on medical device materials and to address the current outbreak with potential public health importance.

Design, synthesis, and evaluation of prodrug antimicrobials to control biofilms

Presenter: **Danica Walsh**[1,3], PhD Student
Co-authors: Tom Livinghouse[1] and Phil Stewart[2,3]
Affiliation: [1]Department of Chemistry & Biochemistry,
[2]Department of Chemical & Biological Engineering,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The majority of microorganisms in nature, including those responsible for hospital-acquired infections and fouling of industrial processing equipment, live in association with surfaces as biofilms. Due to the

secretion of proteins, extracellular DNA and lipopolysaccharides, biofilm communities are encased in a robust matrix which reduces their susceptibility to antimicrobial agents. *The long term goal of my research project is to develop efficient, prodrug antimicrobial reagents that are able to permeate the biofilm matrix, as well as the cell membrane in order to eradicate biofilm colonization.* This began with the literature review and minimum inhibitory concentration (MIC) evaluation of simple phenolic compounds with antimicrobial activity, followed by functionalization to increase toxicity and lipophilicity. Ester appendages are being placed on select compounds to implement a prodrug design, as is used in cellular dyes such as Calcein AM and pharmaceuticals such as Bolmantalate. As with fluorescent cellular dyes, once inside the cell the ester appendages will be cleaved, and the resulting compound will be negatively charged and trapped within the cell. It is our hypothesize that the efficacy of antimicrobial agents towards biofilms will be efficiently restored through this strategic design and synthesis of derivatives with modulated polarity that are engineered to have high levels of cellular retention upon undergoing a cleavage event in the cell. This new class of prodrugs presents a wide array of potential applications, from the control of biologically induced corrosion to the incorporation into household cleaning products.

SESSION 5: Biofilm Physiology

Investigating single cell growth using drop-based microfluidic incubation

Presenter: **Shawna Pratt**[1,3], PhD Student
Co-authors: Tatsuya Akiyama[1], Geoffrey Zath[2,3], Michael Franklin[1,3], Connie Chang[2,3]
Affiliation: [1]Department of Microbiology & Immunology,
[2]Department of Chemical & Biological Engineering,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Heterogeneity in microbial populations is a key contributor to the resilience of bacteria living in biofilm communities. Traditional microbiology and bulk studies excel at probing population level dynamics; however, these studies give limited insight into heterogeneity at the individual cell level. A true understanding of single cell dynamics and heterogeneity requires single cell resolution. Drop-based microfluidics is a promising method for evaluating single-cell growth and stress responses. Drop-based microfluidics allows encapsulation of single cells in monodisperse, biocompatible fluid drops and provides an efficient, low-reagent and high-throughput method for conducting single cell bacterial growth studies. Here, an adaptation of drop-based microfluidics for time-lapse monitoring and quantification of single *Pseudomonas aeruginosa* (PAO1 wild type and Δhpf) cell growth is presented. Single bacterial cells are encapsulated in 15μm diameter drops containing nutrient. The drops containing cells are emulsified in an oxygen-rich, fluorinated oil phase and are protected from coalescence by a biocompatible surfactant at the aqueous/oil interface. The cell-laden drops are injected into a microfluidic device that keeps the drops in a stationary, monolayer array that is well-suited for confocal imaging. A method for preparation of the microfluidic device promotes thermodynamic stability and allows for drop stability over incubation periods of 24+ hours. With this advancement, growth of single *Pseudomonas aeruginosa* cells is monitored via time-lapse confocal microscopy. Growth responses in the bacterial strains are quantified using the collected fluorescence based-data. Results demonstrate that cells exhibit heterogenous growth responses between individual cells within a single population. Variation in population heterogeneity is based on the presence of the hibernation promotion factor gene, *HPF*, and the exposure of cells to starvation stress. Varied lag phase times show heterogeneity in growth, which provides insight into the bulk culture observation of overall decreased growth rate in cells exposed to starvation stress. These results demonstrate that drop-based microfluidics provides a novel method for single-cell monitoring of bacterial physiological heterogeneity and responses to stresses.

Heterogeneity in *Pseudomonas aeruginosa* populations

Presenter: **Tatsuya Akiyama**, Postdoctoral Researcher
Co-authors: Michael Franklin, Kerry Williamson
Affiliation: Department of Microbiology & Immunology, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

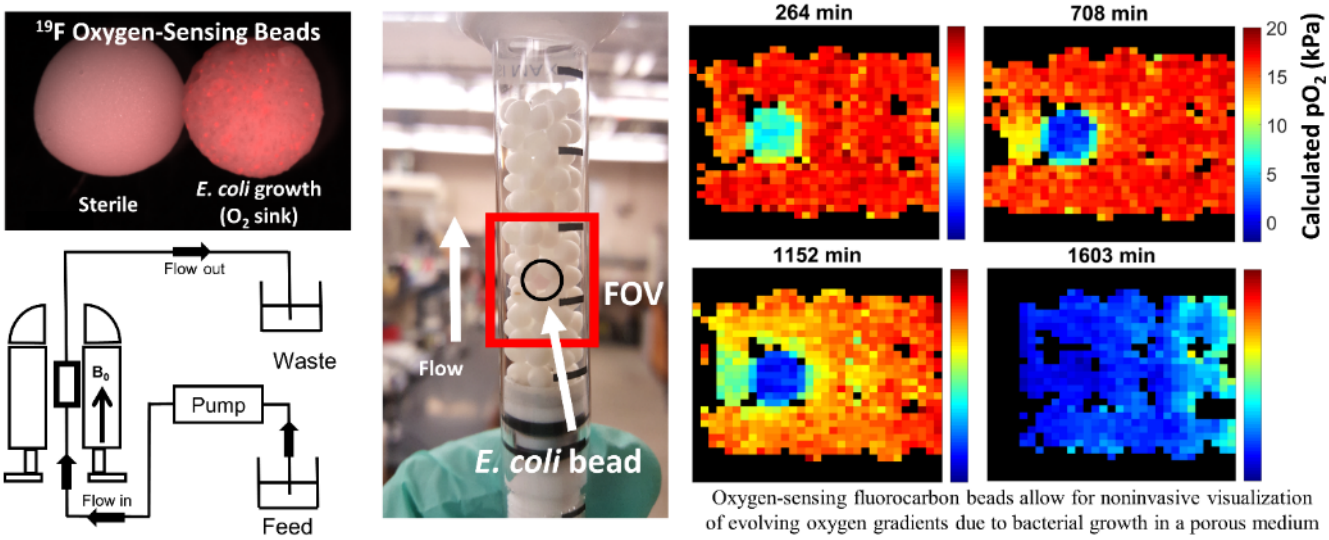
Physiological heterogeneity of biofilm subpopulations dictates certain biofilm functions. For example, antibiotic tolerance of biofilm-associated cells differs between the actively metabolizing bacteria and the dormant cells, with the dormant cells persisting through antibiotic treatments. While dormant, microbes must maintain integrity of the macromolecules that are necessary for resuscitation when conditions become favorable for regrowth. Recently, we identified a ribosome-associated protein, hibernation promoting factor (HPF), that is important for maintaining ribosomal integrity in dormant *P. aeruginosa* cells. HPF was necessary for optimal resuscitation of *P. aeruginosa*. Single-cell level studies of ribosomal RNA abundances in dormant cells indicated that there is heterogeneity in ribosome content during starvation-induced dormancy. This heterogeneity was consistent with the ability of individual cells to recover from dormancy. Factors associated with microbial dormancy, including ribosome-preserving factors, are potential targets for controlling antibiotic tolerant cells within biofilms.

Spatiotemporal mapping of oxygen in a microbially-impacted packed bed using ^{19}F nuclear magnetic resonance oximetry

Presenter: **Jeffrey Simkins**, PhD Student
Co-authors: Philip Stewart, Joseph Seymour
Affiliation: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Bacterial biofilms, aggregates of bacteria encased in a self-secreted matrix of metabolic products, are ubiquitous in industrial, environmental, and clinical settings and, in every case, oxygen gradients are a critical parameter in biofilm behavior. However, measurement of oxygen distributions in biofilms is often intractable due to limitations of traditional methods (e.g. microelectrode). In complex porous systems, which best approximate the actual growth environments of real biofilms (e.g. human wound tissue, soil, wastewater granules), this difficulty becomes especially pronounced. In the present work we use ^{19}F Nuclear Magnetic Resonance Oximetry to quantitatively track the distribution of oxygen in a model porous media system, a packed bed column, inoculated with bacteria. ^{19}F NMR Oximetry, which has been used in medicine to measure oxygenation in blood, tissues, and tumors, exploits the linear dependence of spin-lattice relaxation rate R1 of fluorocarbons on local oxygen concentration. We have designed oxygen-sensing beads comprising an emulsion of the fluorocarbon perfluorooctylbromide (PFOB) dispersed in alginate gel. These beads are used as the solid matrix of the packed bed column and oxygen distribution is tracked over time after inoculation with *E. coli* or *S. epidermidis*. These data are used to identify oxygen reaction kinetics and generate a map of rate constants, model reaction diffusion phenomena to extract intrinsic growth parameters, and characterize differences in oxygen transport and flow modification for distinct bacterial species. In addition, changes in oxygen uptake in response to antibiotic (gentamicin) exposure are documented and discussed.

