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Presentation Abstracts

SESSION 1: Fungal Biofilms

A reproducible protocol for growing relevant filamentous fungal biofilms for industrial consumer product applications

Presenter: Julia Kerrigan[1], Associate Professor of Mycology
Co-Authors: Jordon Gruber[2], Charles A. Pettigrew[3], Deborah Mulligan[3], Kevin I.T.Wright[4]
Affiliation: [1]Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, USA.
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Fungal biofilms are ubiquitous in the environment and their detrimental importance continues to be established relative to biodegradation (spoilage, malodor) and potential health impacts (respiratory sensitization, irritation). Standard approaches to their remediation in the built environments generally involve the combined use of cleaning and antifungals to control fungal growth as an initial preventative intervention step, reducing the risks to health resulting from extended exposure. Establishing the efficacy of disinfectants relative to fungi is often based on laboratory protocols utilizing processed fungal cultures (hyphae and spores) which do not reflect the filamentous fungal biofilms present in the built environment. While standardized, these methods exclude recognized biofilm-related factors which may confer resistance to treatments. Here we describe a first stage, reproducible protocol for establishing biofilms on a standardized glass surface under low shear conditions using a drip flow reactor - utilizing a common filamentous fungus, *Aspergillus niger*. By evaluating the progressive phenotypic changes involved in biofilm development, which reflects growth in the natural environment, this laboratory model could be used to evaluate the dynamic efficacy of antifungal actives. A similarity between *A. niger* and models describing the biofilm growth of yeasts and filamentous fungi was observed. There were recognizable phases of development, starting with the early stages of biofilm formation, which involves spore attachment, germination, and hyphal tip growth. Next was the production of interwoven hyphae and extracellular polysaccharides that allows for consolidation of attachment to the glass surface within the liquid environment. The mature biofilm contained a mucoid hyphal matrix and aerial sporogenous cells that produced spores in mass. The development of a repeatable filamentous fungus biofilm growth system, understanding the morphological steps involved in biofilm formation, should enable future studies on how to further prevent and remove them.

Relevance of fungal biofilms: An industrial perspective

Presenter: Tony A. Rook, Sr. Manager, Microbiology Resource Center
Affiliation: The Sherwin-Williams Company, Cleveland, OH, USA.

The prevention of fungal defacement is important to many industrially relevant materials such as paints, plastics, leather, wood, foam, and carpets. Colonization of filamentous fungal contamination of these materials can lead to a depletion of surface aesthetics. When left untreated heavy fungal colonization can lead to biodeterioration of the materials which may lead to product failures. In addition to surface defacement, fungal contamination can impact the integrity of the chemicals, such as the bulk storage of surfactants or biofuels. Historically, fungal defacement of industrially relevant surfaces has been studied using traditional microbiology methods. A review of the current test methods used for assessing strategies to prevent fungal contamination of industrially relevant surfaces will be presented. The relevance of studying filamentous fungal defacement and/or biodeterioration of industrially relevant materials as a biofilm development event will be presented, while reviewing how biofilm methods may be adapted to improve the study of these phenomenon.

A review of lab protocols for fungal biofilm studies and our path forward

Presenter: Diane K. Walker, Research Engineer
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

A literature review was conducted to identify laboratory methods that researchers are using to study fungal biofilms. While much focus has been on bacterial biofilms, the impact of fungal biofilms in industry, as well as in human health, has led to greater interest in these under-studied members of the micro-community.

It is not always easy to find the right balance between field relevancy and practicality when developing laboratory methods. Fungal biofilms can vary greatly depending on the conditions under which they are grown (i.e., types of nutrients, material/substrate, duration, temperature, etc.). Studies can be especially challenging when the objective is to determine a product's antimicrobial efficacy and to have confidence that a promising product demonstrates the same effectiveness when in actual use.

In an effort to address a number of these issues, the goal of this talk is to provide an overview of currently used methods with respect to their ability to:

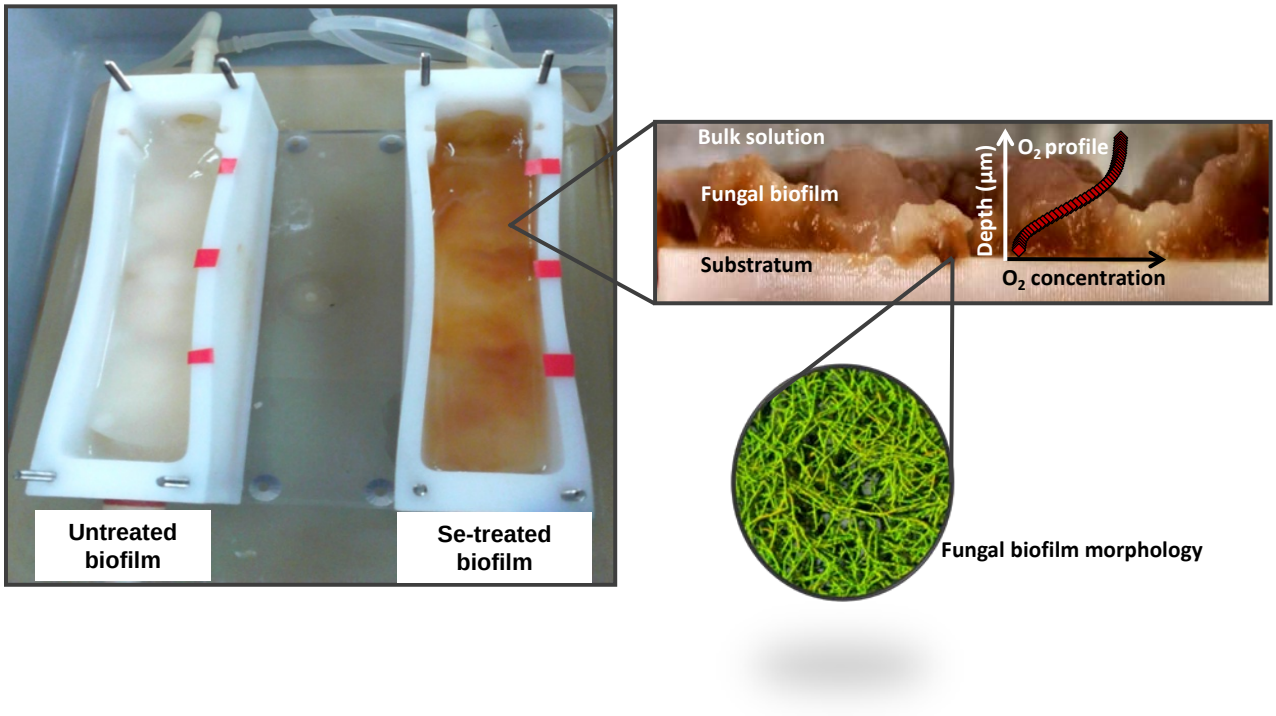
- Grow a reproducible interspecies fungal/bacterial biofilm
- Establish a timeline of biofilm formation for treatment application
- Conduct a combination approach to analysis (i.e., weight, dyes, assays, microscopy, etc.)

This presentation will also propose approaches for growth and analyses of fungal biofilms that, with the input of our Industrial Associates, can initiate further discussion on a path forward for the development of fungal biofilm methods.

Effect of selenite on the morphology and respiratory activity of *Phanerochaete chrysosporium* biofilms

Presenter: Erika Espinosa-Ortiz, Postdoctoral Research Associate
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The temporal and spatial effects of selenite (SeO_3^{2-}) on the physical properties and respiratory activity of *Phanerochaete chrysosporium* biofilms grown in flow-cell reactors, were investigated using oxygen microsensors and confocal laser scanning microscopy (CLSM) imaging. A 25% reduction of the O_2 flux was observed in the fungal biofilm after being exposed to a SeO_3^{2-} load of 1.67 mg Se L^{-1} h^{-1}, for 24 h. Moreover, compaction and densification of the hyphal arrangement was observed in the biofilm as a result of long-term exposure (4 days) to SeO_3^{2-}, resulting in thinner biofilms compared to biofilms grown in the absence of SeO_3^{2-}. To the best of our knowledge, this is the first time that the effect of SeO_3^{2-} on the aerobic respiratory activity of fungal biofilms is described.



Citation: Espinosa-Ortiz, E.J., Pechaud, Y., Lauchnor, E., Rene, E.R., Gerlach, R., Peyton, B.M., van Hullebusch, E.D., Lens, P.N.L. (2016). Effect of selenite on the morphology and respiratory activity of *Phanerochaete chrysosporium* biofilms. *Bioresource* Technology. 210:138-145. DOI: j.biortech.2016.02.074

Volatile organic compounds of a filamentous fungal mat at varying oxygen conditions

Presenter: Heidi R. Schoen[1,2], PhD candidate
Co-authors: W. Berk Knighton[3], Brent M. Peyton[1,2]
Affiliations: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering, and
[3]Department of Chemistry & Biochemistry, Montana State University,
Bozeman, MT, USA.

Filamentous fungal mats produce numerous volatile organic compounds (VOCs). VOCs are often metabolites, which provide information on the metabolic state on the mat. Many fungal VOCs are bioactive and act to inhibit bacteria, other fungi, insects and herbivores, as well as being target aroma and biofuel compounds. Production of fungal VOCs may be related to primary metabolism, such as fermentative products, or secondary metabolites. Secondary metabolites are not directly required for cellular growth (e.g. antibiotics), and many bioactive VOC secondary metabolites are not produced by fungi under standard culturing conditions. This work explores the VOC production of a filamentous fungal endophyte, Nodulisporium isolate TI-13, known to produce volatile primary metabolites, like ethanol and acetoin, and secondary metabolites, like benzaldehyde, cineole, 3-methyl-1-butanol, and phenylethyl alcohol, which are all aroma and biofuel target compounds. The fungus was grown as a mat in a solid state reactor with the agricultural byproduct beet pulp as the substrate. Four oxygen conditions were evaluated for induction of secondary metabolite production. VOC production was monitoring using proton transfer reaction-mass spectrometry, which is a soft ionization method used for quantifying gas phase volatiles. Production of primary metabolites, especially ethanol, and secondary metabolites was significantly modulated between oxygen conditions. The predominant VOCs produced varied based on oxygen condition, with the highest concentrations of most VOCs being produced under anoxic (10/21) or microaerophilic (8/21) conditions. Microaerophilic conditions induced the largest concentration increases in secondary metabolites produced by the fungal mat.

***Candida* and *Malassezia* yeasts in biofilms**

Presenter: Garth James, Associate Research Professor, Chemical & Biological Engineering
Co-authors: Kelly Kirker, Steve Fisher, Laura Boegli, Makayla Eickelberg, Erika Avera, and Elinor deLancey Pulcini
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Candida and *Malassezia* are yeast genera that are part of the normal human microbiome, but can also be opportunistic pathogens. *Candida albicans* can cause oral, vaginal, and blood stream infections, particularly in immunocompromised individuals. In addition, *C. albicans* forms biofilms on contact lenses and cases, dentures, and medical devices such as central venous catheters. Infections associated with these biofilms cause considerable morbidity and mortality. *Malassezia furfur* is a lipophilic yeast, which inhabits sebaceous areas of human skin. *M. furfur* is associated with dandruff and may also cause or exacerbate skin diseases, including tinea versicolor and seborrheic dermatitis. We have grown *C. albicans* biofilms on hydrogel contact lenses using a modified drip-flow biofilm reactor. These biofilms contained 4.3-5.7 log CFU/lens (mean 5.7 log CFU/lens) and the hyphal morphotype of *C. albicans* predominated in these biofilms. We have also grown mixed kingdom biofilms containing bacteria and fungi from saliva in a CDC biofilm reactor equipped with Lucitone (denture base) or hydroxyapatite coupons. These biofilms contained a variety of fungal species including ascomycetes and several species of *Candida*, including *C. albicans*. Fungi of the yeast morphotype predominated in these biofilms. *M. furfur* was grown as single-species biofilms or in co-culture with the lipophilic bacterium, *Propionibacterium acnes*, to represent common microorganisms in sebaceous areas of human skin. These biofilms were grown in a modified colony drip-flow biofilm reactor on acellular dermal matrix material. A most probable number technique was developed for the enumeration of *M. furfur*. This model was used to evaluate common topical treatments for acne vulgaris. Overall, these studies indicate that yeasts of medical importance can readily be grown as single-species biofilms or in mixed-kingdom biofilms with bacteria.

SESSION 2: Industrial Biofilms

Copper: An effective anti-microbial?

Presenter: Colin Anderson, R&D Director
Co-authors: Neal Blossom, Director of Global Environmental and Regulatory Affairs
Affiliation: American Chemet Corporation, East Helena, MT, USA.

Copper as a metal has been used for eons to control unwanted organisms that negatively impact a wide variety of human activities. For more than two centuries it has played a major role in the prevention of fouling on the underwater hulls of commercial shipping and pleasure craft, having originally given rise to the term "copper bottomed" when it was used on wooden ships in the 19th century. It is currently used globally to control certain diseases that damage agricultural crops, and it is being increasingly recognized as an antimicrobial agent that can help prevent the spread of infections in hospitals. However, its use in medical facilities has been limited due to the high cost of installation. This presentation provides data showing that there are effective, but lower cost, copper-containing materials available, that could assist in the battle against unwanted microorganisms if made more widely available.

