VIRTUAL MEETING

BIOFILM TECHNOLOGIES

PATHWAYS TO PRODUCT DEVELOPMENT

February 1–3, 2021

PROCEEDINGS

CENTER FOR BIOFILM CBE ENGINEERING

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Workshop Abstracts

A statistical assessment of standard methods: Case studies of ASTM, CDC, STM & MBEC biofilm methods

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

There are many useful methods and systems used today to study biofilm in the laboratory. One method or system is not better than another, just more relevant for the particular environment of interest. This workshop will review ASTM standardized biofilm methods and describe the desirable characteristics of any biofilm method including ruggedness, responsiveness, repeatability, and reproducibility. Required experimental designs and data analysis approaches to study these attributes will be covered.

CBE online biofilm resources

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

The CBE offers many powerful resources via its website that are designed to be useful to academic researchers, regulatory bodies, and representatives from industry. This brief presentation will provide information for accessing these tools. These include where to find methods training videos, how to access our heavily utilized Images Library, where to find papers authored by CBE researchers and students spanning decades, contact information for CBE faculty and staff and their research areas, a primer on biofilm basics and KSAs, a series of articles focusing on laboratory tests for surface disinfectants.

Poster Abstracts

CBE Poster #770

Date: 02/2021
Title: **Evaluation of the reproducibility of microtiter plate-based biofilm quantification methods**

Authors: **Jontana Allkja**[1,2], Nuno F. Azevedo[1], Albert E. Parker[2,3], Darla M. Goeres[2], Print-aid ring trial team
Affiliation: [1]Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Mathematical Sciences, Montana State University, Bozeman, MT, USA.
Sponsored by: Marie Sklodowska – Curie grant agreement No 722467, Print-Aid project.

Microtiter plate methods are commonly used for biofilm assessment. However, their data have often been difficult to reproduce. A ring trial was performed in 5 different laboratories to evaluate the reproducibility and responsiveness of three biofilm quantification methods in 96-well microtiter plates: crystal violet, resazurin, and CFU counts. Experiments were divided into two main groups: control and treatment. An inter-lab protocol was developed for the study. This was separated into three steps: biofilm growth, biofilm challenge, biofilm assessment. For control experiments, participants performed the growth and assessment steps only. For treatment experiments, all three steps were performed and the efficacy of sodium hypochlorite (NaOCl) in killing *S. aureus* biofilms was evaluated. In control experiments, on the $\log_{10}$-scale, the reproducibility SD was 0.44 for crystal violet, 0.53 for resazurin, and 0.92 for CFU counts. In the treatment experiments, CFU counts had the best reproducibility with respect to responsiveness, making it the more reliable method to use in an antimicrobial efficacy test. This study showed that the microtiter plate is a versatile and easy-to-use biofilm reactor which exhibits good repeatability and reproducibility for different types of assessment methods.

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CBE Poster #771

Date: 02/2021

Title: **Persistence of a locally isolated cyanobacterial biofertilizer and its effects on the soil crust microbiome**

Authors: **Hannah Goemann**[1,2], Rebecca Mueller[1], Brent Peyton[1,3]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: NSF EPSCoR, WAFERx

Interest in biofertilizers as sustainable alternatives to chemical fertilizers has developed in recent years as the negative environmental impacts of chemical fertilization such as groundwater contamination and decreased soil quality are becoming increasingly evident. Nitrogen-fixing cyanobacteria are promising biofertilizers as they provide both nitrogen and carbon to the soil, and if successfully established, may reduce long-term fertilizer inputs. However, the effect of biofertilizers on the soil microbiome is largely unknown and unexplored, despite the vital roles soil microorganisms play in driving major soil nutrient cycles. Therefore, we sought to understand how a locally isolated nitrogen-fixing cyanobacterial biofertilizer (CBF) affected crop growth, nutrient cycling, and the soil microbiome over a three-year field study (2018-2020) with perennial bioenergy crops switchgrass and tall wheatgrass. Of particular interest is the influence of the locally isolated *Nostoc sp*. biofertilizer on the cyanobacterial community of the soil microbiome as well as the persistence of the biofertilizer following application. The persistence of the biofertilizer may be indicative of biocrust formation which could increase its value as an agricultural application. Formation of cyanobacterial biocrusts has been utilized as a restoration strategy for degraded soils due to increased soil aggregation by cyanobacterial exopolysaccharides as well as increased nutrient and moisture retention. In addition, determining biofertilizer establishment is vital to decreasing long-term N and C inputs. Here we present microbial community data representing the effects of two years of biofertilizer application on the bacterial community of the soil crust at our Post Research Farm field site. The field study is located at the MSU Arthur H. Post Research Farm near Bozeman, MT where each year we conducted soil sampling from April-September on plots either fertilized with a locally isolated *Nostoc* sp. CBF, urea, or left unfertilized. High-throughput sequencing on the Illumina MiSeq platform of 16S and LSU rRNA gene sequences was used to capture the bacterial/archaeal and fungal soil communities respectively, while soil chemical analysis monitored changes in C, N, and pH. We found evidence of CBF persistence across seasons without strong changes to the soil microbiome composition. In addition, our data support the ability of CBF to replace synthetic fertilization while maintaining plant biomass production.