(See Figure 1 on next page)



Oxygen-sensing fluorocarbon beads allow for noninvasive visualization of evolving oxygen gradients due to bacterial growth in a porous medium

CRISPR-mediated defense and bacteriophage counter-defense in *P. aeruginosa*

Presenter: **MaryClare Rollins**, Research Associate
Co-Authors: Blake Wiedenheft, Assistant Professor
Affiliation: Department of Microbiology & Immunology, Montana State University, Bozeman, MT, USA.

Viruses that infect bacteria (i.e. bacteriophages) are the most abundant biological entities on earth, and the selective pressures they impose have profound impacts on the composition and behavior of microbial communities in every ecological setting. Our work aims to understand the mechanisms that bacteria use to defend themselves from phage infection, and the counter-defenses that phages use to subvert bacterial immune systems. The adaptive immune system in *Pseudomonas aeruginosa* relies on a 350 kDa CRISPR RNA (crRNA)-guided surveillance complex that binds foreign DNA and recruits a trans-acting nuclease for target degradation. I will present structures of this complex bound to two different bacteriophage-encoded anti-CRISPR proteins that suppress the immune response. This work help explains how bacteria mount an immune response and how viruses evolve to subvert these immune systems.

SESSION 6: Biofilm Imaging

CBE imaging capabilities: An overview

Presenter: **Heidi J. Smith**, Postdoctoral Research Associate
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The diversity of advanced imaging instrumentation within the CBE Imaging Core allows for a wide range of sample types (e.g. from thin cryosections to fully hydrated biofilms, from 2D to 3D, from disturbed to intact) to be imaged. The CBE imaging core houses light/epifluorescent microscopes, stereoscopes, confocal microscopes, a laser micro-dissection microscope, an optical coherence tomography system, and confocal Raman microscopes. Each imaging technique has advantages, however, it is also important to understand the optical and analytical constraints of each imaging platform when designing an experiment. This talk offers information on different imaging capabilities within the CBE with an emphasis on the applicability of different imaging techniques to a wide range laboratory and field

samples. Information on sample requirements (e.g. penetration depth, sample preparation, and sample state) will be summarized for instrumentation within the CBE imaging core. Currently we are adding to these capabilities by integrating new imaging techniques that target active microbial populations and identify specific compounds of interest (e.g. BONCAT and stable isotope probing). Microscopy and imaging technology are rapidly evolving fields and the future of imaging within the CBE is focused on developing correlative microscopy techniques that will establish inter-connectivity between individual analysis platforms.

Quantification of biofilm characteristics from images with error bars

Presenter: **Al Parker**, Biostatistician
Affiliation: Center for Biofilm Engineering, Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA.

Imaging technologies have revolutionized the potential to identify and characterize biofilms in 3D down to the microscale. Unfortunately, quantitation and statistical analyses of the biofilm characteristics from images have lagged behind, especially when imaging thick biofilms. In thick biofilms, fluorescence attenuates markedly as a function of depth into the biofilm. By directly modeling this attenuation, we show how to quantify biofilm characteristics from a 3D image, with error bars, using either the method of maximum likelihood or Bayesian maximization (that is different than Otsu's method used by COMSTAT and ImageJ). Importantly, the quantification does not dependent on a user selecting a threshold for the image; however, a maximum likelihood (or Bayesian) estimate of the threshold is provided by the modeling process. An immediate consequence of this work is to generate efficient imaging designs (e.g., pixelations and number of z-slizes) for capturing images that optimally inform biofilm characteristics of interest.

Characterizing biofilms using centrifuge force microscopy

Presenter: **Thomas B. LeFevre**, PhD Student
Co-author: James N. Wilking
Affiliation: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilms are microbial communities that form on surfaces and are implicated in a wide variety of intractable medical and industrial problems. A better understanding of biofilm adhesion strength, and its variation between surfaces and species, is desired. We are using a Centrifuge Force Microscope (CFM) to measure adhesion strengths on a cellular and macroscopic level. The CFM utilizes a microscope-camera assembly inside a centrifuge to observe the effects of artificial gravity on a biofilm sample. When the biofilm detaches from its surface, the camera records the speed at which the centrifuge was running, which determines the force applied. Another application of the CFM is single-molecule force measurement. Two common biofilm extracellular matrix components are polysaccharides and amyloid fibers. The CFM can measure the mechanical properties of amyloid fibers that have been chemically bound to a surface. A microbead is attached to the end of each fiber, and effective gravity pulls on the microbead to stretch the fiber, determining its elastic modulus. Furthermore, polysaccharides bound to microbeads can be brought into contact with the amyloid fibers and then pulled away with effective gravity. Measuring the interaction forces between polysaccharides and amyloid fibers offers insight into how biofilms utilize this interaction for maintaining structural integrity.

SESSION 7: Industrial Biofilms

Biofilms in industrial water handling systems: An overview of real-time bio-monitoring and system performance

Presenters: **David Vela**, Senior Research Scientist, and **Angela Delegard**, Research Scientist
Co-Authors: Adrian Denvir[1] and Chiachi Hwang[2]
Affiliation: [1]Chem-Aqua, Mohawk R&D Labs, Irving, TX, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Within the last decade researchers have gained a significant understanding of the role of industrial biofilms in many sectors including water treatment, oil and gas, food processing and manufacturing. Biofilms are the preferred habitat for microorganisms; in this environment they are protected from harsh chemical treatments, shear stress, and predation. Biofilms create conditions that cost industry billions of dollars annually through heat transfer loss, decrease in equipment lifetime, and increased greenhouse gas emissions. Additionally, biofilms in industrial water lines and other systems can harbor harmful pathogens. The types of bacteria responsible for these complications vary from system to system and are influenced by factors such as chemical treatment, environmental conditions, and water quality. Chem-Aqua has developed the bioDART, a novel real-time biomonitoring device designed to monitor the potential for biofilm formation in industrial cooling systems and closed loops. After the initial proof of concept validation was completed in a collaborative effort with MSU's CBE, the systems were deployed to 28 unique industrial water systems across the United States for testing. These industrial systems provided diverse geographical and temporal biofilm sample sets monthly. These data allowed us to establish a dynamic Biofouling Index and Biofouling Rate unique to each system. Additional biofilm analyses from each sample included heterotrophic plate counts, carbohydrate measurements, DNA quantification, and next-generation sequencing (NGS). The NGS work is a collaboration with the CBE. Metagenomic sequence analysis was utilized to provide a new understanding of the variations in bacterial populations existing in industrial biofilms. Data revealed from the analysis provides insight on the phylogenetically and metabolically distinct species present in the biofilms. This will aid in the development of new anti-biofilm strategies for industrial water treatment systems.

Microbial defacement of building materials

Presenter: **Erika J. Espinosa-Ortiz**[1], Postdoctoral Research Associate
Co-authors: Paul Sturman[1], Robin Gerlach[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Microbial growth on building materials (walls, floors, ceilings) may result in *(i)* (mostly cosmetic) defacement of surfaces, *(ii)* degradation of materials, which could result in major maintenance costs, and *(iii)* human health hazards including increased risk of asthma and allergies in indoor environments. The most commonly detected microorganisms on indoor building materials are fungi and bacteria, whereas a more diverse microbial community has been observed on outdoor building materials including fungi, bacteria (actinomycetes and cyanobacteria), algae and small animals (protozoa, rotifers and nematodes). We have begun to investigate the colonization of different materials by fungi and bacteria as well as the subsequent biofilm development. Material biodeterioration was evaluated by accelerated tests in an environmental chamber, designed to be operated as a high humidity/partially-wetted system. Coupons of common building materials including wood, plastic and glass, coated with commercially available indoor and outdoor paints, were inoculated with spores of *Aureobasidium pullulans* (a common mold fungus) and *Pseudomonas putida* (a bacterium commonly found in soil and water). The coupons were incubated at room temperature (23±1°C) and relative humidity >95%; periodic wetting of the samples with growth medium was performed via a misting system. Microbial proliferation and material defacement were analyzed weekly for four weeks. Digital image processing was used as a tool for assessment and analysis of material defacement; this method has the potential for a less subjective and more reproducible procedure to determine microbial coverage on surfaces compared to the current standard relying on simple human visual perception. The building materials used, and the operational conditions tested in this study favored fungal growth over bacterial establishment. The proposed environmental chamber could be used to test the biodeterioration of building materials under a broad range of operational conditions (relative humidity, temperature, simulation of rain events, light intensities, etc.) that can influence microbial proliferation and biofilm development.