Integrated molecular, physiological and in silico characterization of two extremophilic *Halomonas* isolates

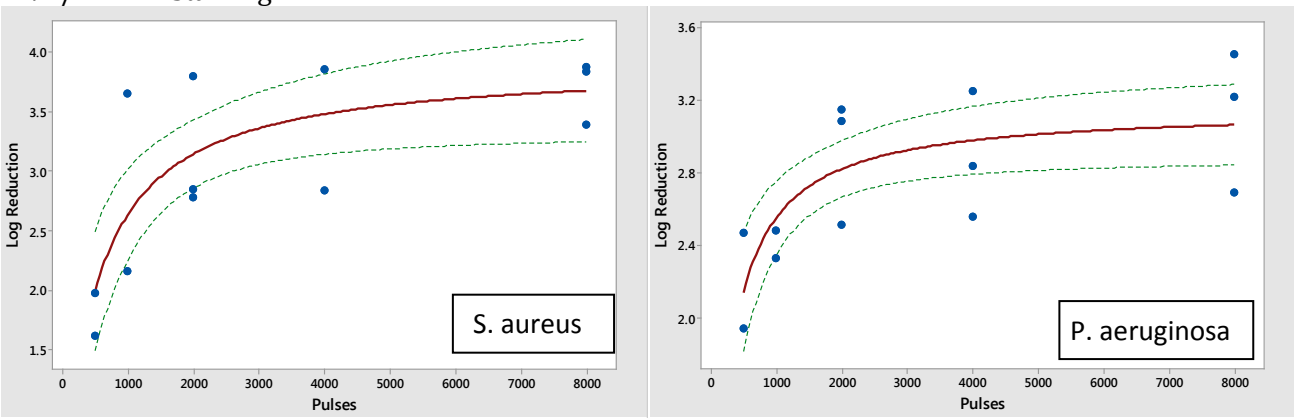
Presenter: Ross Carlson[1,2], Professor
Co-authors: Chuck Pettigrew[3], Principal Scientist
Affiliation: [1]Department of Chemical & Biological Engineering, and
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Procter & Gamble Company, Mason, OH, USA.

A collaborative, industry-CBE research team studied two haloalkaliphilic bacteria via molecular, physiological, and in silico metabolic pathway analyses. Genomes from the organisms, designated *Halomonas* BC1 and BC2, were sequenced; 16S ribosomal subunit-based phylogenetic analysis revealed a high level of similarity to each other and to *Halomonas meridiana*. Both strains were moderate halophiles with near optimal specific growth rates (≥60% max) observed over <0.1 to 5% (w/v) NaCl and pH ranging from 7.4 to 10.2. Isolate BC1 was further characterized by measuring uptake or synthesis of compatible solutes under different growth conditions; in complex medium, external glycine betaine was accumulated while ectoine was synthesized in salts medium. Transcriptome analysis of isolate BC1 grown on glucose or citrate medium measured differences in glycolysis- and gluconeogenesis-based metabolisms, respectively. The annotated BC1 genome was used to build an in silico, genome-scale stoichiometric metabolic model to study catabolic energy strategies and compatible solute synthesis under gradients of oxygen and nutrient availability. The theoretical analysis identified energy metabolism challenges associated with acclimation to high salinity and high pH. This collaborative industry-academic study documents central metabolism data for the industrially and scientifically important haloalkaliphile genus *Halomonas*, providing a knowledge base for research of applied relevance.

Acoustic pressure shock wave technology successfully disrupts medical and non-medical 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., Alpharetta, GA, USA.
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Acoustic Pressure Shock Wave Technology (APSWT) was evaluated for in vitro effects on medical biofilms formed by Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria and also for removal of biofilms comprised of marine microorganisms. Biofilms were grown on polycarbonate coupons in a CDC Biofilm Reactor using methods similar to ASTM E2562-12. Coupons were exposed to 500 to 8,000 pulses, at 4 shocks/second and at the highest energy setting (E6) for the PACE Control Console (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*, 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*, a 2 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 marine microorganisms a 4 log reduction was achieved with 1000 pulses, a 3.9 log reduction with 2000 pulses and a maximum 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 shock wave device 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 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 3: Biofilm Methods

Methods to assess biofilm prevention on surface modified urinary catheters

Presenter: Darla Goeres, Associate Research Professor, Chemical & Biological Engineering
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

In the battle to reduce medical device and implant related infections, prevention of bacterial colonization of surfaces is a logical strategy. Bacterial colonization of a surface is a precursor to biofilm formation. If bacteria are not able to attach to a surface, a biofilm cannot form. Biofilm is the etiological agent of many implant and device related infections and once established, microorganisms in biofilm can be up to a 1000 times more tolerant to antibiotic therapy. Often the best treatment strategy is removal of the implant or device at a high socioeconomic cost to the patient.

Health-care associated infections (HAIs) are a major burden due to associated morbidity, mortality, and costs and have been identified as a priority of the Department of Health and Human Services. Catheter associated urinary tract infections (CAUTI) are the most prevalent of the device-related HAIs and the prevalence of CAUTI has not improved despite scientific advancements in the understating of UTI and a variety of potential improvements for urinary catheter technology. An estimated 12-16% of patients will receive a urinary catheter during hospitalization and approximately 50% of patients catheterized for more than one week will develop a urinary tract infection. There is currently a glaring lack of reliable *in vitro* standard test methods for evaluating the efficacy of anti-biofilm technologies that could be used to prevent CAUTI and other device-related infections.

The Burroughs Wellcome Foundation recently awarded the CBE a five year grant to develop and validate a quantitative *in vitro* method that will enable regulators to efficiently evaluate the efficacy of surface modified urinary catheters. The grant's aims are:

1) Identify key parameters to include in the in vitro method.
2) Develop a quantitative method that simulates relevant use conditions.
3) Optimize the method using ruggedness testing.
4) Validate in an inter-laboratory study.
5) Submit the validated method for acceptance as an approved ASTM standard method and to the FDA's Medical Device Development Tools (MDDT) program.
6) Correlate the standard method test results with existing clinical trial data for two different products.

This presentation will report on the first step in the methods development process, which is to identify the most influential parameters to incorporate in an *in vitro* method designed to assess surface modified urinary catheters. This list will then be used to assess the catheter methods most commonly used by researchers in the published literature.

EPA's Regulatory Update: Use of the Single Tube Method to support biofilm claims for antimicrobial products

Presenter: Rebecca Pines, Biologist
Co-authors: Stephen Tomasino, Ph.D., Senior Scientist
Affiliation: U.S. Environmental Protection Agency, Microbiology Laboratory Branch, Ft. Meade, MD, USA.

To support the registration of an antimicrobial product with a public health claim, registrants are required to submit efficacy data to the United States Environmental Protection Agency (EPA) using standard laboratory methods. EPA is planning to propose the use of the Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilm to add a biofilm claim to a registered antimicrobial product with an existing hospital disinfectant claim.

Biofilms are grown in the CDC biofilm reactor on borosilicate glass coupons. The Single Tube Method utilizes these biofilm-laden coupons to evaluate the efficacy of an aqueous antimicrobial product, resulting in the generation of a log reduction value (the quantitative difference between the number of viable bacteria recovered from control coupons compared to treated coupons).

The standard operating procedures (SOPs) used by EPA's Microbiology Laboratory Branch (MLB) for growing biofilm in the CDC reactor and for evaluating disinfectant efficacy using the Single Tube Method are adapted from ASTM standard methods E2562-12 and E2871-13, respectively. These SOPs have been evaluated extensively over the course of four collaborative studies since 2014 – three for *Pseudomonas aeruginosa* and one for *Staphylococcus aureus*. The study conducted in 2014 on *P. aeruginosa* yielded unexpected levels of variability in log reduction values for the high efficacy treatments. Discussions with collaborators revealed increased variability resulting from inadvertent contact and splashing of the coupon-associated inoculum onto the inner walls of the reaction tube during coupon deposition. This discovery led to the development of a splashguard in early 2015 and its subsequent verification by collaborators later that year. With this improvement now in place, EPA launched its final method performance study with *P. aeruginosa*, which included the splashguard, in late 2015. The results from the 2015 method performance study inform the performance standard outlined in EPA's upcoming "Interim Guidance to Assess the Efficacy of Antimicrobial Pesticide Products Intended to Control Public Health Biofilms."

The guidance document includes proposals for a maximum contact time, required mean log density of bacteria in biofilm per coupon, required number of tests, a product performance standard, and labeling guidance. An update will be provided on the status of the guidance document.

In order to communicate the proposed process for registering antimicrobial products with biofilm claims, EPA is planning to provide the procedures for growing *P. aeruginosa* or *S. aureus* biofilms and for evaluating them against antimicrobial products, the proposed regulatory guidance, and relevant collaborative study materials on a docket for public review. After comments are collected, EPA plans to finalize the procedures and provide the revised methods for use.

Using statistical confidence and power to assess performance standards for biofilm claims using the Single Tube Method

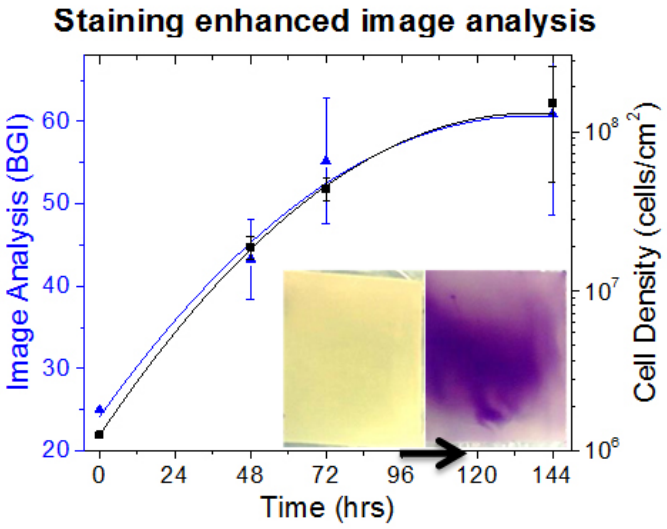
Presenter: Al Parker[1, 2], Assistant Research Professor, Bio-statistician
Affiliation: [1]Department of Mathematical Sciences, and
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

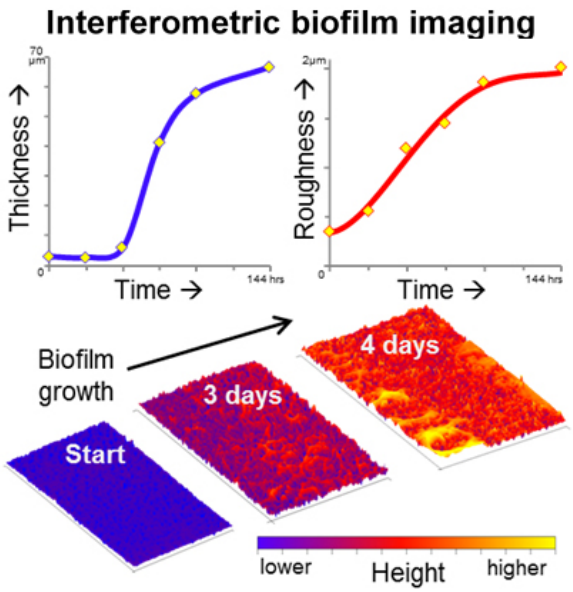
A performance standard (PS) for an antimicrobial test method defines an acceptable outcome for an antimicrobial product being tested. The PS also specifies the number of tests that must be performed, the number of laboratories required to conduct the tests, and the test microbes used. The specifications set by the PS can be evaluated by two desirable and quantifiable statistical characteristics: (1) the confidence level of the PS, which is the percentage of ineffective products that the PS correctly fails; and (2) the power of the PS, which is the percentage of excellent products with high efficacy that the PS correctly passes. In this talk, this statistical approach is applied to assess PS's for anti-biofilm products using the single tube method. The single tube method may eventually be used by EPA to register products with anti-biofilm claims.

Quantifying biofilm development and structure with image analysis and high resolution 3D imaging

Presenter: Curtis Larimer, Research Scientist
Co-authors: Eric Winder, Jon Suter, Matt Prowant, Michelle Brann, Robert Jeters, Jiyeon Park, Shane Addleman, George Bonheyo
Affiliation: Pacific Northwest National Laboratory, Richland, WA, USA.

The soft structure and water-like bulk properties of hydrated biofilms make them difficult to characterize by any means, especially in a non-destructive manner. We have developed two quantitative assays for biofilms and biofouling—one for quick field assessment and one for high resolution lab-based research. For the field assay we created a biomolecular stain mixture intended to highlight early development of biofilms so quantitative information can be captured with ordinary macro-scale photographs. A novel image analysis algorithm was developed to objectively and quantitatively measure biofilm accumulation from digital images of the stained surfaces, and results compared favorably with independent measurements of total organic carbon and cell counts from each sample. This simple method facilitates assessment of spatial heterogeneity of a biofilm across a surface and can be scaled easily from small test coupons to large areas. We also developed a new method for measuring and monitoring the thickness and topology of live biofilms using white light interferometric microscopy. This imaging method provides large area (~3-5 mm) 3D topological maps of biofilm surfaces with vertical resolution of just 3 nanometers. A microfluidic flow cell was designed to support dynamic imaging and quantitative surface metrology. Applications of both methods in biomedical, industrial, and life science research will be discussed.





Osmotic pressure-induced rupturing of gastrointestinal organoids

Presenter: James Wilking[1, 2], Assistant Professor
Affiliation: [1]Department of Chemical & Biological Engineering, and
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

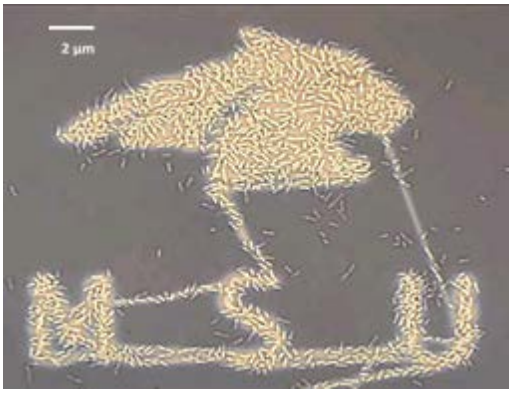
Organoids are millimeter-scale tissues that replicate the structure and function of naturally formed organs. They are grown in the lab from tissue biopsies or the directed differentiation of stem cells. Gastrointestinal organoids serve as a model system for the gut and offer potential in microbiome research, regenerative medicine, and drug formulation testing. Gastrointestinal organoids are roughly spherical in shape, with an inner volume of aqueous liquid enclosed by a tissue shell. While this structure appears to be adequate for short-term experiments, it is not clear how the lack of luminal transport impacts organoid viability. Here, we describe the use of time-lapse microscopy to follow human gastrointestinal organoid dynamics. Strikingly,

we observe that gastrointestinal organoids periodically rupture. We demonstrate that this popping behavior is due to the buildup of an internal osmotic pressure and show that the addition of appropriate compounds to the media may be used to reduce the frequency of rupture events and extend the time available for organoid experiments.