Scale in water systems: A biomineral?

Presenter: **Adrienne Phillips**[1,3], Assistant Professor
Co-authors: Darla Goeres[2,3], Anne Camper[1,3]
Affiliation: [1]Department of Civil Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Scale in water distributions systems leads to flow restrictions, reduced heat transfer efficiency, increased costs due to higher energy needs, and may lead to entrapment of biofilms inside the calcium or magnesium deposits. Scale formation and control has been long studied and was prominent in the early work at the Center for Biofilm Engineering. However, $CaCO_3$ scaling is still a problem for industry and municipalities. New information about increasing atmospheric CO_2 concentrations, a warming climate and gaps in knowledge about $CaCO_3$ formation in water systems makes this a timely topic. In this presentation, the topic of scale deposits will be re-visited with a focus on water chemistry leading to scale formation, the role of microbes and biofilms in nucleation of minerals, common methods to control scale in water systems, and the fate of microbes after anti-scaling treatment. Recent work on a laboratory method that grows a biofilm with scale deposits to study prevention, eradication (treatment) and the efficacy of biocides in scaled systems will be presented.

Poster Abstracts

Industry Posters

Date: 07/2018
Title: **Formation of *Pseudomonas aeruginosa* biofilm in PTFE tubing according to ISO/TS 15883-5 annex F standard**
Authors: **Bruno Haas**, Marie-Claude Gagnon, Philippe Labrie
Affiliation: STERIS Canada ULC, Québec, QC, Canada.
Sponsored by: STERIS Corporation

Flexible endoscopes, including nasoendoscope, are increasingly used in minimally invasive surgeries. Endoscopes contaminated with pathogenic bacteria, generally biofilm, can be the cause of device-related nosocomial infections. These devices represent a particular challenge for cleaning and disinfection because of their complexity, design, and inability to withstand high temperatures. Endoscopes reprocessing is crucial to prevent biofilm formation within the lumens. The first step of reprocessing is the manual cleaning of the endoscope to remove most of the organic and microbial load by flushing and brushing the surfaces and lumens of the devices. The second step is a manual or automated disinfection and sterilization. Since flexible endoscopes are heat-sensitive, they are chemically disinfected. ISO/TS 15883-5 Annex F standard describes a method to form biofilm in PTFE tubes. Biofilm-contaminated tubes are then used in surrogate devices mimicking the internal design of flexible endoscopes. These surrogate devices are then processed in automated endoscope processors to validate cleaning and chemical disinfection of biofilm in endoscopes. STERIS Corporation designs and manufactures automated endoscopes processing systems. The present study describes the ISO/TS 15883-5 Annex F method used to validate cleaning of flexible endoscopes biofilm in compliance with ISO standards.

Date: 07/2018
Title: **Evolution of biofilm methodologies: Choosing the model**
Authors: **Christopher J. Jones**[1], M. Ryan Mettetal[1], Binjie Xu[1], Albert E. Parker[2], Ethan E. Mann[1]
Affiliation: [1]Sharklet Technologies, Inc. Aurora, CO, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Sharklet Technologies, Inc.

Sharklet Technologies has identified a specific microtopography that modulates bioadhesion. This pattern is utilized to limit bacterial adhesion and surface fouling in a host of medical and consumer devices. Throughout the years, Sharklet has developed many biofilm and fouling models in order to test and validate prototype devices. These models include simple *in vitro* testing of bioadhesion and complex animal models of biofouling. Additional parameters include flow/static, acute/chronic, and colonization/biofilm development. Here, we highlight several of the wide variety of biofilm tests developed, highlighting the benefits and drawbacks of each method. Collectively, these approaches provide a variety of methods which allow selection of an appropriate model for each of the devices being tested with the ultimate goal of generating reproducible data to determine the efficacy of devices compared to the existing products.

Center for Biofilm Engineering Posters

CBE Poster #721

Date: 07/2018
Title: **Growing and treating a multispecies biofilm with modifications to an ASTM international standard test method**
Authors: **Madelyn Mettler[1,3]**, Kelli Buckingham-Meyer[3], Lindsey Lorenz[3], Diane Walker[3], Darla Goeres[1,3], Paul Sturman[3], Al Parker[2,3]
Affiliation: [1]Department of Chemical & Biological Engineering,
[2]Department of Mathematical Sciences,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: CBE Industrial Associates

Biofilms commonly encountered in healthcare, industry, food processing, hospitality, and home settings, just to name a few, are complex systems composed of multiple bacterial species. Therefore, it is of interest to investigate these more complex biofilms in a research lab, including how multispecies biofilms respond to antimicrobial agents. This poster presents the results of a study conducted in the Standardized Biofilm Methods Laboratory that tested a multispecies biofilm composed of *Pseudomonas aeruginosa, Flavobacterium spp.,* and *Klebsiella pneumoniae* grown in the CDC Biofilm Reactor against a low and high concentration of sodium hypochlorite. The log reductions calculated for the multispecies biofilm will be compared to log reduction achieved for a single species biofilm.

CBE Poster #722

Date: 05/2018
Title: **Nitrogen fixation in a syntrophic coculture alters biofilm structure and function**
Authors: **Kristen A. Brileya[1]**, Isaac R. Miller[1,2], Alison L. Osborn[1,2], Matthew W. Fields[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Microbiology & Immunology, Montana State University, Bozeman, MT, USA.
Sponsored by: ENIGMA

Free-living diazotrophic bacteria and archaea are key players in global nitrogen cycling, making fixed nitrogen available in anoxic systems with significant contribution to dinitrogen flux from the atmosphere. Current research on nitrogen fixation in biofilm is dominated by examples from root-associated bacteria. In this study, a previously characterized syntrophic coculture of *Desulfovibrio vulgaris* and *M. maripaludis* was evaluated for potential to fix nitrogen under syntrophic growth conditions and in continuous culture biofilm. Extensive work has been done on the regulation of nitrogen fixation in *M. maripaludis*, while it is less studied in *D. vulgaris*, and coculture. Little attention has been directed to the role of nitrogen limitation and fixation in the SRB:methanogen syntrophy that is perched at the edge of thermodynamic constraints. The purpose of this work was to determine the feasibility of nitrogen fixation in coculture biofilm and infer the role of both members. We predicted that diazotrophic biofilm structure and composition would be distinct from ammonium-fed biofilm. Coculture growth rate was reduced in batch planktonic diazotrophic studies, and biomass yield was low, presumably due to poor transport of N_2 gas to the aqueous phase. Continuous culture in a biofilm reactor afforded constant sparging with N_2, and mixing, which resulted in biofilm formation along with a robust planktonic community. Biofilm structure was notably different under diazotrophic growth

compared to ammonia conditions as visualized with electron microscopy, with a thin biofilm that appeared to be arranged to allow for gas transport. Relative abundance of *D. vulgaris* and *M. maripaludis* in the biofilm was similar to intermediate aged ammonium-grown biofilm, while the planktonic ratio was reversed with 61% *M. maripaludis* and 39% *D. vulgaris* for a comparable amount of total planktonic protein. Biofilm protein represented just under 10% of the total reactor biomass under N_2 fixation, while ammonium-grown biofilm accounted for 80% of the total reactor biomass. The total ratio of *D. vulgaris*: *M. maripaludis* was 1:1 compared to a ratio of 2.5:1 in ammonium-grown conditions. The results suggest that the required energy burden of nitrogen fixation lowers biofilm biomass allocation and promotes more equalized carrying-capacity for the two biofilm populations.