Montana Nanotechnology Facility: A powerful resource for biofilm science and engineering

Presenter: David Dickensheets[1,2], Professor and Director
Affiliation: [1]Department of Electrical & Computer Engineering and
[2]Montana Nanotechnology Facility, Montana State University, Bozeman, MT, USA.

By engineering structures on the nano or micro scale, we can more intimately control the interaction between biofilms and the surfaces they inhabit. From simple nanotexturing to control hydrophobicity, to the patterned growth of activated binding sites for single bacteria and small population studies, to the development of full microfluidic incubators or other flow systems, nanofabrication can offer powerful new tools for biofilm engineering as well as basic science. This talk will highlight some of the nanofabrication and characterization capabilities available within the Montana Nanotechnology Facility, and show some examples of microfluidic devices and other structures for biofilm research that have been fabricated here.



Microbacteria Bobcat, created after patterning substrate using dip-pen nanolithography.

SESSION 4: Multi-Species Biofilms

The ecology of nitrification in water systems: A consortium of organisms and metabolisms

Presenter: Anne Camper[1,2,3], Regents Professor and Associate Dean
Affiliation: [1]Department of Civil Engineering,
[2]Center for Biofilm Engineering, and
[3]College of Engineering, Montana State University, Bozeman, MT, USA.

Chloramine use in drinking water has become an industry practice to reduce the formation of chlorinated disinfection by-products. However, the ammonia introduced by the decay of chloramine can lead to nitrification which results in the production of nitrite and nitrate, and may also contribute to corrosion problems in premise plumbing. To address these issues, research was conducted using simulated copper and plastic premise plumbing systems with periods of flow and stagnation and fed varying levels of ammonia and organic carbon as humic substances.

Initial work demonstrated that indigenous organisms established nitrifying conditions after a period of 6 months. Control of nitrification was attempted through changes in added copper, chlorite, and chloramine levels. Nitrification was remarkably robust throughout these changes. Statistical analyses of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) profiles determined that the active bacterial populations were different in the two systems. The assemblage of the organisms was also different from that of the influent population, suggesting that material and ammonia/organic carbon concentration affect the population. No known ammonia oxidizing bacteria were detected suggesting the role of different group for ammonia oxidation.

Fluorescence *in situ* hybridization (FISH) detected archaea in the biofilm from both reactors. Archaeal 16S rRNA gene sequences were found to be phylogenetically affiliated with known archaeal ammonia oxidizers. Two archaeal *amo*A sequences were amplified from the system as determined by DGGE. Bacterial abundances were comparable in the two systems but archaeal abundances were higher in the PVC reactor suggesting material effect on the overall microbial population composition and density. Subsequent work investigated the kinetics of ammonia oxidation of this mixed microbial community. A first order reaction rate higher than what is typically expected for other nitrifying communities in oligotrophic environments was obtained.

Acknowledgment: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Forced cooperation leads to improved productivity in a multispecies biofilm

Presenter: Laura Camilleri [1,2,4], PhD Candidate
Co-authors: J. Kuehl[3,4], A. Mazurie[2], B. Bowen[3,4], C. Petzold[3,4], A. Deutschbauer[3,4], T. Northen[3,4], and M. W. Fields[1,2,4]
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[3] Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
[4] ENIGMA (http://enigma.lbl.gov/)

In the absence of sulfate as an electron acceptor for *Desulfovibrio vulgaris* Hildenborough and the addition of the hydrogenotrophic methanogen, *Methanococcus maripaludis*, the two cell types become interdependent via previously proposed product inhibition syntrophy, and crossfeeding of by-products allows a cooperative syntrophic relationship to be established. We have recently demonstrated that *M. maripaludis* exhibits taxis toward hydrogen, or hydrogenotaxis, as well as showing that biofilm helps optimize the carrying capacity of the two populations. In order to better understand the interactions between *M. maripaludis* and *D. vulgaris* Hildenborough, RNA-Seq and deuterium-labeled proteomics was used to characterize the coculture biofilm as compared to the planktonic mono- and co- culture states. Our results suggest that key steps in methanogenesis are down-expressed for *M. maripaludis* and electron transfer related genes are down-expressed for *D. vulgaris* Hildenborough. Many of the up-expressed genes include hypothetical proteins but also include cell surface modifications, communication via small metabolites, N-cycling, and metal homeostasis. This is in direct contradiction with results published for work done with similar coculture systems in the planktonic growth mode, and the results suggest mechanisms that enable the biofilm to increase productivity as well as increase carrying-capacity via resource sharing in a methanogenic biofilm.

Acknowledgment: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research US Dept of Energy Contract No: DE-AC02-05CH11231

Biofilms enhance survival in extreme environments

Presenter: Heidi J. Smith[1, 2], Postdoctoral Research Associate
Co-authors: Amber Schmit[1,3], Rachel Foster[4,5], Sten Littmann[5], Marcel Kuypers[5], Christine Foreman[1,3]
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[2] Land Resources & Environmental Sciences, and
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Bremen, Germany.

Organisms found in Antarctica have been shown to possess a variety of mechanisms to persist under low temperatures, desiccation, freeze thaw events, and UV radiation. A less explored stress response in cold temperature organisms is biofilm formation; the formation of biofilm in other organisms from a wide range of environments has shown that biofilm is formed in response to imposed environmental stressors. In this study we show via confocal laser scanning microscopy that microbial communities on glacial surfaces in Antarctica persist in biofilms. Overall, ~35% of the cryoconite sediment surfaces were covered by biofilm. Nanoscale secondary ion mass spectrometry measured significant enrichment of ^{13}C and ^{15}N above background in both *Bacteroidetes* and filamentous cyanobacteria (i.e., *Oscillatoria*) when incubated in the presence of ^{13}C-NaHCO3 and $^{15}NH_4$. This transfer of newly synthesized organic compounds was dependent on the distance of heterotrophic *Bacteroidetes* from filamentous *Oscillatoria*. We conclude that the spatial organization within these biofilms promotes efficient transfer and cycling of nutrients. Further, these results support the hypothesis that biofilm formation leads to the accumulation of organic matter on cryoconite minerals, which could influence the surface albedo of glaciers.

Effects of chlorhexidine treatments on single and mixed species biofilms of *Streptococcus mutans* and *Lactobacillus acidophilus* as well as *S. mutans* and *Actinomyces naeslundii*

Presenter: Rosa Oliveira[1], Postdoctoral Researcher
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[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The ideal methodology to test in vitro biofilm antimicrobial susceptibility is to use a model that simulates the in vivo environment. A drip flow reactor (DFR) is a good choice to mimic the oral environment because of the continuous low fluid shear that simulates salivary flow and clearance. However, the use of this reactor has not been validated using oral bacteria and an antimicrobial agent. The primary aim of this study was to validate the use of the DFR to form biofilms involving oral microorganisms. The secondary aim was to use the DFR to evaluate the growth and chlorhexidine susceptibility of biofilms composed of 1) *Streptococcus mutans* ATCC 25175 and *Lactobacillus acidophilus* ATCC 4356 2) *S. mutans* ATCC 25175 and *Actinomyces naeslundii* ATCC 12104. Biofilms grew on hydroxyapatite coated glass slides, with BHI broth at 10 mL/h flow rate supplemented with 0.2 or 0.5% sucrose depending on the species used. The DFR was incubated for 24 h at 37 °C/5% CO_2. Biofilms were treated with 0.2% chlorhexidine (CHX) or 0.9% NaCl for 2 min. Bacterial viability was determined by agar culture method and by confocal laser scanning microscopy (CLSM) using the Live/Dead Viability kit. DFR validation was analyzed by the unpaired t test (α=0.05). Interaction between bacterial species was analyzed by two-way ANOVA and Tukey tests (α=0.05). Chlorhexidine treatment affected both biofilms at the same proportion despite distinct initial concentrations of *S. mutans* mono-cultures. No interaction between the two factors studied (treatment solution and culture condition) was found in *S. mutans* and *L. acidophilus* biofilms. However, viability was significantly reduced by CHX treatment. *L. acidophilus* in mono-cultures grew significantly less than *S. mutans* cultures. On the other hand, an

interaction between the two factors studied was found for *S. mutans* and *A. naeslundii* mixed cultures. *S. mutans* in mixed cultures with *A. naeslundii* showed an increased resistance to CHX. The present study showed the applicability of DFR for growing oral biofilms and testing antimicrobial agents. Significant interactions were found between *S. mutans* and *A. naeslundii* but not between *S. mutans* and *L. acidophilus*.

SESSION 5: Wound Biofilms

Biofilm-related oxygen consumption in wounds

Presenter: Garth James[1] Associate Research Professor, Chemical & Biological Engineering
Co-authors: Alice Ge Zhao[2], Marcia Usui[2], Robert A Underwood[2], Hung Nguyen[3], Haluk Beyenal[3], Elinor deLancey Pulcini[1], Alessandra Agostinho Hunt[4], Hans C Bernstein[5], Philip Fleckman[2], John Olerud[2], Kerry S. Williamson[1], Michael J. Franklin[1], and Philip S. Stewart[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Division of Dermatology, Department of Medicine, University of Washington, Seattle, WA, USA.
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Biofilms have been implicated in delayed wound healing, although the mechanisms by which biofilms impair wound healing are poorly understood. Many species of bacteria produce exotoxins and exoenzymes that may inhibit healing. In addition, oxygen consumption by biofilms and by the responding leukocytes, may impede wound healing by depleting the oxygen that is required for healing. In this study, we used oxygen microsensors to measure oxygen transects through *in vitro*-cultured biofilms, biofilms formed *in vivo* within scabs from a diabetic (db/db) mouse wound model, and *ex vivo* human chronic wound specimens. The results show that oxygen levels within mouse scabs had steep gradients that reached minima ranging from 17-72 mmHg on live mice and 6.4-1.1 mmHg on euthanized mice. The oxygen gradients in the mouse scabs were similar to those observed for clinical isolates cultured *in vitro* and for human *ex vivo* specimens. To characterize the metabolic activities of the bacteria in the mouse scabs, we performed transcriptomics analyses of *Pseudomonas aeruginosa* biofilms associated with the db/db mice wounds. The results demonstrated that the bacteria expressed genes for metabolic activities associated with cell growth. Interestingly, the transcriptome results also indicated that the bacteria within the wounds experienced oxygen-limitation stress. Among the bacterial genes that were expressed *in vivo* were genes associated with the Anr-mediated hypoxia-stress response. Other bacterial stress response genes highly expressed *in vivo* were genes associated with stationary-phase growth, osmotic stress, and RpoH-mediated heat shock stress. Overall, the results support the hypothesis that bacterial biofilms in chronic wounds promote chronicity by contributing to the maintenance of localized low oxygen tensions, through their metabolic activities and through their recruitment of cells that consume oxygen for host defensive processes.

Chronic wounds in diabetic patients: Biochemical association between skin microbiome and metabolic landscape

Presenter: Mary Cloud B. Ammons, Assistant Research Professor
Affiliation: Department of Chemistry & Biochemistry, Montana State University, Bozeman, MT, USA.

Treatment of chronic, diabetic ulcers is a major socioeconomic burden with an estimated $58 billion annually in medical costs. Chronic, non-healing wounds contribute significantly to the suffering of diabetic patients with mild to severely compromised immune systems and result in nearly a quarter of diabetics enduring at least one limb amputation within their lifetime. The American Diabetes Association estimates that by 2050 there will be over 350 million diabetics in the United States correlating to nearly 80 million diabetics with at least a single limb amputation as the result of a non-healing wound**.** In diabetic ulcers, the normal wound healing process becomes dysregulated and stalls at the inflammatory stage. Colonizing bacteria biofilms are a major contributor to delayed healing; however how the metabolic phenotype of the colonizing biofilm influences the wound healing process remains unknown. This gap in knowledge inhibits the development of effective treatment protocols, resulting in amputation being the current standard of care.

Our research indicates that small molecule metabolite profiles are strongly associated with microbiome profiles within the chronic wound environment, indicating that the diabetic ulcer landscape is shaped by the metabolic activity of the colonizing bacteria. In addition, recent findings have demonstrated that bacterial metabolites can regulate inflammation, suggesting that, in chronic ulcers, small molecule metabolites derived from the colonizing bacterial biofilm may contribute to the dysregulation of the healing process, stalling the wound at the inflammatory stage. To address the critical need for developing evidence-based treatment protocols, we seek to **develop a statistical algorithm of diagnostic and prognostic indicators that can be incorporated into a treatment management plan executed directly at the bedside by nursing staff**.

This research project utilized a longitudinal, systems biology approach to better understand the biological factors that contribute to the development, progression, and resolution of chronic diabetic ulcers using debridement samples collected from pre-diabetic and diabetic patients presenting at the Wound and Hyperbaric Clinic at Bozeman Deaconess Hospital. To capture the metabolic profiles of the diabetic ulcers, we used high-throughput NMR, which is particularly adept for the clinical setting as metabolite profiles were obtained from crude debridement samples and were directly identified and quantified. We found that the metabolic landscape varied over time, had limited variation between patients, and reflected bacterial investment in secondary metabolism. To capture the evolving microbiome profiles, we used high-throughput 16s rRNA next generation sequencing. This allowed us to identify the metabolically active bacteria within the ulcers that contributed to the metabolic landscape. We found that the microbiome profiles varied significantly patient to patient, but remained relatively stable over time within each wound. To lay the foundation for clinical application, we integrated the metabolite and microbiome profiles into the clinical metadata extracted from the electronic medical records (EMR) with the long-term goal of developing quantifiable algorithms that could complement subjective symptom assessments for patient-centered, evidence-based treatment protocols.