CBE Poster #723

Date: 07/2018
Title: **Spatio-temporal characterization of strontium partitioning during MICP under continuous flow**
Authors: **Neerja Zambare**[1,3], Ellen Lauchnor[2,3], Robin Gerlach[1,3]
Affiliation: [1]Department of Chemical & Biological Engineering,
[2]Department of Civil Engineering,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana Research and Economic Development Initiative (MREDI)

Bacterially driven reactions such as ureolysis can induce calcium carbonate ($CaCO_3$) precipitation, a process known as Microbially Induced Calcium carbonate Precipitation (MICP). Subsurface ureolytic microorganisms are increasingly being considered for MICP-based groundwater remediation. Long term sequestration of heavy metal contaminants such as strontium (Sr) can be achieved via partitioning of the heavy metals into $CaCO_3$ precipitates produced by MICP. Controlling the amount and distribution of the $CaCO_3$ precipitates, and the degree of metal partitioning in the precipitates is necessary in order to use this "co-precipitation" technology in potential groundwater treatment applications. Precipitate mass and distribution can be controlled by manipulating fluid flow and saturation states. This was demonstrated by MICP and Sr co-precipitation experiments performed in flat, porous media flow reactors. Effects of flow rates and calcium concentrations on Sr co-precipitation were studied. Additionally, a modified flow cell was used to assess spatio-temporal characteristics of Sr co-precipitation. Calcium removal rates correlated linearly with the calcium mass flow rates into the flow cell, suggesting that under the conditions tested, MICP was limited by calcium transport. Highest calcium precipitation and Sr removal was achieved at the lowest volumetric flow rate and calcium concentration tested. Lower calcium concentrations resulted in higher Sr partitioning. With distance into the flow cells, precipitate size increased accompanied by a decrease in the strontium-to-calcium content of the precipitates. Distance from the inlet and time proved to be significant factors influencing urea utilization, calcium precipitation and Sr co-precipitation.

CBE Poster #724

Date: 07/2018
Title: **Characterizing the adhesion and rheology of biofilms using centrifuge force microscopy**
Authors: **Thomas B. LeFevre**, James N. Wilking
Affiliation: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilms are microbial communities that form on surfaces and are implicated in a wide variety of intractable medical and industrial problems. A better understanding of biofilm adhesion strength, and its variation between surfaces and species, is desired. We are using a Centrifuge Force Microscope (CFM) to measure adhesion strengths on a cellular and macroscopic level. The CFM utilizes a microscope-camera assembly inside a centrifuge to observe the effects of artificial gravity on a biofilm sample. When the biofilm detaches from its surface, the camera records the speed at which the centrifuge was running, which determines the force applied. Another application of the CFM is single-molecule force measurement. Two common biofilm extracellular matrix components are polysaccharides and amyloid fibers. The CFM can measure the mechanical properties of amyloid fibers that have been chemically bound to a surface. A microbead is attached to the end of each fiber, and effective gravity pulls on the microbead to stretch the fiber, determining its elastic modulus. Furthermore, polysaccharides bound to microbeads can be brought into contact with the amyloid fibers and then pulled away with effective gravity. Measuring the interaction forces between polysaccharides and amyloid fibers offers insight into how biofilms utilize this interaction for maintaining structural integrity.

CBE Poster #725

Date: 07/2018
Title: **An optimization approach to light-based 3D printing of vascularized hydrogels**
Authors: **Aaron D. Benjamin**[1,2], Reha Abbasi[1,3], Madison Owens[1,3], Robert J. Olsen[1,3], Danica J. Walsh[1,4], Thomas B. LeFevre[1,3], James N. Wilking[1,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Mechanical & Industrial Engineering,
[3]Department of Chemical & Biological Engineering,
[4]Department of Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA.
Sponsored by: N/A

Hydrogels are soft, water-based gels with widespread applications in personal care products, medicine and biomedical engineering. Many applications require structuring the hydrogel into complex three-dimensional (3D) shapes. For these applications, light-based 3D printing methods offer exquisite control over material structure. However, the use of these methods for structuring hydrogels is underdeveloped. In particular, the ability to print hydrogel objects containing internal voids and channels is limited by the lack of well-characterized formulations that strongly attenuate light and the lack of a theoretical framework for predicting and mitigating channel occlusion. Here we present a combined experimental and theoretical approach for creating well-defined channels with any directional orientation in hydrogels using light-based 3D printing. This is achieved by the incorporation of photoblocker and the optimization of print conditions to ensure layer-layer adhesion while

minimizing channel occlusion. To demonstrate the value of this approach we print hydrogels containing individual spiral channels with centimeter-scale length and submillimeter-scale cross-section. While the channels presented here are relatively simple, this same approach could be used to achieve more complex channel designs mimicking, for example, the complex vasculature of living organisms. The low cytotoxicity of the gel makes the formulation a promising candidate for biological applications.

CBE Poster #726

Date: 10/2017
Title: **Overview of experimental systems and approaches supporting *in situ* mineral precipitation research and development at the Center for Biofilm Engineering-Montana State University**
Authors: **Robin Gerlach**[1,2], Adrienne Phillips[1,3], Al Cunningham[1,3], Randy Hiebert[1,4]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[4]Montana Emergent Technologies, Butte, MT, USA.
Sponsored by: ENIGMA

The Center for Biofilm Engineering (CBE) at Montana State University has a long, successful history of investigating biofilm and mineral precipitation processes in subsurface environments. This poster summarizes many of the experimental approaches the CBE has taken to develop field-suitable technologies.

CBE Poster #727

Date: 07/2018
Title: **Immobilization of the urease enzyme to increase its thermal stability**
Authors: **Zach Frieling**[1,2], Gerlach Robin[1,2], Phillips Adrienne[1,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Department of Energy and the State of Montana

Biomineralization is a very promising technology being developed at the Center for Biofilm Engineering for various engineering applications. Biomineralization is the formation of a precipitate, namely calcium carbonate, induced by microbes that can be used to remediate toxic metals, plug very small pores, seal fissures, and many more. The application of this technology can be sealing of permeable wellbores to prevent further leakage for example in the, storage of supercritical CO_2 and to stop the leaking of methane from wells into the atmosphere. There are several unique ways to induce biomineralization either using bacteria, a plant-based source of urease enzyme, or even through high temperature thermal degradation of urea. The bacteria and plant-based enzyme both have limited use in the subsurface as the temperature increases beyond 70°C they start to degrade. The degradation of the enzyme happens at much lower temperature than the temperature where thermal degradation of urea occurs (100°C). As a result, there is a temperature gap, between 70-100°C in which it is difficult to promote biomineralization via the urea hydrolysis pathway. This technology is often applied in the subsurface where the temperature can rise above the threshold that the bacteria and enzymes work yet sit below where the reaction can occur spontaneously. This places a limitation on how biomineralization may be utilized and therefore creates a new area to be researched. This research focuses on developing methods to overcome the temperature gap and to improve the usefulness of biomineralization for

subsurface conditions where the temperature may be between 70-100°C. This is done by immobilizing enzymes onto surfaces to stabilize the enzyme and aid it in retaining activity at elevated temperatures. Immobilization in this case is placing the bacteria or enzyme in a gel or adsorbing the enzyme to a surface to protect them from thermal degradation. Immobilization can shield the bacteria and enzyme furthering the use of biomineralization so that it can be used at temperatures that normally could not have been reached. This results in a more effective and efficient use of biomineralization that could lead to even more innovative ways in which it is utilized.

CBE Poster #728

Date: 06/2018
Title: **Functional screening of a metagenomic library for the detection and isolation of novel thermostable enzymes from an alkaline hot spring in Yellowstone National Park**
Authors: **Noelani Boise**[1,3], Dana Skorupa[2,3], and Brent Peyton[2,3]
Affiliation: [1]Department of Land Resources & Environmental Sciences,
[2]Thermal Biology Institute,
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: N/A

Though extremophiles are known to be valuable in a multitude of industrial applications, to date few extremophilic Archaea and Bacteria have been successfully cultured in the laboratory. Microorganisms found in geothermal environments contain unique and robust enzymes that perform optimally under extreme levels of pH and temperature. These thermoenzymes are necessary in industrial applications such as plant biomass to fuel conversion systems and industries like paper and pulp manufacturing, where critical biocatalysts must function under extreme operational conditions. In this study, a metagenomic library was constructed using genomic DNA derived from a thermoalkaline hot spring (70°C, pH 8.6) in Yellowstone National Park. Functional screening assays were subsequently initiated to identify desirable thermoenzyme-encoding genes. This technique overcomes known limitations with microbial cultivation by using high-throughput assays to screen thousands of clones for desired functionality. Work involving functional screening assays is ongoing in this study. High-value enzymes of interest currently being screened for include: xylanases, amylases, laccases, pectinases, cellulases, lipases, esterases, and oxidoreductases. Once clones with the desired activities listed above are identified, they will be sub-cloned, expressed, and characterized for their temperature and pH stability, as well as substrate specificity. This functional-based screening method has yet to be employed on a hot spring from Yellowstone National Park, despite the fact that Yellowstone is home to the highest concentration of geothermal features on Earth (>14,000). If successful, work here will increase the number of high-pH adapted thermoenzymes available for use in a variety of biocatalyzed processes operating under harsh environmental conditions.

CBE Poster #729

Date: 07/2018
Title: **Effect of coal rank and ^{13}C algae amendment concentration on microbial methane production**
Authors: **George Platt[1,2,3]**, Katie Davis[1], Elliott Barnhart[1,3], Robin Gerlach[1,2]
Affiliation: [1] Center for Biofilm Engineering,
[2] Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3] United States Geological Survey, Helena, MT, USA.
Sponsored by: Department of Energy (DOE), United States Geological Survey (USGS)

Biogenic coalbed methane (CBM) is produced by the activity of *in situ* microbial communities that catalyze the conversion of coal to methane in shallow, anoxic coalbeds. The research presented here aims to determine the effect of coal rank on microbial methane production using anaerobic enrichments that mimic *in situ* conditions. Coal samples of different rank varying from lignite to low volatile bituminous coal were obtained from the Argonne National Laboratory's Premium Coal Sample Program (Argonne, IL, USA) and the Powder River Basin (near Birney, Rosebud County, MT, USA). Enrichments were prepared using filtered formation water and native microbial communities from the Powder River Basin (PRB). It was determined that coal rank is loosely correlated with microbial methane production, with lower rank coals (subbituminous) generally producing more methane (µmol methane /g coal) than higher ranked coals (Low Volatile Bituminous). It was found that the algae extract amended enrichments containing High Volatile Bituminous coal, Low Volatile Bituminous coal, and subbituminous B coal resulted in statistically different total methane production ($p=0.02$, $p=0.01$, $p=0.05$, respectively) than their unamended analogous. Additional studies were conducted by amending different amounts of coal with concentrations of ^{13}C-labeled algae (*Chlorella sorokiniana*, strain SLA-04) to determine whether an increase in amendment amount correlated with an increase in methane production. Headspace gas composition was monitored using gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). Selected enrichments were destructively sampled for microbial community analysis while others were preserved for future desorption studies. Additionally, it was found that increasing amendment concentration and coal mass did not result in a proportional increase in headspace and aqueous methane production. Desorption studies will be performed to quantify the total methane production.

CBE Poster #730

Date: 06/2018
Title: **Simultaneous water recycle, nutrient reclamation, and high-lipid production for aquaculture**
Authors: **Isaac R. Miller[1,2]**, Wendy M. Sealey[3], and Matthew W. Fields[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
[3]Bozeman Fish Technology Center, United States Fish and Wildlife Service, Bozeman, MT, USA.
Sponsored by: National Science Foundation Graduate Research Fellowship Program (NSF GRFP)

Aquaculture is an important source of protein for human consumption and it is predicted that production will vastly increase over the next decade. Therefore, the viability of aquaculture as a source of protein is heavily reliant on identifying both sustainable freshwater sources and sustainable and cost-

effective ingredients for fish feed production. Aquafeeds are traditionally composed of fish oil harvested from wild fish populations, however, these resources are rapidly dwindling due to overfishing. The incorporation of fish oil into aquafeeds is preferred because it is a concentrated source of essential fatty acids and imparts the final protein product with high levels of heart and brain healthy fatty acids. The rising costs of fish oil due to diminishing natural resources calls for a rapid decrease in the use of fish oil harvested from wild populations as the source of lipids in aquafeeds. Microalgae are increasingly being looked to as an alternative feed source as they are naturally high in DHA and EPA. Other alternative feedstocks, specifically terrestrial plant oils such as soy or flax, contain precursor fatty acids which cannot be efficiently converted to DHA and EPA. An investigation of the response to plant-based feeds reported that there are significant shifts in species richness and diversity in the intestinal fish microbiota when aquafeed changes. Manipulation of the gut microbiome in salmonids affects the production of key enzymes, resulting in altered feed digestibility and growth factors in the host. Ultimately, alternative aquafeeds are successful when the natural microbiota of the fish is minimally altered. Because wild fish derive essential fatty acids directly and indirectly from algae, it is hypothesized that algal diets will be more effective in preserving the microbiome than other plant diets. Contemporary aquaculture practices demand immense freshwater resources which further supports the need to identify alternative sources and production practices for aquafeed generation. Currently, clean water required by aquaculture becomes waste, containing nitrates and phosphates at levels requiring treatment prior to release into freshwater systems. The development of a nutrient recycling system, where algae are cultivated in the effluent waste from the fish culture system, would drastically reduce the environmental costs associated with aquaculture water treatment. Culturing microalgae in wastewater is an innovative way to convert toxic levels of biological waste into biomass for feedstock, and shows promise in maximizing production per volume of water and limiting waste per mass of fish. The overarching hypothesis for this proposed work is that algae, which naturally produce EPA and DHA, can be grown cost-effectively in a nutrient recycling system which will promote higher lipid accumulation and thus better potential for use in fish feed.

CBE Poster #731

Date: 07/2018
Title: **Moving towards continuous evolutionary studies with microfluidics**
Authors: **Humberto S. Sanchez[1,2]**, Zackary Jay[1], Connie B. Chang[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Chemical & Biological Engineering Department, Montana State University, Bozeman, MT, USA.
Sponsored by: Defense Advanced Research Projects Agency

Current microfluidic technologies have enabled researchers to precisely study individual cells. This is achieved by creating discrete aqueous droplets surrounded by oil and stabilized with a surfactant. By tuning the flowrates and cell concentrations the desired amount of cells in said droplets can be achieved as well as loading other solid particles. From these processes the field has expanded so that serial passaging and single cell sequencing can be achieved as well as PCR amplification and even detection and droplet sorting. All of the above operate at frequencies in the kilohertz range making them incredibly high throughput. However these technologies can still be improved as the surfactants required are expensive to purchase and they are mostly operated using syringe pump systems that can be difficult to manipulate and not easily adapted to vessels used in laboratories across the world. From an engineering perspective microfluidic technologies can be improved and scaled up with modular, pressure driven systems, studying how the droplets change in response to pressure and temperature changes, and adapting reagent delivery systems for single cell sensing. All of this can expand the number

of users in the field of microfluidics and potentially expand the number of evolutionary studies being performed so that more discoveries can be made in other fields.

CBE Poster #732

Date: 06/2018
Title: **Potential functional diversity of microbial communities in alkaline hot spring**
Authors: **Rebecca Mueller**[1,2], Dana Skorupa[1,2], Bekah Anderson[1,3], Brent Peyton[1,2,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Thermal Biology Institute,
[3]Deparment of Chemical & Biological Engineering, Montana State University, Bozeman MT, USA.
Sponsored by: Keck Foundation

High temperature, alkaline hot spring ecosystems have been under-studied to date. Due to the high levels of alternate electron acceptors and low levels of organic nutrients, these ecosystems have been shown to host microbial taxa with atypical metabolic pathways, and the extreme abiotic conditions likely select for organisms that produce enzymes that have applications to biotechnology, such as thermostable cellulases. Using high-throughput sequencing techniques, we examined the taxonomic and potential functional diversity of the microbial communities in the Five Sisters, a chain of five hot springs with alkaline pH (8.3-8.6) and high temperatures (71<T<85 °C). Genomic analyses using both targeted sequencing of the 16S rRNA gene and metagenomics showed that these springs were dominated by the Archaea (54% relative abundance), with a high proportion of poorly understood phyla, including the candidate phylum Aigarchaeota. Analysis of putative protein families identified *a priori* as having potential application to biofuel production, including cellulases, laccases and nitrilases, showed that a high diversity of the microbial phyla contained these genes. Lignocellulose decomposition was linked primarily to members of the Bacteria, but a high proportion of nitrilases were classified to the Archaea, suggesting they have strong effects on nitrogen cycling within these systems. We also identified a number of CRISPR protein families, primarily linked to Bacteria, but with a high number classified as Aigarchaeota. Together, these findings indicate that hyperthermic, alkaline hot springs host unique microbial communities, and are reservoirs for products, such as thermostable enzymes, that have potential applications to biotechnology.