Predictive multiscale modeling of microbial biofilm consortia

Presenter: Ross P. Carlson[1,2], Professor
Co-authors: Matthew W. Fields[1], Luke Hanley[3], Michael A. Henson[4]
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Biofilms are ubiquitous in medical, environmental, and industrial contexts. The majority of naturally occurring microbes grow as mixed species biofilms. These biofilm consortia are comprised of a large number of cellular phenotypes with complex interactions and self-organize into three-dimensional structures. While foundational to the majority of microbial life on the planet, the basic design principles of biofilm consortia are still poorly understood. We believe that a combination of computational and experimental tools is needed to address the challenge of characterizing, predicting and treating these fundamental systems.

The overarching goal of this project is to develop an experimentally driven, predictive multiscale model that generates quantitatively accurate predictions of biofilm formation dynamics, species distributions and responses to perturbations. The biofilm model will be formulated by combining genome-scale metabolic reconstructions of individual species with reaction-diffusion equations for nutrient, metabolic byproduct and antibiotic transport through the biofilm. The biofilm model will be multiscale with respect to both time and length scales, with the metabolic models linking individual genes to cellular dynamics and the consortia model linking individual cells to community dynamics. The research will be developed using a medically relevant, three species chronic wound model system. Treatment of chronic wounds costs the US in excess of $25 billion per year and the costs are anticipated to grow rapidly due to the rise in diabetes and obesity. The specific aims of the proposed research are: (1) construct and evaluate a multiscale metabolic modeling framework for multispecies consortia biofilms in a dynamic, spatially resolved format; (2) develop and implement spatially resolved biofilm analytical methods to quantify physiologies of consortia and monoculture biofilms to inform and validate the computational model; and (3) predict and test spatially resolved metabolic responses to culturing perturbations including antibiotic treatments with iterative loops of hypothesis refinement.

Bacterial fitness determinants in chronic wound infections – Correlation with *in vitro* biofilm fitness

Presenter: Sarah J. Morgan[1,2], Postdoctoral Fellow
Affiliation: [1]Department of Microbiology and
[2]Department of Genome Sciences, University of Washington, Seattle, WA, USA.

P. aeruginosa is found in biofilm-like aggregates in chronic wounds and other chronic infections, raising the possibility that biofilm-related phenotypes explain bacterial behavior in chronic infections. However, many questions remain. For example, it is not known if biofilm growth *in vivo* obviates the need for conventional virulence functions; if biofilm-formation functions identified *in vitro* are important *in vivo;* or which *in vitro* biofilm models best represent *in vivo* wound conditions. We addressed these questions using clinical isolates from human wounds, comprehensive genomic screens (tn-seq) in a murine diabetic wound model, and *in vitro* experiments.

We found that clinical chronic wound isolates were frequently defective for secreted proteases, rhamnolipid production, and pili-mediated motility. Consistent with this result, inactivation of acute virulence factors did not affect bacterial fitness in murine chronic wounds. A screen of transposon mutants (tn-seq) identified gene inactivations that did compromise bacterial fitness in wounds. Interestingly, genes affecting wound fitness were predicted to mediate disparate metabolic and stress-resistance functions, suggesting that multiple environmental conditions affect fitness in experimental wounds.

We tested a set of 30 mutants with differing fitness in the chronic wound (ranging from 22X more fit to over 100X less fit than wild type) in stress conditions that may be present in chronic wounds. Many of the mutants that had low fitness in the mouse were defective in anaerobic growth, or resistance to membrane and oxidative stress. Furthermore, none of the mutants with high wound fitness exhibited sensitivity to these stresses. Together, these findings indicate that anaerobic, oxidative, and membrane stress are critical for survival in chronic wounds.

We used the same test set of *P. aeruginosa* mutants to determine if the genetic determinants of bacterial fitness in wounds overlapped with those needed in *in vitro* biofilms. A comparison of the mutant's fitness in wounds and microtiter plate biofilms showed no correlation. However, there was a strong correlation ($p<0.0001$) between the fitness of mutants in chronic wounds and the colony biofilm model (developed by P. Stewart and colleagues). These experiments indicate that bacterial fitness in wounds may depend upon the capacity for anaerobic growth, oxidative and membrane stress resistance. Furthermore, the colony biofilm model may be useful for identifying the bacterial functions that mediate chronic wound infections.

SESSION 6: Device-Related Biofilms

Understanding *E. coli* biofilms on urinary catheters: Are there CAUTI-specific characteristics?

Presenter: Maria Hadjifrangiskou, Assistant Professor
Co-authors: Allison R. Eberly, Kyle A. Floyd, Jonathan Schmitz, Charles Stratton
Affiliations: Vanderbilt University Medical Center, Departments of Pathology, Microbiology & Immunology; Urologic Surgery, Nashville, TN, USA.

Catheter-associated Urinary Tract Infections (CAUTIs) have been on the rise over the last 5 years in the United States. Uropathogenic *Escherichia coli (*UPEC), the primary causative agent of community-acquired and catheter-associated urinary tract infections (UTIs) form extracellular and intracellular biofilms during infection. A self-produced extracellular matrix that is virtually impenetrable to antibiotics encases biofilm-associated bacteria. Combined with the steady rise in antibiotic-resistant UPEC strains, biofilm formation presents a serious health problem, especially in the case of CAUTI.

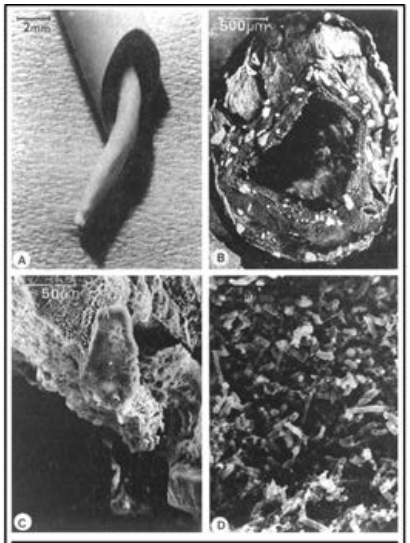

Figure 1 (taken from *Donlan R M , and Costerton J W Clin. Microbiol. Rev. 2002;15:167-193*). Biofilms often occlude urinary catheters. Cut section of a urinary catheter collected from a patient, revealing a worm-like structure occluding the lumen; (B) low-power scanning electron micrograph of a freeze-fractured cross-section of a blocked catheter; (C) crystalline formations on the outer surface of a freeze-dried preparation of material blocking the catheter; (D) fixed and critical-point-dried specimen showing that, below their crystalline coats, the catheter casts are composed of a mass of cocci and bacilli.

Figure 1 depicts an extreme case, where the bacterial biofilm occluded the catheter lumen. However, in the majority of cases, bacterial CAUTI biofilms are hard to detect visually, given that they can span as little as 15-20 microns in depth (Fig. 2B, micrograph). **As a result, the properties of the bacterial communities that give rise to the majority of CAUTI cases remain poorly characterized, because the bacteria that reside in biofilms on the catheter are discarded along with the removed catheter.** Catheters constitute the largest reservoir for infection, allowing bacteria that seed the catheter to ascend to the bladder and kidneys. Dispersal from the catheter biofilm facilitates continuous dissemination of bacteria to the urinary tract, a process that greatly contributes to the increasing risk of catheter-associated urosepsis. A challenge for the development of new therapies has been the poor understanding of the molecular details that combined, drive CAUTI pathogenesis. **Furthermore, knowledge is lacking as to whether or not CAUTI-distinguishing characteristics exist in UPEC isolates.**

In collaboration with the clinical microbiology laboratory and the urology outpatient clinic at Vanderbilt University Medical Center (VUMC), we have collected a large array of urinary isolates, including strains isolated directly from contaminated catheters. Analyses of growth and biofilm-forming properties of these isolates revealed that catheter-associated isolates display overall slower biofilm kinetics, compared to isolates that were obtained from voided urine, or the blood. Regardless of the different kinetics, the majority of isolates displayed enhanced colonization under conditions in which aerobic respiration can be used, including during growth in 4-6% oxygen that is the concentration of oxygen typically dissolved in urine. We are currently combining classical genetics approaches with the technology of matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) to further scrutinize biofilm formation by CAUTI *E. coli* isolates. Our goal is to identify oxygen-regulated biofilm factors and pathways that will provide insights into CAUTI and UPEC biofilm pathogenesis and will serve as potential anti-biofilm targets, and determine whether CAUTI-defining features exist and may arise as a function of the catheter environment.
Impact: Together the proposed studies will ascertain the molecular details that drive catheter-associated biofilms in response to oxygen and other catheter-specific environmental cues.

Strategies to treat intracellular and biofilm forming *Staphylococcus aureus* in orthopedic infections

Presenter: Devendra Dusane, Postdoctoral Fellow
Co-authors: Kyrouac, D; Petersen, I; Calhoun, J; Ellis, T; Stoodley, P.
Affiliation: Department of Microbial Infection & Immunity; Department of Orthopedics; The Ohio State University, Columbus, Ohio, USA.

The increasing resistance of bacteria to conventional antibiotics and the challenges posed by bacteria leads to chronic and recurrent infections. In orthopedic infections, bacteria may be protected from commonly prescribed antibiotics by forming biofilms or growing intracellularly within osteoblasts. Antimicrobial agents in conjunction with antibiotics, effective in the treatment of both intracellular and extracellular pathogens are needed. We hypothesize that non-antibiotic compounds can be used in conjunction with antibiotics to clear both intracellular and biofilm bacteria found in osteomyelitis. SAOS-2 osteoblast-like cell lines were infected with green fluorescent protein (gfp) labelled *S. aureus*. Extracellular planktonic bacteria were killed using gentamicin, and once the antibiotic was removed, seeding from the infected osteoblasts formed extensive biofilms. Antibiotics (vancomycin, dicloxacillin) and non-antibiotic compounds carbonyl cyanide m-chlorophenyl hydrazone (CCCP), bone morphogenetic protein (BMP-2) and sodium fluoride (NaF) were evaluated individually and in combination for their ability to kill intracellular bacteria. Checkerboard assay using these compounds showed lowering of antibiotic MIC with CCCP in combination with antibiotics. Antibiotics at MIC had no effect on reducing the intracellular *S. aureus*, however at 10x MIC, significant elimination of intracellular *S. aureus* was observed. Interestingly, vancomycin was ineffective in treating the intracellular bacteria even at 10x MIC. Confocal laser scanning microscopy (CLSM) showed increase in permeabilization of osteoblasts to antibiotics in presence of the non-antibiotic compound. Efficacy of antibiotics to inhibit growth of intracellular and biofilms of *S. aureus* was enhanced in presence of CCCP. BMP-2 and NaF had no effect on growth of *S. aureus* either alone or in combination with antibiotics. In conclusion, combined application of antibiotics and non-antibiotic agents could help in the treatment of intracellular and biofilm bacteria associated with osteomyelitis.

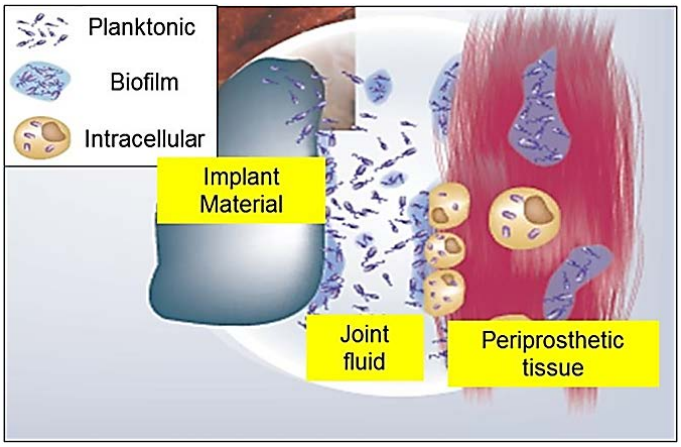

Figure 1. Emerging picture of periprosthetic infection showing planktonic, biofilm and intracellular bacterial population present during osteomyelitis.

Preclinical wound biofilm models: Establishment, consequences, and topical antimicrobial effects

Presenter: Eric Roche, R&D Manager
Co-Authors: Paul Renick, Dan Fitzgerald, Emma Forrest, Shannon Tetens, David Earnest, Jillian McMillan, Brett Kiedaisch, Sarah Ramsay, Egeenee Daniels, Dennis Carson, and Lei Shi
Affiliation: Smith & Nephew, Fort Worth, TX, USA.

Bacterial biofilms have been found to be present in the majority of chronic wounds, but prospective clinical data demonstrating the importance of biofilm to delayed healing and guiding anti-biofilm therapy are lacking. By pooling control data from porcine studies with MRSA infection, we were able to demonstrate that a pig-passaged strain that increased the biofilm character of the infection also increased healing delay in the model. A variety of other studies in recent years using rodent and rabbit models have supported the concept biofilms contribute to wound chronicity. Given bacteria in biofilms are highly tolerant of most antimicrobials, what data can guide choice of anti-biofilm wound therapy? Cadexomer Iodine (CI) was recently reported to have superior efficacy against *P. aeruginosa* biofilms in an ex vivo porcine skin model. We confirmed the strong performance of CI against *P. aeruginosa* biofilm using colony and drip flow in vitro wound biofilm models. Similar in vitro efficacy of CI was further demonstrated against mature S. aureus biofilms using these models. In addition, rapid kill of mature *S. aureus* and *P. aeruginosa* colony biofilms was visualized by confocal microscopy using live/dead fluorescent stains. The superior in vitro staphylococcal biofilm efficacy of CI was demonstrated to include MRSA using multiple biofilm models with log reduction, live/dead, and metabolic endpoints. Comparator antimicrobial treatments, including silver-based dressings used throughout, and other active agents used in individual models, produced only limited effects against the mature biofilms. Given the promising in vitro biofilm activity, CI was tested in an established mouse model of MRSA wound biofilm. CI had significantly greater impact on MRSA biofilm in mouse wounds than silver dressings or mupirocin based on gram-stained tissue sections and quantitative microbiology from tissue samples. The superior efficacy for CI in these in vitro and in vivo biofilm models suggests Cadexomer Iodine topical therapy may represent a better choice to address established bacterial biofilm in chronic wounds.

Poster Abstracts

Academic Posters (non-CBE)

Title: **Creating worst case biofilm conditions in a CDC Reactor**
Date: 07/2016
Authors: **Rebecca Bright, Amanda Deal**, Dan Klein, and Paul Lopolito
Affiliation: STERIS Corporation, Saint Louis, MO, USA.