CBE Poster #733

Date: 07/2018
Title: **Development of minimum information guidelines and standardized methods for biofilm experiments**
Authors: **Jontana Allkja**[1,2], Darla Goeres[2], Nuno Filipe Azevedo[1]
Affiliation: [1]LEBAPE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Marie Sklodowska-Curie Action—H2020-MSCA-ITN-2016 grant agreement No 722467 (PRINT-AID)

Print-Aid is a European training network initiative for the development of personalized anti-infective medical devices combining printing technologies with antimicrobial functionality. An important part of Print-Aid is the development of minimum information guidelines and standardized methods related to biofilm experiments. Minimum information guidelines instruct researchers and journal reviewers on

the minimum information that should be included in a scientific paper, for the experiments to be independently reproduced. Our guideline focuses on spectrophotometric and fluorometric methods of biofilm assessment and more specifically their use in microtiter plate experiments. Moreover, a ring trial between several laboratories across Europe and the US is being organized, to standardize some of the more common microtiter plate methods of biofilm assessment. More precisely, the evaluation of *Staphylococcus aureus* biofilm prevention by two different antibiotics through crystal violet, resazurin and Colony Forming Unit (CFU) count methods.

CBE Poster #734

Date: 06/2018
Title: **Neutrophil clearance of nascent *Staphylococcus aureus* biofilm**
Authors: **Brian A. Pettygrove[1,2]**, Kyler B. Pallister[2], Jovanka M. Voyich[2], Philip S. Stewart[1,3]
Affiliation: [1]Center for Biofilm Engineering
[2]Department of Microbiology and Immunology,
[3]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana State University Office of Research and Economic Development

S. aureus is a common cause of nosocomial infections on implanted biomaterial surfaces. Mechanisms of immune evasion in the early stages of *S. aureus* biofilm formation are not yet clear. The objective of this work was to determine parameters important in the ability of host defenses to eradicate contaminating microorganisms from a biomaterial surface and thereby prevent establishment of a biofilm-based infection. Clearance of newly attached *S. aureus* bacteria from a serum-coated glass surface by human neutrophils was investigated *in vitro* using time-lapse confocal scanning laser microscopy and quantitative image analysis. In control experiments in which the surface was inoculated with bacteria but no neutrophils were added, attached bacteria grew rapidly (mean specific growth rate 0.80 ± 0.17 h^{-1}) and formed discrete aggregates. When neutrophils were added to the system, they migrated on the surface and discovered, phagocytosed, and killed bacteria. If too few neutrophils were present, some bacteria remained undiscovered and continued to grow into clusters. Initial neutrophil surface densities of approximately 7,000 cells per cm^2 were required for effective surveillance and bacterial destruction. Under these conditions, the log reduction in viable bacteria compared to control wells without neutrophils was 2.11 ± 0.55, with some experiments resulting in non-detectable survivors. To assess the importance of timely neutrophil recruitment, bacteria were given a 4 h head start before adding neutrophils. Bacterial aggregates up to 120 μm^2 in area formed in these experiments and the larger of these aggregates were recalcitrant to neutrophil clearance. Without the head start, a log reduction in viable bacteria of 1.57 ± 0.82 was observed, with several experiments again resulting in non-detection of survivors. When bacteria were given the head start, only a 0.30 ± 0.23 log reduction was observed. The ability of neutrophils to adhere to the surface and exhibit functional mobility was severely impaired when heat-inactivated serum was used, suggesting a critical role for complement proteins in mediating surface-associated neutrophil function. Without proper interaction with the material surface, phagocytosis of bacterial clusters was almost entirely prevented. In control experiments, neutrophils in normal autologous serum were able to reduce bacterial burden by 1.75 ± 0.89 logs. When heat-inactivated serum was utilized, the log reduction in bacteria dramatically decreased to 0.08 ± 0.14, indicating that little killing of *S. aureus* occurred. These results suggest that neutrophils have the potential to eliminate a nascent biofilm, but they must be recruited in sufficient numbers and quickly enough to achieve this outcome.

CBE Poster #735

Date: 07/2018
Title: **Introduction of hand hygiene and disinfection practices to reduce bioburden in suburban athletic training rooms**
Authors: **Lisa Bowersock[1]**, Al Parker[1], Jim Arbogast[2], Chelsea Conley[2], Mark LaBelle[3], John Rapko[4]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]GOJO Inc., Akron, OH, USA.
[3]Case Western/University Hospitals, Cleveland, OH, USA.
[4]Technical Consultant, Saint Louis, MO, USA.
Sponsored by: GOJO Inc.

The science and practice of infection prevention has traditionally been applied in hospitals and other medical facilities where risks of transmission of infectious agents are notoriously high. Despite the fact that high school and collegiate athletes are healthy and fit, transmission of bacterial infections is a known risk to athletes within team facility settings. While it is accepted that proper hand-hygiene and facility cleaning can limit the spread of pathogens, many trainers and athletes do not have adequate practices. By better understanding the environments shared by athletes, small interventions have the potential to yield dramatic improvements in public health. Through instituting new cleaning products and practices in the training rooms of two high schools and two colleges, we hope to better understand how to decrease the bioburden of high-risk surfaces. Various surfaces within the training rooms of two high schools and two colleges in the US were tested for bacterial pathogen risk using multiple technologies: two ATP meters (Hygiena® ATP Meter and Charm® ATP Meter) and conventional spread plating (Aerobic Plate Counts in CFU/g and presence/absence of MRSA, VRE, *S. aureus*, *Enterococcus sp*., *E. coli*, and Coliforms). The surfaces in each of the training facilities were tested in September 2017 to establish a baseline before any intervention. The same surfaces at the same facilities were tested at three subsequent time points during implementation of a graduated intervention plan: in November 2017 after hand hygiene products were given to the employees of the facilities; and in February and May 2018 after further education was given to the training center employees on the use of the cleaning products. Pooled across all the schools and surfaces, there was a statistically significant decrease in bacterial load after the intervention (Hygiena® meter p-value = 0.03, APC p-value=0.01). However, the Charm® ATP measurements and the presence/absence results of the various bacterial species did not show a statistically significant decrease in bioburden after introducing the new products and cleaning practices.

CBE Poster #736

Date: 06/2018
Title: ***In-vitro* testing of bacterial attachment and biofilm formation on different breast implant outer shell surfaces**
Authors: **Garth A. James[1]**, Laura Boegli[1], John Hancock[2], Lisa Bowersock[1], Albert Parker[1], and Brian M. Kinney[3].
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Establishment Labs, Santa Barbara, CA, USA.
[3]University of Southern California, Beverly Hills, CA, USA 90212.
Sponsored by: Establishment Laboratories

Breast augmentation is the most common cosmetic surgical procedure in the United States (300,378 in the US in 2017). This is in addition to breast reconstruction procedures after cancer treatment (106,295). Bacterial biofilms have been implicated in breast implant complications including capsular contracture and the rare cancer, breast implant-associated anaplastic large-cell lymphoma (BIA-ALCL). However, the role of biofilms in these diseases remains unclear. Implants with textured surfaces have become popular due to better maintenance of position and aesthetic properties. Surface texture influences the both the responses of human cells, such as macrophages, and biofilm formation. Textured implants have been shown to decrease capsular contracture with subglandular placement. However, BIA-ALCL has been associated primarily with certain textured implants. In this study, biofilm formation by *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Ralstonia pickettii* were compared for four surface textures relative to a smooth control surface. The textured surfaces were from SmoothSilk®/SilkSurface® (Silk), VelvetSurface® (Velvet), Siltex®, and Biocell® implants, while the smooth surface was from Natrelle® implants. The roughness and surface area of each material was assessed using non-contact profilometry. Surface areas increased with roughness and were similar among the three least rough implants (Smooth, Silk, and Velvet) and among the roughest implants (Siltex and Biocell). Bacterial attachment (2 hours) and biofilm formation (24 hours) were evaluated using a CDC Biofilm Reactor, with analysis by viable plate count (VPC) and confocal scanning laser microscopy (CSLM). Overall, VPC indicated there was significantly more bacterial attachment and biofilm formation on the Siltex and Biocell implants than the Silk or Velvet implants; although there were differences between species and time points. CSLM confirmed more bacterial attachment and the formation of thicker biofilms on the implants with rougher surface textures. Thus, highly-textured breast implant surfaces can harbor greater bacterial loads than implants with smoother surfaces.

CBE Poster #737

Date: 06/2018
Title: **Drop-based microfluidic polymerase chain reaction methods**
Authors: **Geoffrey Zath[1,2]**, Zack Jay[1], Connie Chang[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: DARPA

The power of drop-based microfluidics promises reduced biological assaying times and greater sample throughput than standard laboratory procedures. For example, a common technique such as Polymerase Chain Reaction (PCR) can be adapted to work at the microfluidic scale. Here we present two microfluidic techniques for droplet digital PCR (ddPCR) and drop-based quantitative PCR (qPCR).