Multiple critical parameters exist for disinfection of hard surfaces including the biocidal agent, biocide concentration, contact time, contact temperature, microbial population and type, soil level, surface characteristics, water quality and other factors. In this series of studies, the authors examined how various surfaces and microorganism types effected the ability to disinfect and clean biofilm. The surfaces that were examined included EPDM rubber, PTFE Teflon™, Buna rubber and UMHW polyethylene. CDC reactors were used to develop a *Pseudomonas aeruginosa* biofilm on coupons made of each surface type according to ASTM E2562-12. The ASTM E2871-13 single tube method was followed for harvesting and testing coupons against formulated alkaline detergent at 1% (v/v) and a formulated hydrogen peroxide/peracetic acid sterilant at 20% (v/v). The cleaning procedure utilized a low action immersion cleaning method and swabbing technique for measurement of total organic carbon (TOC) before and after product contact. The results illustrated that surface-type was not the most significant contributing factor on disinfectant efficacy. Both products evaluated had similar log reductions independent of surface type, with all trials resulting in a >6 $\log_{10}$ average reduction. In a similar series of studies utilizing polycarbonate coupons, a *Bacillus cereus* generated biofilm consisting of vegetative and endospore phenotypes illustrated a challenge to using a single disinfectant step. The cleaning and disinfectant efficacy results from *P. aeruginosa* and *B. cereus* biofilm testing displayed minor variation in surface-types but significant differences between products and microbial species tested.

Title: **Combating microbial biofilms with a combination of enzymes and antibiotics**
Date: 07/2016
Authors: **Phillip J. Brumm**[1,2]
Affiliation: [1]C5-6 Technologies LLC, Fitchburg, WI, USA.
[2] Varigen Biosciences, Madison, WI, USA.

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator protein, a gated ion channel. The current median age of survival for individuals with CF is approximately 38 years. Over 80% of CF mortalities are attributable to respiratory failure from chronic bacterial infections of the lungs, most commonly caused by *Pseudomonas aeruginosa* Individuals with CF have impaired mucociliary clearance which results in airway mucus plugging]. This creates hypoxic microenvironments, forcing invasive microbial species to adapt by forming biofilm, which is highly tolerant to most forms of antibiotic treatment. There are currently two types of drug treatments directed at respiratory infections for cystic fibrosis. The first drug treatment uses recombinant human DNase (Dornase Alfa). The second drug treatment uses antibiotics such as tobramycin, a frontline drug to kill the *Pseudomonas aeruginosa* (Pae) residing in the lung. Even in combination, these two drugs are unable to clear chronic Pae infections. A critical barrier to progress in combatting these chronic *Pseudomonas* infections that affect CF patients is a physical barrier – the biofilm produced by the cells makes them resistant to clearing by either the body's natural defenses or by antibiotic treatment.

We have discovered a novel enzymatic approach to disrupting Pae biofilm through an innovative conceptual framework of attacking the biofilm as one would attack the cell wall of a plant. The combination of three innovations allowed the rapid identification of several candidate enzymes capable of preventing biofilm formation and, with antibiotics, eliminating biofilm. Using a high throughput microplate assay, we identified eight candidate enzymes that are able to hydrolyze heat-fixed Pae biofilms. In further testing, three of them were shown to inhibit the formation of Pae biofilms in culture, disrupt Pae biofilm in the presence of tobramycin, and are not toxic to mammalian CF-derived airway cells. This approach has potential to improve treatments for a variety of biofilm-based microbial infections.

Title: **Preventing bacterial adhesion with a paintable superhydrophobic lubricant infused composite coating**
Date: 07/2016
Authors: **Curtis Larimer**[1], Chris Barrett[1], Jonathan D. Suter[1], Xiye Xiong[2], Chuck Smallwood[1], and George T. Bonheyo[3], R. Shane Addleman[1]
Affiliation: [1] Pacific Northwest National Laboratory, Richland, USA.
[2] University of Pittsburgh, Pittsburgh, USA.
[3] Marine Science Laboratory—Pacific Northwest National Laboratory, Sequim, USA.

Coatings that prevent biofilm formation could have far-reaching impact in healthcare, medicine, and industry. A polymer nanomaterial composite coating that combines two biomimetic approaches—a lotus leaf like texture with the slippery surface of a pitcher plant—is currently in development at PNNL. The coating has low surface energy and self-healing surface that prevents bacterial adhesion. A blend of inexpensive, nontoxic biocompatible components can be sprayed or painted on a surface to form superhydrophobic structure. A low-energy lubricant is infused throughout the structure, resulting in a stable slippery liquid interface. Nanoporous material in the coating aids in retention of the lubricant, extending useful lifetime of the slippery liquid surface.

Title: **Wireless heating of *Pseudomonas aeruginosa* biofilms for *in situ* implant mitigation**
Date: 07/2016
Authors: **Erica Ricker**[1], Ann O'Toole[1], Trigg Bader[1,2], Bryce Hundley[1], and Eric Nuxoll[1]
Affiliation: [1] Chemical and Biochemical Engineering, University of Iowa, Iowa City, IA, USA.
[2] Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA.
Sponsored by: Bioprocessing National Institutes of Health Fellowship, American Heart Association, and National Science Foundation

Each year in the United States tens of thousands of people have medical implants removed due to bacterial biofilm infections growing on the device. These biofilms form on the surfaces of the implants and are difficult for doctors to treat and for the immune system to eradicate. Since antibiotics and the immune system are not sufficiently effective at eradicating these biofilms, the current standard of care is for a surgeon to perform invasive surgery to remove the implant and surrounding infected tissue, creating significant local tissue damage and a prolonged healing process. The patient then undergoes a high course of antibiotics until the infection is gone, followed by another surgery to implant a new device. This second implantation has twice the risk of infection as the first implantation. The current method for dealing with bacterial biofilm implant infections has a low patient quality of life and incurs billions of dollars in health care costs in the U.S. alone.

We have proposed a new approach to solve this problem: localized, wireless heat shock to kill the biofilm in situ without surgical intervention. By coating the implant with a polymer encasing iron oxide nanoparticles, an alternating magnetic field can induce remote heating that conductively transfers to the surface of the implant where the biofilm infection is located. Surprisingly little is known about the effect of sub-autoclave thermal exposure on the viability of biofilms. To intelligently design mitigation coatings, the degree of

bacterial reduction must be correlated to both temperature and exposure time. To this end, Pseudomonas aeruginosa biofilms grown in a drip flow reactor were exposed to temperatures of 37°C to 80°C for exposure times ranging from 1 minute to 30 minutes. Temperature had a much greater effect than exposure time and followed a modified Arrhenius equation,

$$\left(\frac{CFU}{cm^2}\right) = \left(\frac{CFU}{cm^2}\right)_0 - [0.079 + 0.044\log(t)](T - 37)$$

where $\left(\frac{CFU}{cm^2}\right)_0$ is the amount of colony forming units (CFU) found after a 37°C thermal shock, t is the exposure time in minutes, T is the thermal shock temperature in degree Celsius, and $\left(\frac{CFU}{cm^2}\right)$ is the resulting viable cell count after the thermal shock. To determine the viability of this equation for a variety of P. aeruginosa biofilms, we grew the biofilms in more stressful conditions and various media, and then exposed each biofilm to an elevated temperature. We found that the equation was effective at predicting the amount of remaining viable cells at the higher temperatures, with up to six orders of magnitude decrease in viable cells; however, the minimum temperature for bacterial reduction was significantly higher, above 50°C for these hardier biofilms. In addition, preliminary results have shown that the combined effect of heating with antibiotics may improve the efficacy of mitigating a biofilm in situ.

These studies break ground on a new approach to dealing with medical implant infections, starting with fundamental investigations on how well heat will kill a biofilm, progressing to more complex studies on the interplay of multiple strategies. The understanding of the effect of heat on biofilms will help expand the available techniques for treating biofilms on implants and other surfaces that cannot reach the higher temperatures and pressures currently used for sterilization, allowing for sterilization of materials that melt or deform under conventional conditions. The introduction of such a novel coating in combination with antibiotics could obviate thousands of surgeries, improve patient quality of life, and save billions of dollars spent on ex-plantation, recovery, and re-implantation.

Title: ***P. Aeruginosa* proteomics for models of multispecies biofilms**
Date: 07/2016
Authors: **Yeni P. Yung**[1], Ross Carlson[2], Luke Hanley[1]
Affiliation: [1] Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA.
[2] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Pseudomonas aeruginosa is an opportunistic gram negative bacteria that is commonly found with other bacteria in chronic wounds. Understanding the interspecies interaction in such multispecies biofilms is essential for devising effective therapies. The expression of transport and other membrane proteins can be used to follow metabolic pathways and elucidate mechanistic behavior of multispecies biofilms. We apply cellular membrane subfractionation of *P. aeruginosa* isolate from a chronic wound patient, followed by shotgun liquid chromatography tandem mass spectrometry-based proteomics. Preliminary results from this extraction and proteomic analysis identified over 300 membrane proteins. Included in the transport proteins so identified are the tripartite multidrug transporter mexAB-oprM efflux responsible for multidrug resistance, siderophore receptor involved in inorganic ion transport, ABC transporters, and transporters of nucleotides, lipids, carbohydrates and amino acids. We will discuss how such proteomic data can be used to inform computational models of multispecies biofilms.

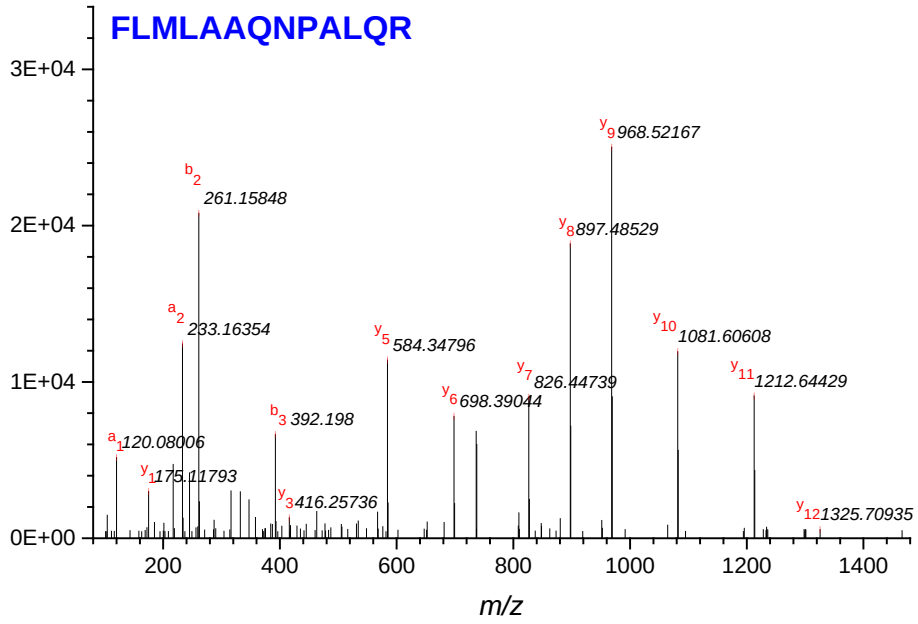

Figure 1: Liquid chromatography tandem mass spectra of protein MexB recorded on a high resolution mass spectrometer.

Center for Biofilm Engineering Posters

CBE Poster #650

Title: **Elementary flux mode analysis of irradiance-induced stress acclimation strategies in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1**
Date: 07/2016
Authors: **Ashley E. Beck**[1,2], Hans C. Bernstein[2], Ross P. Carlson[2]
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[2] Pacific Northwest National Laboratory, Richland, WA, USA
Sponsored by: PNNL, NSF

Irradiance plays a central role in regulating phototrophic metabolisms, including the metabolism of photoautotrophic cyanobacteria. Oxygenic cyanobacteria are critical primary producers in most aquatic ecosystems and have become industrially relevant as bioprocess hosts for biofuels and secondary metabolite synthesis. Here, the model thermophilic cyanobacterium Thermosynechococcus elongatus BP-1 was studied for metabolic acclimation strategies to irradiance-induced stress using elementary flux mode analysis. Metabolic stress was considered in conjunction with the availability of dissolved inorganic carbon and fixed nitrogen as well as the inhibitory effects of metabolic byproducts. Physiologies and their associated byproduct secretion profiles were analyzed over a gradient of irradiances. Formate was predicted to be the most competitive fixed carbon byproduct under stress conditions, a result interpreted in terms of metabolic pathways. Additionally, this work details the experimental determination of biomass macromolecular composition (carbohydrate, DNA, lipid, protein, RNA) for stoichiometric models, which is an often undervalued activity.