Through ddPCR we have quantified the viral copy number of our influenza A virus (IAV) stock without the need for a standard. By encapsulating a dilution series of our stock virus in 65 pL drops and performing PCR within that volume, we can count drops where amplification has occurred (bright) versus those where it has not (dim) on a fluorescent microscope. We then fit the bright/dim drop ratio to a Poisson distribution to determine the average starting copies in each drop. The ddPCR results match closely with our initial estimate of stock virus from a commercial qPCR machine ($1.5x10^9$ versus $1.2x10^9$ copies/mL). While our ddPCR method is a valid technique for the absolute quantification of a bulk sample (e.g. 96 well plate format), we are unable to use it with drop samples (e.g. studying IAV infectivity in drops). For our drop samples, we are developing a drop-based qPCR method for quantifying IAV populations at a single drop resolution. This will allow us to study cell-to-cell heterogeneity of IAV infection dynamics over multiple virus generations that has not been possible prior. We are currently optimizing our drop-based qPCR method to be used with real world samples and have data from experimental controls from our stock IAV to present. In the end, this method will allow for real time fluorescent sample detection at >500 Hz with the ability to multiplex up to three different probes with a separate reference dye channel. The two methods presented here are a sample of techniques and technologies available in our microfluidics lab in the Center for Biofilm Engineering.

CBE Poster #739

Date: 07/2018
Title: **ChickenSplash! Exploring the health concerns of washing raw chicken**
Authors: **Cati Carmody[1,2]**, Benjamin Grodner[1,2], Scott McCalla[3], James Wilking[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT USA.
[2]Department of Chemical & Biological Engineering,
[3]Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA.
Sponsored by: NSF/USP/INBRE

In 2003, the FDA created an advertising campaign warning cooks not to wash raw chicken due to the potential for microbial transmission through splashing liquid. The cooking community responded negatively, and the FDA eventually reversed their recommendation due to lack of scientific evidence. Despite the public controversy, since that time, there have been no significant findings reported in the literature. Our study aims to determine whether or not microorganisms can be transferred in drops and to characterize the conditions of fluid flow that contribute to splashing. To conduct this study, we use a combination of high-speed imaging, image tracking software, and microbiology techniques.

CBE Poster #740

Date: 07/2018
Title: **Engineering human gut tissues in the lab**
Authors: **Barkan Sidar[1,2]**, Andy T. Sebrell[3], Brittany R. Jenkins[3], Jason R. Spence[4,5], Diane Bimczok[3], Seth Walk[3], Henry Fu[6], James N. Wilking[1,2]
Affiliation: [1] Department of Chemical & Biological Engineering,
[2]Center for Biofilm Engineering,
[3]Department of Microbiology and Immunology, Montana State University, Bozeman, MT.
[4]Department of Internal Medicine, Division of Gastroenterology,
[5]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA.
[6]Mechanical Engineering Department, University of Utah, Salt Lake City, UT, USA.
Sponsored by: NSF, NIH, Gates Foundation

Human organoids are three-dimensional, millimeter-scale human tissues that replicate much of the structure and function of naturally formed organs. These tissues have a variety of potential applications in biotechnology, including drug formulation testing, regenerative medicine and microbiome research. Despite their potential applications, knowledge of how growth, material transport and mechanical properties influence organoid structure is lacking. The main goal of my research is to understand and optimize the structure of gastrointestinal organoids to improve their viability and reliability as model systems. To achieve this, I use a combination of time-lapse microscopy, image analysis and modeling to develop an understanding of organoid growth and development. Knowledge gained from this work may provide insight into water transport mechanisms across the epithelial tissue, which are poorly understood.

CBE Poster #741

Date: 04/2018
Title: **Remediation of mine tailings using microbially induced calcite precipitation**
Authors: **Ellen Lauchnor[1,3]**, Whitney Kieffer[1,2], Emily Stoick[1,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Undergraduate Scholars Program

Some of the chief waste products of mining are tailings, consisting of finely pulverized rock after the ore has been extracted. The presence of large tailings piles at abandoned mine sites introduces the potential for metals within the tailings to leach into rainfall and runoff and contaminate surrounding soils and groundwater. A possible solution to this problem is the use of microbially induced calcium precipitation (MICP) to stabilize the mine tailings. MICP makes use of natural bacterial processes to promote the precipitation of calcium carbonate as a mineral seal or protective layer. The objective of this study was to investigate the feasibility of using MICP to promote $CaCO_3$ formation on the surface of tailings piles, thereby stabilizing tailings from erosion and reducing heavy metal leaching. Tailings were collected from the Carpenter-Snow Creek Mining District in Central Montana and treated with additions of solutions containing bacteria, urea and dissolved calcium for a week. In this work, a native bacterial community with ureolytic capability was utilized to induce MICP. Results indicate that re-inoculation of bacterial cultures was necessary to promote MICP in tailings. With additional optimization, this could be a promising technology for preventing erosion and leaching of mine tailings.

CBE Poster #742

Date: 06/2018
Title: **Spatiotemporal profile of calcium carbonate minerals in biofilms**
Authors: **Sobia Anjum,** Robin Gerlach
Affiliation: Department of Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Fulbright and the Department of Energy

Bacterial biofilms are communities of bacterial cells that attach to surfaces and produce extracellular polymeric substances (EPS). EPS act as scaffold that allows cells to attach to surfaces but can also limit the growth of bacteria and their extracellular reactions. The bacteria used in this study are *Escherichia coli* MJK2 and *Sporosarcina pasteurii* ATCC11859, and the reaction of interest is ureolysis. Ureolysis is the hydrolysis (breakdown) of urea resulting in an increase in pH and induction of calcium carbonate precipitation in the presence of dissolved calcium. The overall reaction can be summarized as follows:

$$(NH_2)_2CO + 2H_2O + Ca^{2+} \rightarrow 2(NH_4^+) + CaCO_3(s)$$

This calcium carbonate precipitation is also called biomineralization. Biomineralization has applications in soil stabilization, subsurface barrier establishment, sealing of well bores, concrete remediation and water remediation. The spatiotemporal organization and size of the minerals can play a significant role in the process. Therefore, it might be important to understand the formation of these minerals at the microscale. In this study the process of biomineralization was observed at the microscale by growing *E. coli* strain MJK2 and *S. pasteurii* strain ATCC11859 biofilms in Drip Flow Reactors (DFRs). The biofilms were observed using confocal scanning laser microscopy and Field Emission Scanning Electron Microscopy (FE SEM). The images showed that nucleation and precipitation varies with the type of bacteria used, and the availability of calcium chloride and urea. Maximum attachment was observed in the presence of both, calcium chloride and urea, relative to situations that contained only urea or only calcium chloride. The spatial profile for the developed minerals was observed in 3D using confocal scanning laser microscopy. An understanding of these processes will guide us in improving the biomineralization process for various applications. Future research will characterize the mechanical properties of these biofilms to understand strength and durability in prospective industrial applications.

CBE Poster #743

Date: 06/2018
Title: **Effects of isolated biosurfactant producers on coal biodegradation to methane**
Authors: **Rita Park[1,2],** Matthew Fields[1,2], Adrienne Phillips[1,3], Margaux Meslé[1]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Microbiology & Immunology,
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Undergraduate Scholars Program

Coal dependent methanogenesis (ie. methane (CH_4) production from coal) is a source of energy production that has been receiving increased attention recently. It is evident that the rate-limiting step of this process is the bioavailability of the coal (Barnhart et al., 2016). As biosurfactants are amphipathic molecules mainly produced by microorganisms including bacteria, yeast, and fungi, their possession of both hydrophobic and hydrophilic components allow them to exhibit a variety of surface activities, some of which help to solubilize hydrophobic substrates (Desai and Banat, 1997; Kokare et al., 2007; Kokare,

Chopade, and Mahadik, 2009). In this proposal, the role of biosurfactants in the presence of coal and their impact on the production of methane will be explored. This experiment is part of the Department of Energy (DOE)-funded Microbially Enhanced Coal Bed Methane (MECBM) project, which studies methanogenesis in shallow coal beds of the PRB located in southwestern Montana. The methane produced in these subsurface coal beds is almost exclusively biogenic (Strapoc et al., 2011) and accumulates from the activity of *in situ* microbial populations that are involved in coal degradation and methane formation (Barnhart et al., 2013, 2016). The MECBM project intends to improve *in situ* production of CBM at an economic scale for extraction and use as an energy source for homes. In this proposal, the use of a biosurfactant producing microorganism to increase biodegradation of coal to CH_4 will be explored by observing the effects of three different, unknown biosurfactant producers, in the presence of coal in laboratory cultures.

CBE Poster #744

Date: 07/2018
Title: **Thermal stability of urease produced by *Sporosarcina pasteurii***
Authors: **Arda Akyel[1,2]**, Adrienne Phillips[1,3], and R. Gerlach[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: US Department of Energy

Urease produced by the bacterium *Sporosarcina pasteurii* was tested at a range of temperatures (20 to 80°C) to assess its kinetics and thermal stability. *S. pasteurii* has been shown to be unable to grow at temperatures above approximately 40°C, however it has been demonstrated that the enzyme can still remain active. We observed that the enzyme half-life decreases with increasing temperature while enzyme activity increases. When both enzyme inactivation and activity are considered, 60°C was determined to be the optimal temperature for the ureolysis reaction during a two-hour timeframe, which is deemed relevant for field deployment. We have developed a method to produce urease using *S. pasteurii*, thermally inactivate it and thus make it available for enzyme-induced calcium carbonate mineral precipitation (MICP) technology implementation in the field.

CBE Poster #745

Date: 07/2018
Title: **MICP in the field: enhancement of wellbore cement integrity and permeability modification**
Authors: **Catherine M. Kirkland[1,2]**, Abby Thane[1], Randy Hiebert[1,3], Robert Hyatt[3], Johannes Hommel[4], Jim Kirksey[5], Alfred B. Cunningham[1,2], Robin Gerlach[1,6], Lee Spangler[7], and Adrienne J. Phillips[1,2]
Affiliation: [1] Center for Biofilm Engineering,
[2]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[3]Montana Emergent Technologies, Butte, MT, USA.
[4]Institute for Modelling Hydraulic and Environmental Systems, University of Stuttgart, Germany.
[5]Loudon Technical Services LLC, Charleston, WV, USA.
[6]Department of Chemical & Biological Engineering,
[7]Energy Research Institute, Montana State University, Bozeman, MT, USA.
Sponsored by: US Department of Energy

Microbially-induced calcite precipitation (MICP) is being widely researched as an emerging technology for subsurface engineering applications including sealing defects in wellbore cement and modifying the permeability of rock formations [1]. Our study team, including Montana State University's Energy Research Institute and Center for Biofilm Engineering, the University of Stuttgart, Montana Emergent Technologies, and Loudon Technical Services, recently used the ureolytic bacteria, *Sporosarcina pasteurii*, to promote calcium carbonate precipitation in an existing oil well, Rexing #4, in Indiana. The Rexing #4 well was previously used as an injection well to sweep residual oil to production wells. Several years ago, injection pressure was lost, and the well was removed from service. Subsequent well logging measurements suggested that, rather than entering the target injection formation, injectate was traveling up the wellbore through defects in the well cement to a sandstone thief zone approximately 50 feet above the target formation. The goal of the field demonstration project at the Rexing #4 well was to use MICP to reduce permeability in the thief zone and cement defect to restore injection pressure and return Rexing #4 to service as an injection well. Conventional oil field methods were used to deliver MICP-promoting fluids downhole to the treatment zone approximately 2288 feet below ground surface (bgs), including a slickline dump bailer and injection of water through the tubing string to push the biomineralization fluids into the formation. The injection strategy consisted of one 15 L bailer of microbial culture followed by two bailers of calcium medium. By Day 6 and after 25 inoculum injections and 49 calcium medium injections, the injectivity of the system had decreased by approximately 70%. Injection tests conducted approximately two weeks after the end of the field demonstration yielded injectivity values consistent with those recorded at the end of Day 6.

CBE Poster #746

Date: 06/2018
Title: **Activity partitioning in an archaeal-bacterial co-culture biofilm**
Authors: **Laura Camilleri[1,2]**, B.P. Bowen[4,5], C.J. Petzold[4,5], R. Hatzenpichler[3], T.R. Northen[4,5], and M.W. Fields[1,2]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Microbiology & Immunology,
[3]Department of Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA.
[4]Lawrence Berkeley National Laboratory,
[5]ENIGMA, Berkeley, CA, USA.

Symbiosis is widespread throughout the biosphere with well-studied examples in and across all three domains of life. In communities of bacteria and archaea, mutualism is typically referred to as syntrophy ("eating together") whereby products of one metabolism serve as substrates for another metabolism. The syntrophy between sulfate-reducing bacteria (SRB) and methanogenic archaea is of interest because these guilds both play crucial roles in many different anaerobic environments. In the absence of sulfate as an electron acceptor and the addition of the hydrogenotrophic methanogen, *M. maripaludis*, the two cell types are interdependent via previously proposed product inhibition syntrophy, and cross-feeding of by-products allows a cooperative syntrophic relationship to be established. In monocultures, only *D. vulgaris* Hildenborough readily forms biofilm, and the topography of the biofilm is relatively flat and thin. However, the co-culture biofilm is evenly interspersed with *M. maripaludis*, and is thicker and filled with topographical features such as ridges, spires, and valleys. In order to better understand the interactions between *M. maripaludis* and *D. vulgaris* Hildenborough, deuterium-labeled proteomics and BONCAT microscopy was used delineate activity states of the two biofilm populations. Deuterium-labeled proteins were observed in both populations, and the *D. vulgaris* labeled proteins were enriched in proteins involved in carbon oxidation and electron transfer while the *M. maripaludis* proteins were enriched in carbon dioxide processing and methane generation. Interestingly, BONCAT labeling was observed for both organisms grown as monocultures under sulfate-reducing or hydrogen-utilizing conditions, respectively. However, under co-culture biofilm conditions, only *D. vulgaris* was detected to be BONCAT active, yet methane was being actively produced. In addition, when an active methanogenic co-culture biofilm was perturbed with a constant sulfate influx, the methanogen population not only persisted but increased and remained viable. The data suggest that a mutualistic co-culture biofilm partitions activity to optimize carbon processing and energy conservation.

CBE Poster #747

Date: 06/2018
Title: **Classification and application of biosurfactants produced by polar microbes**
Authors: **Ben Trudgeon[1,2,4,5]**, Markus Dieser[2,3] and Christine M. Foreman[2,3]
Affiliation [1] Department of Civil Engineering,
[2]Center for Biofilm Engineering,
[3]Department of Chemical and Biological Engineering,
[4]Honors College,
[5]Undergraduate Scholars Program, Montana State University, Bozeman, MT, USA.

Surfactants are chemicals that can alter the properties of liquids. They are amphiphilic compounds, meaning they have a water soluble polar group attached to a water insoluble hydrocarbon chain. This allows them to greatly reduce the surface tension of water and other liquids, and aid in the creation of emulsions between two liquids. Surfactants are used commonly in many industries, and their widespread application can pose possible risks to the environment because of their toxicity and inability

to biodegrade. Biosurfactants, however, are surfactants produced by microorganisms that are biodegradable and safe for the environment. The chemical composition of the biosurfactants produced by microbes can vary dramatically between organisms, making some microbes vastly more attractive for certain uses than others. In this project, microbes previously isolated by the Foreman Research Group from polar regions are being tested for their ability to produce different biosurfactants. Since these microbes are naturally found in extremely cold environments, they are more likely to possess the ability to produce biosurfactants that are effective at cold temperatures. After the ideal biosurfactant-producing organisms are identified, the biosurfactants produced by each organism are then screened and classified. Once the biosurfactants have been screened and classified, they can then be tested for possible industry and bioremediation uses. This project focuses more closely on the application of the produced biosurfactants in hydrocarbon remediation.

CBE Poster #748

Date: 07/2018
Title: **Cyanobacteria biofertilizer on the frontier of agricultural sustainability**
Authors: **Hannah Goemann[1,2]**, James Vallie[1,3], Brent Peyton[1,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Microbiology and Immunology,
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: NSF OIA EPSCoR Track II

As global agricultural demands are predicted to increase exponentially in coming years, there is an expected concurrent demand for increased sustainability in agricultural practice and production. Chemical fertilizer production for agriculture is an extremely energy intensive process and is among the greatest sources of greenhouse gas emissions. As such, innovative, alternative fertilization practices are needed to reduce environmental impacts while maintaining agricultural productivity. Biological soil crusts comprised of nitrogen-fixing cyanobacteria have been shown to increase nutrient availability for cropped plants, and improve soil water holding capacity, while also reducing erosion and nutrient runoff. These effects have been shown in recent greenhouse and arid, uncultivated land studies but little is known regarding the viability or productivity of exogenous cyanobacterial biofertilizers in agricultural field settings. In addition, the impact of a living biofertilizer on the resident soil microbial community is relatively unstudied yet vital to the sustainable management of soil microbial ecosystems. Therefore, this study aims to bridge this knowledge gap by coupling biofertilizer treatment to switchgrass crop production and monitoring the soil microbial community through metagenomic analysis and changes in soil physicochemical characteristics.