CBE Poster #671

Title: **Stoichiometric analysis of primary autotrophy and biomass turnover in a thermoacidophilic iron oxidizing archaeal community**
Date: 06/2015
Authors: **Kristopher A. Hunt**[1,2], Ryan deM. Jennings[3,4], William P. Inskeep[1,3,4], Ross P. Carlson[1,2,3]
Affiliation: [1]Center for Biofilm Engineering,
[2]Department of Chemical & Biological Engineering,
[3]Thermal Biology Institute, and
[4]Department of Land Resources & Environmental Sciences,
Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation Integrative Graduate Education and Research Traineeship Program in Geobiological Systems (DGE 0654336) and Emerging Frontiers in Research and Innovation 0937613 at Montana State University

Microbial communities are responsible for the majority of global nutrient cycling, making them prime targets for controlling greenhouse gas production and eutrophication. However, the complexity of most naturally occurring microbial communities limits their tractability due to the large number of species and interactions. Extreme temperature and pH environments, like those found in Yellowstone National Park geothermal springs, typically reduce community species diversity; these relatively simple communities represent ideal model systems for studying primary and secondary nutrient fluxes through multiple trophic levels. An aerobic, thermoacidiphilic archaeal biofilm community, which grows at 60-70°C and pH 2-4, was modeled using metagenomics data, direct *in situ* measurements and novel stoichiometric modeling approaches. The most abundant autotroph in the system, *Metallosphaera yellowstonensis* MK1, was modeled as an obligate aerobe which oxidizes iron(II) and various reduced sulfur species while respiring on limiting oxygen; MK1 primary productivity was modeled to constrain the potential community compositions and fluxes. The most

abundant heterotroph in this system, *Geoarchaeota* archaeon OSPB-1, modeled recycling of nutrients acquired by MKI via primary producer biomass degradation. This study represents the first stoichiometric analysis of nutrient / biomass recycling in a natural microbial community. Characterization of this geothermal system illustrates constraints of electron donors and acceptors on community energetics and nutrient recycling

CBE Poster #673

Title: **Promoting lipid accumulation in *Chlorella vulgaris* UTEX395 using nitrogen limitation and bicarbonate amendment under different nitrogen regimes**
Date: 07/2016
Authors: **Matthew Jackson**[1,2,4], Ashley Berninghaus[1,2,4], Todd Pedersen[1,2,4], Robert Gardner[3,4], Robin Gerlach[1,2,4]
Affiliation: [1] Center for Biofilm Engineering, and
[2] Department of Chemical & Biological Engineering, Montana State University Bozeman, MT, USA.
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[4] Algae Biomass Organization Member
Sponsored by: U.S. Department of Energy – Advancements in Sustainable Algal Production (ASAP) DE-EE0005993

Algal cultivation requires water and nutrients (N and P), both of which may be limited in different geographic regions. The use of low quality waste streams with high nutrient content may offset the pressure placed on water and fertilizer resources. However, it is first necessary to understand how algae growth is affected by the different nitrogen species present. In addition, the use of bicarbonate amendment at nitrogen depletion for enhanced lipid accumulation is well understood for algae cultures including *Chlorella vulgaris*, but has not been demonstrated with algae cultivated using nitrogen species other than nitrate. In the current study we evaluated the growth and lipid accumulation for *Chlorella vulgaris* UTEX395 using a variety of nitrogen regimes (nitrate, ammonium, urea, and a combination of the three). We also evaluated how a bicarbonate amendment at the time of nitrogen depletion affected lipid accumulation under the different nitrogen conditions. We found that UTEX 395 was able to grow using all of the nitrogen regimes evaluated. Nitrogen was consumed most rapidly in the ammonium and mixed nitrogen conditions, however similar growth rates were achieved for all cultures except those cultivated using urea as the sole nitrogen source. Cultivation using urea resulted in slower growth and the cells appeared smaller and more yellow. Lipid accumulation using the bicarbonate amendment at nitrogen depletion was similar for all nitrogen conditions.

CBE Poster #674

Title: **Bulk and molecular level characterization of organic matter in glacial ice**
Date: 06/2016
Authors: **Juliana D'Andrilli**[1,2], Heidi J. Smith[2,3], Christine M. Foreman[1,2]
Affiliation: [1]Department of Chemical & Biological Engineering,
[2]Center for Biofilm Engineering, and
[3]Department of Land Resources & Environmental Sciences, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation Antarctic Sciences (NSF ANT-1141936)
National Science Foundation Division of Materials Research (NSF DMR-11-57490)

Polar ice cores are a powerful tool for reconstructing the timing and extent of past changes in Earth's climate, and for documenting changes in atmospheric levels of greenhouse gases. Once thought devoid of life, glacial ice is now recognized as a habitat for microorganisms and a potentially significant reservoir of organic material (OM). Temporal trends in OM quality from the early Holocene ice extending back to the Last Glacial

Maximum (LGM; 6,000-27,000 years BP 1950) of the West Antarctic Ice Sheet Divide core (WDC) were measured by fluorescence spectroscopy (EEMS). A three component PARAFAC model was validated indicating a dominance of microbial OM origin in all climate periods. Increased OM fluorescence intensities coincided with elevated concentrations of Ca, Mn, and Sr in the LGM, and also with volcanic activity in the early Holocene and LGM. Taken together, ice core OM was dominated by labile, protein-like fluorescence signatures for the 21,000 year record implying that microbially derived OM is the largest contributor to the chemical nature of the OM. A subset of Antarctic ice cores from the Byrd Station (~20,458 years BP 1950) and the replicate WDC (~14,530 years BP 1950) were collected to characterize the OM molecular composition by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). Organic constituents contained $C_cH_hN_nO_oS_s$ molecular species over broad O/C ratios, although data from the Byrd core have less oxygenated and more hydrogen saturated species. Antarctic Byrd and WDC OM contain an abundance of lipid-, protein-, and amino sugar-like labile chemical species derived from microbes. Bulk level EEMS analysis of both ice core OM samples corroborate the molecular level characterization results determined by FT-ICR MS.

CBE Poster #675

Title: **Bacterial disinfection, model development and experimental validation**
Date: 07/2016
Authors: **Sepideh Ebadi**, Nick Cogan, Tom Keller
Affiliation: Department of Mathematics, Florida State University, Tallahassee, FL, USA.

Bacterial infections cause many chronic diseases such as tuberculosis, meningitis and pneumonia. These diseases may not respond to treatment with antibiotics. Bacteria evade antibiotics using a variety of tolerance mechanisms such as modifying their genotypic or phenotypic expression. They can also protect themselves in structured communities referred to as biofilms. In order to outsmart the bacteria we must have a better understanding of these tolerance mechanisms.
The focus of this study is on the dynamics between phenotypes. Understanding the changes in the persistent bacterial population before and after antibiotic challenge is of primary importance for creating treatment methods.

In this research we intend to bridge the gap between experimental and theoretical/mathematical models and to deliver brighter intuitions to experiments that describe several current hypotheses regarding phenotypic expression.

Finding the best applicable set up to eliminate the bacteria by comparing our theoretical model against experimental data, is our main goal. This will then be used to develop and quantify treatment methods. This poster demonstrates two different approaches towards understanding bacterial tolerance. The first is based on numerical analysis of the model. It represents three phases of the bacterial populations: Susceptible, Stationary and Persisters, and provides results which closely match to the data. The results also are a better match comparing to the previous studies. The second approach provides the best parameter set for the model using optimization.

CBE Poster #676

Title: **Detecting microbially induced calcite precipitation (MICP) in a model well-bore using downhole low-field NMR**

Date: 07/2016
Authors: **Catherine M. Kirkland**[a,b], Sam Zanetti[a], Adrienne J. Phillips[a,e], Elliot Grunewald[c], David O. Walsh[c], Sarah L. Codd[a,d,]
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Sponsored by: National Science Foundation, U.S. Department of Energy

Microbially induced calcite precipitation (MICP) has been widely researched in recent years due to its relevance for engineering applications including subsurface barriers for hydrodynamic control and sealing of reservoir cap rocks [1]. Subsurface applications of MICP are inherently difficult to monitor non-destructively and with spatio-temporal resolution. Nuclear magnetic resonance (NMR), however, is commonly used to characterize the pore size distributions, porosity, and permeability of subsurface geologic formations [2]. These are the same physical properties affected by MICP, indicating that NMR well-logging tools may have potential for monitoring subsurface engineering applications of MICP. This investigation used a low-field NMR well-logging probe to monitor MICP in the pore spaces of a sand-filled radial-flow bioreactor. Signal amplitude and T_2 relaxation were measured over an 8-day experimental period to identify the change in signal response due to MICP. No significant changes were recorded during the 3-day control period. Following inoculation with the ureolytic bacteria, *Sporosarcina pasteurii,* and subsequent injections of urea and calcium substrate pulses for 4 days, the NMR measured water content in the reactor decreased by approximately 24%. T_2 relaxation distributions bifurcated from a single mode centered at approximately 785 ms into a very fast decaying population (T_2 less than 10 ms) and a larger population with relaxation times greater than 1000 ms.

The reduction in signal amplitude indicates that pore water was displaced by calcite precipitation in the voids. Furthermore, the longer T_2 relaxation times suggest that calcite formation on the quartz sand mineral surface reduced the surface relaxivity, ρ, by shielding paramagnetic impurities from the pore fluid. The combination of changes in pore volume and surface minerology accounts for the changes in the T_2 distributions following MICP. Destructive sampling and subsequent analysis with ICP-MS and gravimetric methods confirmed an evenly-distributed porosity reduction of approximately 16% due to calcite precipitation. These results indicate that the low-field NMR well logging probe is sensitive to the physical and chemical changes caused by MICP in a laboratory bioreactor.

References

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2. L.L. Latour, R.L. Kleinberg, P.P. Mitra, C.H. Sotak, *J. Magn. Reson*., Series A, 1 (1995) 83-91.

CBE Poster #677

Title: **Monitoring community ecology in wastewater treatment lagoons for the production of algal biodiesel**
Date: 06/2016
Authors: **Tisza Bell**[1], L. Doig[1], R. Gerlach[1], B.M. Peyton[1], and M.W. Fields[1]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: U.S. Department of Energy

The large-scale production of algae for biofuel synthesis has the potential to help alleviate current energy problems. The vast majority of research on algal biofuel production has been conducted on single species isolates in closed systems that are costly to maintain. In contrast, open ponds are estimated to be significantly less expensive to run than closed systems. However, cultivation in open systems presents several challenges that include susceptibility to colonization by bacteria, archaea, viruses, and other eukaryotic species. Currently, realistic methods do not exist to simultaneously control positive and negative microbial interactions in large, open systems. In an effort to better understand the possible interactions during growth of eukaryotic, photoautotrophs for algal lipid production in large, open systems, we monitored the microbial community, geochemistry, and fatty acid methyl ester (FAME) content of 5 wastewater treatment lagoons over the course of a year (Figure 1).

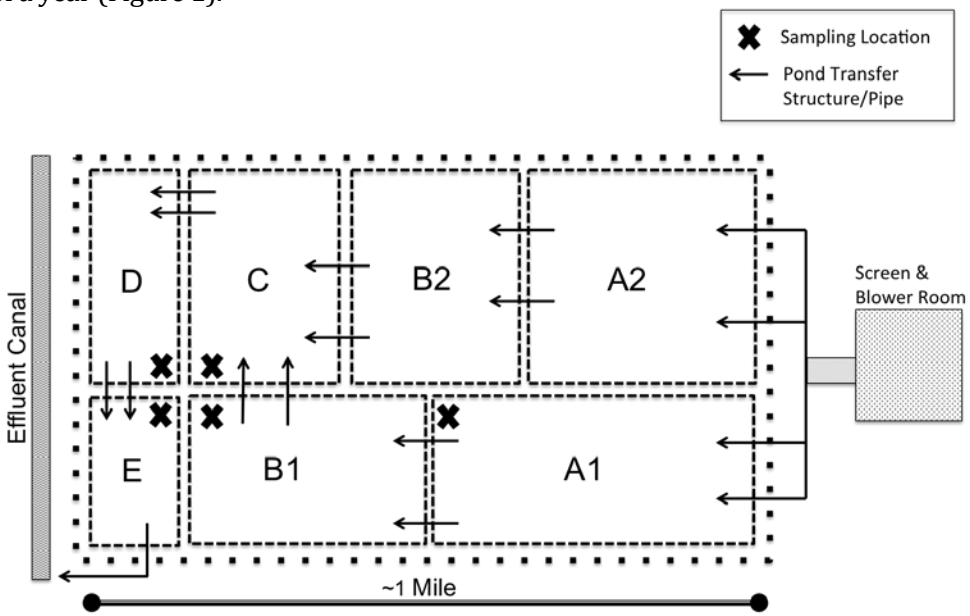

Figure 1: Aerial schematic of the lagoons at the City of Logan Wastewater Treatment Plant. Arrows indicate the flow of water when all lagoons are in use. X's signify the sampling location.

DNA was sequenced from all three domains in addition to viral DNA specific to eukaryotic algae. Each lagoon demonstrated a unique community profile even when taxa were classified at the coarsest scale. Significant correlations were observed between community members, chemical variables, and FAME concentrations. In contrast to previous findings, we observed high levels of FAME (up to 48% w/w) in some lagoons. High FAME concentrations were significantly correlated to ammonia, nitrite, TKN, pH, phosphate, and phosphorus (R^2= 0.87). However, we found that results were substantially influenced by how FAME concentrations were quantified, either as %w/w or volume (g/L). FAME quantified by volume (g/L) correlated with higher biomass concentrations (R^2= 0.78) whereas high FAME as %w/w coincided with lower cell densities (Figure 2).

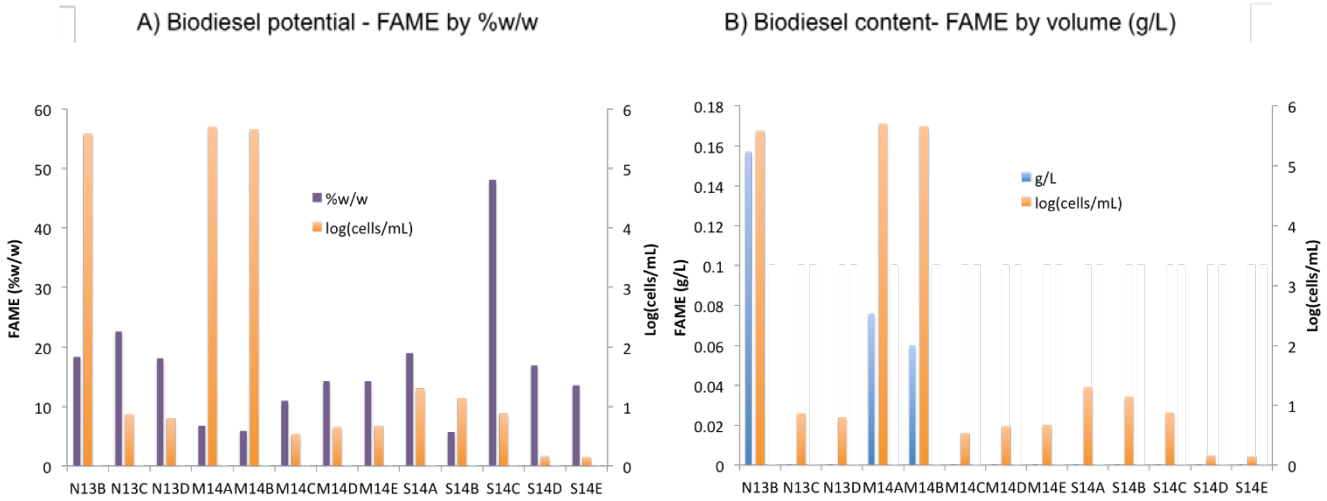

Figure 2: The log of algal cells per mL and fatty acid methyl esters quantification for each time point as A) weight (%w/w) and B) volume (g/L). In A) September 2014 Lagoon C had the highest FAME value by %w/w, but when cell number was included in the calculation B) November 2013 Lagoon B had the highest FAME value by volume.

Thus, high biomass concentrations were more important than algal phylogeny and provided for a higher overall biofuel content (g/L). High FAME volumes also correlated to the abundance of particular viral OTU (R^2=0.2) and could have been a byproduct of viral take over and augmentation of algal host lipid metabolism. Our findings suggest the feasibility of algal biofuel production using wastewater lagoons and shows both positive and negative interactions within the diverse microbial community. The resulting data can provide significant insight into biomass and/or lipid accumulation in an open system.

CBE Poster #678

Title: **Metabolically mediated biofilm-induced programmed cell death in keratinocytes**
Date: 07/2016
Authors: **Sage Schiller**[1]**, Garth A. James[2], Brian Tripet[1], Brian Eiler[1], Valérie Copié[1], Mary Cloud B. Ammons[1]*, Kelly R. Kirker[2]*
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*Corresponding authors **Presenting author
Sponsored by: K01GM103821 (MCBA)
R03AR060995 (MCBA, KRK, SS)
3P20RR02437-02S1 (MCBA, BT, BE, VC)
MSU FYRE Program (SS)

Staphylococcus aureus, a Gram-positive bacterium, is a facultative anaerobe which causes opportunistic infections in the immunocompromised. *S. aureus* can be cultured as two different phenotypes: biofilm and planktonic. In order to grow into a biofilm state there needs to be adhesion of cells to a surface, and adhesion between cells to form multilayered clusters of cells. The biofilms are surrounded by a matrix made up of exopolysaccharides, nucleic acids, and proteins and are significantly more resistant to both therapeutic treatment and the endogenous immune system when compared to planktonic cultures. Previously, it has been observed that small molecules secreted by *S. aureus* induce programmed cell death in keratinocytes through distinct mechanisms. We use PCR (polymerase chain reaction) array mapping of the human programmed cell death signal transduction pathways to determine how secreted factors from *S.* aureus biofilms are killing the keratinocytes. By evaluating the pathways, we can determine the difference in programmed cell death of keratinocytes between the *S. aureus* biofilm and planktonic states. Additional experiments include metabolomics profiling of all small molecule metabolites secreted by the biofilm and

planktonic phenotype of *S. aureus* cultures. By correlating how differences in small molecule metabolite profiles determine the biochemical regulation of programmed cell death in host cells, we can further understand how metabolism affects the host innate immune response to wound colonization by opportunistic pathogens such *S. aureus*.

CBE Poster #679

Title: **Nutrient and temperature stress for lipid accumulation in a novel environmental green microalgae**

Date: 06/2016

Authors: **Luisa Corredor**[1,2], J. Nagy[1,2], R. Gerlach[1,2], A. B. Cunningham[1,2], E. Barnhart[3], M. W. Fields[1,2]

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Sponsored by: Fulbright Scholarship Program
U.S. Department of Energy-Advancements in Sustainable Algal Production (ASAP) contract DE-EE0005993

Microalgae as sustainable feedstocks for biofuel production offer great advantages over higher plants such as efficient CO_2 sequestration/photosynthesis, fast growth rates, and wide tolerance to extreme environmental conditions. In addition, some photoautotrophs can be cultivated in low-quality water and tolerate more extreme conditions. Natural gas development in the Rocky Mountain northwest is associated with large amounts of production water that is not useful for agriculture or municipalities but could be used for the production of algal biomass and/or biofuel. Our goal was to isolate and characterize an algal population native to natural gas production water. In addition, numerous studies have been published about model microalgae under different stress conditions, but a body of knowledge is lacking regarding environmental isolates with potential for industrial applications. Nitrogen limitation has been the most commonly reported factor triggering lipid accumulation in microalgae. We have combined nitrate and temperature stresses to evaluate lipid production in an environmental isolate, from the *Chlamydomonadaceae* family, collected from high alkalinity and salinity natural gas production water (CBM-W). The microalga isolate, CBMW, was grown in buffered and unbuffered Bolds Medium with modified nitrate concentrations (0.5 mM, 2 mM and 3 mM) at different temperatures (20°C, 25°C, 30°C, 35°C).

Growth performance was evaluated by cell counts and total chlorophyll extraction. Media filtrates were used to track nitrate concentration changes and lipid production was measured using Nile Red (NR) fluorescence. Fatty acid methyl esters (FAMEs) speciation was quantified by Gas Chromatography-Mass Spectrometry (GC-MS) after biomass lyophilization and transesterification. The optimum conditions for growth and lipid accumulation were 30°C at 0.5 mM of nitrate (40% w/w of FAME per dry cell weight). Lower and higher temperatures in combination with higher nitrate concentrations had much lower biomass and lipid production. Buffered media appeared to inhibit lipid production. The results indicate that CBMW is a fast growing microalga with high tolerance to nitrogen starvation, temperature stress and potential to produce lipids for biofuel production in actual natural gas production water.

CBE Poster #680

Title: **Biomineralization and wellbore integrity in an analog reactor: A microscopic solution to subsurface fluid migration**
Date: 06/2016
Authors: **Drew Norton**[1], Gerlach R[1], Eldring J[1], Thane A[1], Hiebert R[3], Cunningham AB[1], Spangler L[2], Phillips, AJ[1]
Affiliation: [1] Center for Biofilm Engineering and
[2]Energy Research Institute, Montana State University, Bozeman, MT, USA.
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Sponsored by: U.S. Department of Energy DE-FE0024296

The keystone of subsurface fluid storage is the ability to inject and keep fluids deep in underground aquifers for extended periods of time. Fluid migration in these systems can cause failure of operations and lead to unwanted fugitive emissions. Large pressure gradients between the storage reservoir and the surface create a powerful driving force for fluid migration to the surface, thus requiring a strong seal to maintain containment of subsurface fluids. A great risk for seal failure lies in and around the wellbore used to access the aquifer, in particular defects in the interface of the annulus cement and the well casing. While current oil field remediation technologies are effective for a majority of defects, they falter with defects small enough to hinder high viscosity fluid injection, such as the cement slurry fluid. Microbially induced calcite precipitation (MICP) is a new technique that uses low viscosity fluids and microorganisms (~2 μm diameter) to seal these small defects.

In the MICP process, ureolytic microorganisms hydrolyze urea into carbonate and ammonia. When this process occurs in the presence of fluids rich in calcium, the carbonate can react with calcium ions to form calcium carbonate. This calcium carbonate can then precipitate and form a seal that is capable of bridging small fluid migration pathways. To better understand MICP in geometries similar to the down-hole environment, a lab scale analog reactor has been developed to simulate a wellbore surrounded by a cement annulus. With an overall diameter of 4 inches, this lab analog is modeled approximately one-quarter scale from an actual well used for MICP field-testing. Defects of 0.2 to 1.5 mm can be created in the cement annulus and a clear outer casing allows visual observation of calcium carbonate formation in these defects during experiments. A recent MICP treatment experiment performed over eight days in this analog reactor resulted in a three order of magnitude permeability reduction of a channel flow path approximately 0.8 mm in depth due to a calcium carbonate seal. This result shows the promise of MICP technology to complement existing well-cement remediation technologies, sealing small defects and improving the integrity of down-hole gas storage systems.

CBE Poster #681

Title: **Characterization of novel thermophilic archaea from alkaline springs in Yellowstone National Park**
Date: 05/2016
Authors: **Michelle Meagher**[1,2], Dana Skorupa[1,2], Abdelrhman Mohamed[3], Haluk Beyenal[3], Phuc Ha Thi[3], and Brent Peyton[1,2]
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Sponsored by: Thermal Biology Institute & Center for Biofilm Engineering, MSU
Undergraduate Scholars Program, MSU

Currently, few archaeal isolates have been successfully cultured and grown in the laboratory, such that little is known about the metabolic capabilities, nutrient cycling and basic physiological role of these unique and novel organisms in their environment. Previous work has investigated the microbial diversity existing in

several alkaline hot springs in Yellowstone National Park. A 16S based phylogenetic analysis has identified several sequences as belonging to novel populations, including archaeal phyla *Aigarchaeota*, *Korarchaeota*, and *Nanoarchaeum*. The goal of this research is to cultivate and isolate species of Archaea inhabiting these thermoalkaline springs so as to develop a greater understanding of the prokaryotic life present in such extreme environments. Enrichments were set up both in the lab and *in situ* for several alkaline hot springs in the Heart Lake Geyser Basin. Laboratory enrichments used sediment slurries collected from several different springs as inoculum for a matrix of culture conditions. During *in situ* enrichments, electrodes were deployed for 30 days, applying a small voltage across the hot springs. Additionally, negative controls were deployed in each spring with no voltage applied. DNA was extracted from lab enrichment cultures, as well as samples of anode, cathode, and control electrode material. Lab enrichment cultures containing low levels of oxygen displayed apparent growth when observed via fluorescence microscopy. However, these cultures yielded insufficient genetic material for analysis. Metabolite exchange is believed to be an important factor in the growth of these novel organisms, and future lab culturing attempts will include co-culturing methods, using thermophilic bacteria that have previously been identified in these systems. DNA extracted from samples of electrode material from *in situ* enrichments was amplified using PCR, and sent to Washington State University for sequencing. Cathode electrode material from the two hottest springs (>70°C) yielded DNA concentrations far higher than the control samples with no voltage. Pending sequencing results, it appears that a small applied voltage may enrich for certain microorganisms in these extremely high temperature environments.

CBE Poster #682

Title: **Effects of nutrient limitation on *Desulfovibrio vulgaris* biofilm composition, structure, and metal deposition**

Date: 06/2016

Authors: **Lauren Franco**[1,2], S. Wu[3], R. Csencsits[3], M. Joo[3], J. Ivanisevic[4], G. Siuzdak[4], M. Auer[3], M.W. Fields[1,2]

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Sponsored by: ENIGMA (http://enigma.lbl.gov) at LBNL supported by Office of Biological and Environmental Research U.S. Dept of Energy Contract No: DE-AC02-05CH11231

Desulfovibrio vulgaris is a sulfate-reducing bacterium that is commonly observed in anaerobic subsurface environments associated with metal-reducing conditions. Biostimulation of heavy metal-reducing organisms by injecting electron donors into the subsurface can create unbalanced ratios of electron donor to acceptor and here we show that these ratios affect biofilm structure and activity. Samples were analyzed for protein, carbohydrate, and sulfide content, imaged using electron microscopy, and compared via metabolomics. Microscopy revealed the presence of membrane vesicles, extracellular filaments, and extracellular membranous structures. Membranous structures create geometrical pockets in the biofilm that are devoid of bacteria or are sheet-like structures that are heterogeneously distributed throughout the biofilm. Non-osmicated, uranyl-acetate stained biofilm samples revealed an unstained thin core structure, which upon osmication becomes black, indicating that the thin structure is lipid-based. Serial sectioning using lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers. EDS imaging revealed presence of Fe, O and P, but not sulfide, and these results suggested the metal deposits are not solely the result of inorganic chemistry interactions of metals ions with hydrogen sulfide. The biofilm and metal deposition was visualized in 3D with SBF/SEM, and showed a heterogeneous distribution of metal precipitates away from cells. Metabolomic analysis revealed an up-expression of particular fatty acids under electron-acceptor limited conditions compared to balanced conditions and a down-expression of metabolites involved in DNA turnover, N-cycling, and peptidoglycan turnover, and these results indicated that electron

acceptor-limitation may induce an overall stress response that is coordinated with alternative electron transfer mechanisms.

CBE Poster #683

Title: **Novel algal biofilm reactor with harvesting mechanism for enhanced biomass production**
Date: 07/2016
Authors: **Muneeb S. Rathore[1,2],** Karen M. Moll[1,3], Robin Gerlach[1,2], Brent M. Peyton[1,2]
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Sponsored by: U.S. Department of Energy, Fulbright Scholarship Program

Dewatering of algal biomass for biofuel and valuable coproducts production, is one of the most expensive steps in algal biomass processing because it is energy intensive and the initial biomass concentrations are often too low for the processing of algal biomass to be economically feasible. The costs for dewatering range from 20-30% of the total cost of producing the biomass, limiting the application of algal biomass for biofuel and co-products production. Algal biofilm reactors have been proposed for reducing the amount of water present during harvesting compared to the commonly used planktonic growth photo-bioreactors. However, a lack of competitive cultivation and harvesting techniques has limited industrial application of algal biofilm reactors. Most of the existing technologies require reactors to be dismantled for harvesting. In this study, we present a novel, packed bed, Algal Biofilm Reactor (ABR) coupled with aeration driven detachment for harvesting of biomass to minimize the amount of water present in the harvested biomass. *Chlorella vulgaris*, strain UTEX 395, was grown under continuous flow and batch culture conditions in the ABR with different inorganic carbon concentrations at a 14:10 light: dark cycle for up to 22 days. Bold's Basal Medium was used as the growth medium. Biomass concentrations of 1.5 – 3.3 g L^{-1} were obtained in multiple operations, with biomass productivities of up to 1.0 g m^{-2} day^{-1}. As much as 80% of the biomass could be recovered from the reactor during harvesting and the remaining biomass was used as the inoculum for the next growth cycle without the need for re-inoculation with fresh culture.

CBE Poster #684

Title: **Bacterial dormancy in chronic *Pseudomonas aeruginosa* infections**
Date: 07/2016
Authors: **Kerry Williamson[1, 2],** Tatsuya Akiyama[1, 2], Elizabeth Sandvik[1, 2], Sila Yanardag[1, 2], Ben White[1, 2], Amanda Richards[1, 2], Marcia Riesselman[2], Michele Narayan[12], Marziah Hashimi[2], and Michael Franklin[1, 2]
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Sponsored by: National Institutes of Health

Pseudomonas aeruginosa is an opportunistic pathogen that causes severe and life-threatening illnesses, and is associated with pulmonary infections, wounds, diabetic ulcers, and infections of artificially implanted devices. A hallmark of *P. aeruginosa* is its ability to form biofilms. Biofilms are bacterial cells attached to surfaces, encased in their secreted extracellular macromolecules. Bacteria growing in biofilms have enhanced resistance to the host immune response, and are highly tolerant to traditional antibiotic treatments. One reason that biofilms are difficult to eradicate from infections is that the bacteria are in a variety of different physiological states. Some microenvironments in biofilms are rich in nutrients and oxygen, and the bacteria are actively growing in those zones. However, in zones of the biofilm where oxygen or nutrients are limiting,

the bacteria can enter a dormant state. Antibiotics generally target active metabolic processes, and most antibiotics are ineffective at killing the dormant bacteria. Once antibiotics kill the active cells, the dormant cells may resuscitate, resulting in persistent infections. The goal of this research is to understand how bacteria survive during a dormant state, and how they are able to resuscitate from dormancy. By characterizing transcriptional responses of both the active and the dormant cells of *P. aeruginosa* biofilms, we identified several factors that are required for cells to maintain viability during dormancy. When one of these genes (*hpf*) is deleted, the dormant bacteria have reduced ability to resuscitate. Therefore, the product of this gene may be an effective target for killing dormant bacteria. Such a treatment, if effective, could be used in combination with traditional antibiotics to target both active and dormant bacteria.

CBE Poster #685

Title: **A high-throughput, multiplexed microfluidic method utilizing an optically barcoded drop library**
Date: 06/2016
Authors: **Geoffrey Zath,** Connie Chang
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Sponsored by: Montana State University Office of the Provost

The power of drop-based microfluidics promises reduced biological assaying times and greater sample throughput; however, current drop-based microfluidic methods focus on single-input single-output techniques to provide these benefits. In order to achieve truly high-throughput analysis of biological assays, a multiple-input approach must be taken. This work is focused on developing and validating a drop-based microfluidic method that is capable of encapsulating, in parallel, 96 assay samples in drops and optically tracking them in a barcoded drop library. The advantage of the method presented here is its ability to be integrated with current biological assays performed on a 384-well plate. The first step was to fabricate a three-dimensional microfluidic device capable of accepting 96 sample inputs. Second, formation of drops within the device was characterized by creating a state diagram using Capillary and Weber numbers of the two phase flow. Finally, the use of fluorescent microbeads was investigated for the purpose of optically barcoding drops. A barcoding scheme was developed to allow for fluorescent and spatial labeling of 96 wells of a 384-well plate. The three-dimensional microfluidic device was successfully used to encapsulate 50 μm diameter drops from 24 wells barcoded with fluorescent microbeads at a drop formation rate of 3 kHz per well. Fluorescent detection of the barcoded drop mixture was performed at a rate of 200 Hz and density-based clustering algorithm DBSCAN was used to identify barcoded drop clusters from the fluorescent signal data. Validation of this method was achieved by adding known concentrations of fluorescent blue microbeads to barcoded wells and detecting for their presence in barcoded drop clusters. The barcoding method can be expanded to fully incorporate the 96 inputs of the microfluidic device by adding a spatial barcoding component to each quadrant of 24 optically barcoded wells. The results presented here show the microfluidic platform has the potential to be a useful tool in biological assays involved with tracking a large number of samples in a well plate format

CBE Poster #686

Title: **Assessment of sonication for biofilm harvesting**
Date: 07/2016
Authors: **Lindsey Lorenz**, Kelli Buckingham-Meyer, Fei San Lee, Jennifer Summers, Taly Polukoff, Grace Dickerman, Diane Walker, Al Parker, Paul Sturman, Darla Goeres
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Harvesting biofilm from coupon surfaces is a fundamental component of a biofilm method. Harvesting is a two part process, consisting of removing the biofilm from the surface, and disaggregating it into a homogenous cell suspension. Partial removal and/or disaggregation of the biofilm will result in bias, as described by Hamilton et al in a 2009 AOAC publication. Section 11.2.5 of ASTM Method E2871-13 recommends viewing the coupon surface microscopically to address concerns of bias from harvesting issues.

Potential sources of bias include treatments that fix cells to the surface, as well as treatments that facilitate better removal from the surface, in comparison to an untreated control. A third potential source of bias is the sonicating bath used to harvest the biofilm and the exact steps the laboratory follows when sonicating their biofilm samples. For example, a laboratory may use a sonicating bath that is not suitable for biofilm research, may incorrectly place the vials that contain the coupons in the sonicating bath, place too many vials into the bath at once, operate the sonicator at an insufficient water level, or use a sonicator that is not functioning properly.

The SBML conducted a study with the goal of determining if our sonicating water bath was functioning at optimal performance. A *Pseudomonas aeruginosa* biofilm was grown in the CDC reactor and the biofilm was harvested according to ASTM Method E2871-13 and MLB SOP MB-20. After harvesting, coupons were either serially diluted and plated for viable plate counts, or were stained with LIVE/DEAD BacLight and imaged on the confocal microscope. This poster depicts the results of our study, and describes parameters to consider when using sonication as the harvesting method in biofilm studies.

CBE Poster #687

Title: **High throughput analysis of *Pseudomonas aeruginosa* regrowth after starvation**
Date: 07/2016
Authors: **Shawna Pratt[1,2],** Robert Schaefer [1,2], Tatsuya Akiyama[1,3], Michael Franklin[1,3], Connie Chang[1,2]
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Sponsored by: Montana INBRE

Pseudomonas aeruginosa is a model biofilm-forming bacteria, and is implicated in illnesses such as the infection of patients with cystic fibrosis. Like many biofilms, the *P. aeruginosa* subpopulations present within biofilms are exposed to heterogeneous conditions, such as starvation conditions near the solid surface interface of the biofilm, and rich conditions near the biofilm's liquid interface. The genetic traits which impact the ability of cells to survive periods of starvation is of interest in the overall scope of this study. The hibernation promotion factor gene, *hpf*, is linked to the ability of cells to survive under starvation conditions. This gene leads to the presence of HPF proteins which bond to ribosomes during starvation stress and encourage the cell to go into dormancy, preserving the cells' ribosomes. Here, high-throughput microfluidic fluorescence-based detection is used to analyze the regrowth of two fluorescent *P. aeruginosa* PAO1 populations subjected to starvation periods. In this method, dilute bacteria are encapsulated in 15 µm

monodisperse droplets suspended in oil. Fluorescence within the drops is detected using a custom-built microscope stand. The fluorescence emission correlates to the number of fluorescent bacteria in the drops. Use of microfluidics in this application increases the validity of high throughput microfluidic testing as a biological testing method. Our goal is to differentiate single cells in drops that are indicative of dormancy.

CBE Poster #688

Title: **Selenium bioreduction in mine waste rock at cold temperatures**
Date: 07/2016
Authors: **Hannah Koepnick**[1,2], Chiachi Hwang[1], Cagan Ertuna[1,2], Ruya Zare[1,2], Lisa Kirk[3], Ellen Lauchnor[1,4], Brent Peyton[1,2]
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Sponsored by: Teck

Bioremediation is a viable option for treating selenium in water at mining sites; however, microbial activity slows at near-freezing temperatures. The aim of this experiment was to determine whether microbial selenate reduction could occur under simulated site conditions. Batch and column studies of native microbial community capacity to reduce Se in unsaturated surficial waste rock and saturated backfills from Canadian mining deposits show that oxygen and nitrate inhibition is readily overcome via carbon substrate addition. Biofilm in oxygen-exposed groundwater columns developed at 10°C, and showed near complete nitrate reduction followed by selenium removal, with no associated sulfate reduction. No-carbon control columns also showed significant rates of selenium removal. Anaerobic batch tests were conducted using mine waste rock from decommissioned column reactors from a previous experiment, where selenium removal was observed in the column reactors at 10° C. Batch tests were run in triplicate at 5° C and 10° C, with methanol and glycerol used as carbon sources. Results from the batch tests show that selenium removal at 5° C is comparable to that at 10° C when using methanol as the carbon source. Thus, it may be possible to employ bioremediation at the site, and field tests are scheduled to begin in summer 2016.

CBE Poster #689

Title: **Role of pigments in Antarctic bacteria stress response**
Date: 04/2016
Authors: **Shu Ying Wee**[1,2], Laura Fisch[1,3], Heidi Smith[1,4], Christine Foreman[1,2]
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Sponsored by: NSF 0839075, NSF 1141978, Undergraduate Scholars Program

Microorganisms in polar regions are exposed to a variety of environmental stressors including: temperature extremes, pH, nutrient limitation, ultraviolet radiation, and oxidative stress. One adaptation that cold temperature organisms have developed is the ability to regulate membrane fluidity in order to cope with imposed stressors. Membrane fluidity at low temperatures is achieved through the incorporation of pigments, into the membrane. This project sought to determine the role of pigmentation in organisms' environmental stress responses. Six bacterial isolates from the Cotton Glacier supraglacial stream in Antarctica were selected as they contain a variety of carotenoid and non-carotenoid pigments. At mid-exponential growth, cells were exposed to an array of stressors including: acid, alkali, osmotic, oxidative, freezing and heating. Organisms were exposed for 2, 6, 12, and 24 hours after which the percentage survival was calculated. It is hypothesized that the presence of different pigmentation among cold temperature

organisms contributes to the organism's ability to withstand a variety of imposed environmental stressors. Of the six organisms tested, two did not possess pigments while the other four possessed either xeaxanthin, violacein or astaxanthin. The organisms response to the six stressors varied, with the astaxanthin pigmented organism, CG9_6, being the best survivor under osmotic (1M NaCl), alkali (pH 10) and freezing (-80°C) temperatures, with percentage survival of 176±41%, 145±19% and 215±64% respectively at 2 hours. Under oxidative stress (0.1M H_2O_2) all organisms experienced complete mortality. The non-pigmented organisms, CG23_3 and CG23_4, were the only organisms to survive pH 3 and 40°C at 0.453±0.389% and 9.58±0.829% respectively. Analyses are ongoing to determine the relationship between pigmentation and stress response in these organisms. Pigments are important in many biotechnological and pharmaceutical applications, with the demand for naturally occurring, non-toxic, environmentally friendly pigments continuing to grow. The heterogeneity that nature provides in extreme environments can be capitalized upon to uncover novel organisms with industrial benefits.

CBE Poster #690

Title: **Remediation of coal combustion residuals using microbially-induced calcite precipitation**

Date: 07/2016

Authors: **Abby Thane**[1], Adrienne Phillips[1], Eric Troyer[1], Ben Gallagher[2] , Lee Spangler[3]

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Sponsored by: Montana Research and Economic Development Initiative (Montana REDI)

Waste generated by coal-fired power plants (coal combustion residuals or CCRs) has become a growing concern due to its potential impact on environmental and human health. Large ash spills such as the 2008 Kingston Fossil Plant spill in Tennessee have increased the regulatory scrutiny toward the disposal of CCRs. A final rule published by the Environmental Protection Agency places new standards on CCR storage sites to minimize potential groundwater and atmospheric contamination. One potential solution to reduce the environmental risk of CCRs is through the use of microbially-induced calcite precipitation (MICP). In MICP, Sporosarcina pasteurii produces the urease enzyme which promotes the hydrolysis of urea (ureolysis). If Ca2+ is present it will react with the CO32- formed during ureolysis to produce CaCO3 which can be used to bind particulate materials together. Small-scale batch tests were performed to investigate the production of CaCO3 in the presence of CCRs. Samples taken at different time points were analyzed for decreasing urea concentration and increasing pH, both of which are indicative of ureolysis. Data collected from batch testing and presented here shows MICP successfully producing CaCO3 in the presence of fly ash, waste water, and flue gas desulfurization material (gypsum). It was observed that the resulting CaCO3 has the ability to form around the CCR particles, effectively binding them together. This method of turning CCRs into "biocement" has the potential for application in current and future storage facilities to reduce chemical leaching and fugitive dust emissions.

CBE Poster #691

Title: **Electron Donor Limitation Promotes Metal Corrosion by *Desulfovibrio alaskensis* G20 Biofilm**

Date: 08/2016
Authors: **Greg Krantz**[1], K. S. Lucas[1], L. T. Hoang[2], G. Siuzdak[2], M.W. Fields[1]
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Sponsored by: U.S. Department of Energy ENIGMA

Microbially Influenced Corrosion (MIC) is a major concern for industrial ferrous metal pipelines and can result in pipeline failure. Sulfate Reducing Bacteria (SRB) have been implicated in contributing to MIC due to their production of corrosive H_2S gas and elemental sulfur along with metal-microbe interactions. This project focuses on the effects of Electron Acceptor Limitation (EAL) and Electron Donor Limitation (EDL) on biofilm physiology and corrosion rate on various surface types, including 1018 carbon steel, 316 stainless steel, and borosilicate glass. *Desulfovibrio alaskensis* G20 was grown under steady-state conditions in sulfate-reducing biofilm reactors. Under EAL conditions, biofilms on glass and 1018 steel had elevated biomass levels, both in terms of protein and hexose levels. Under EDL conditions, biofilms on 1018 steel had the highest protein and hexose levels. Differential corrosion rates were observed between EDL and EAL conditions on 1018 carbon steel. The results indicated that different ratios of respiration substrates contributed to altered rates of corrosion, and the difference in corrosion rates could not be explained solely by sulfide, acetate, or carbohydrate levels. Protecting the 1018 metal coupon from biofilm colonization while maintaining exposure to sulfide was shown to dramatically reduce corrosion. Metabolomic mass spectrometry analyses combined with XCMS data processing show an increase in lumichrome, a precursor to flavin adenine dinucleotide (FAD), under the EDL condition, suggesting the bacteria are producing FAD for extracellular electron transfer from the metal, and as a means to increase metal corrosion when starved for electron donor.