CBE Poster #772

Date: 02/2021

Title: **Upgrading methane to value-added products using metabolic modeling**

Authors: **Adrienne D. Arnold**[1,2], Ross P. Carlson[1,2,3]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: Molecular Biosciences Program, Montana State University; NSF Award #1736255 (BuG ReMeDEE); DOE Award # DE-EE0008247 / 000

Methanotrophs are organisms that use methane as their sole carbon and energy source. These organisms are found in diverse habitats and play an important role in global carbon cycling. Methanotrophs are also of industrial interest, as they are able to oxidize methane to key chemical feedstocks like methanol and formaldehyde. In this study, core metabolic models of a type I and a type II methanotroph were constructed and analyzed via elementary flux mode analysis and flux balance analysis. Type I methanotrophs assimilate methane via the ribulose monophosphate pathway, while type II methanotrophs use the serine pathway. Metabolic modeling facilitates

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comparison of core carbon and energy metabolism between the methanotroph types. Byproduct excretion in particular was analyzed, as metabolic byproducts determine the industrial potential of methanotrophs and create the possibility of syntrophic community interactions in the environment between methanotrophs and other organisms.

CBE Poster #773

Date: 02/2020
Title: **Understanding microbial interactions in fungal-bacterial biofilms: implications for environmental remediation**
Authors: **Gretchen Gutenberger**[1], Erika J. Espinosa-Ortiz[1], Joseph Golichnik[1], Robin Gerlach[1]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana CREWS

Bacteria and fungi readily grow together in the natural environment (e.g. soil), where they perform critical work to maintain the functioning of the ecosystem, such as breaking down organic matter for nutrient recycling. By engineering systems where pollutant-degrading fungi and bacteria are integrated, as they commonly are in nature, we can potentially create enhanced systems for the degradation of pollutants. Most research reports the use of single cultures or communities of either fungi or bacteria for the remediation of soil and water. However, there is a lack of information on the use of multi-domain cultures, particularly fungal-bacterial biofilms. Mixed cultures are more robust than monocultures and, due to their varying metabolic functions, have a division of labor, which could in turn make them more efficient at removing pollutants from soil or water. Furthermore, there is some evidence that fungal-bacterial systems can have improved removal efficiencies compared to their monocultures. This project aims to establish fungal-bacterial biofilms with pollutant-degrading microorganisms and to better understand the associations and interactions between their microbial partners. This presentation will summarize our efforts to co-cultivate environmentally relevant microorganisms including the bacterium *Pseudomonas* species (*P. putida* or *P. stutzeri*) and the fungus *Phanerochaete chrysosporium* in a continuous drip flow reactor to form fungal-bacterial biofilms. Future experiments to explore the effect of culture conditions (e.g. order of inoculation, temperature, and pH) on fungal-bacterial biofilm development will also be discussed. The obtained fungal-bacterial biofilms in this study will be used for the bioremediation of selenium in acid mine drainage in future projects.

CBE Poster #774

Date: 01/2021
Title: **Sustainable biomineral composite adhesives through ureolysis-induced calcium carbonate precipitation**
Authors: **Sobia Anjum**[1,2], Kendall Parks[1,2], Robin Gerlach [1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA; [2]Chemical and Biological Engineering, Montana State University, Bozeman MT, USA.
Sponsored by: Fulbright, National Science Foundation, and the US Department of Energy

Adhesives are essential to automobile, aerospace, electronics, and wood industries. Most adhesives currently in the market use petroleum products and volatile organic compounds (VOCs) as raw materials. Both can be harmful to human health and the environment, raising concerns for both workers and users. These concerns have created a demand for more sustainable adhesives, leading to a trend towards green adhesives in global sealants and adhesives markets. Most of the safer and more sustainable adhesives are bio-based, natural adhesives. Some of the prominent green adhesives are soy and starch adhesives making up 50% of the green adhesives market, with applications in construction, paper, and packaging. Other organic bio-based adhesives include albumin, casein, beeswax, gum Arabic, soybean proteins, and starch for use in specialized applications. However, the use of bio-based adhesives is limited due to their sensitivity to water, low adhesive strength compared to synthetic adhesives, and limited usability on a number of surfaces (their use is limited to mostly wood and paper). To expand the functionality of bio-based adhesives, improve adhesive strength and decrease water sensitivity, a natural adhesive worth exploring is a biomineral composite. The biomineral composite consists of organic polymers and microbially produced calcium carbonate. Calcium carbonate is produced in place during the curing process using a mechanism referred to as ureolysis-induced calcium carbonate precipitation (UICP). UICP occurs according to the following

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reaction and can be promoted by bacterial cells *(MICP: microbially induced calcium carbonate precipitation)* or through the free enzyme *(EICP: enzymatically induced calcium carbonate precipitation)*.

$$H_2N\text{-}CO\text{-}NH_2(\text{urea}) + 2H_2O + Ca^{2+} \xrightarrow{urease\ enzyme} 2NH_4^+ + CaCO_3(s)$$

The calcium carbonate formed as a result of this reaction along with microbial cells (or free enzyme), its products, and organic additives is referred to as a biomineral composite. To expand the application range of bio-based adhesives beyond wood and paper in this work, the substrates tested are glass and steel. So far, guar gum and soy protein have been used as additives to make biomineral composites. We show that the adhesive strength of the composite produced with soy protein is greater than guar gum composites, soy protein or guar gum alone. MICP composites with and without additives have been previously studied for a broad array of applications, including soil stabilization, concrete remediation, creating subsurface barriers and remediation of radionuclides. Field applications of this process by our lab group have shown the potential of using MICP with lower cost bulk chemicals. We are building on this knowledge to develop novel composites for adhesive and similar applications.

CBE Poster #775

Date: 02/2021
Title: **Standard assay development for coronavirus survival and disinfection**
Authors: **Kelly R. Kirker**, Garth A. James, Matthew W. Fields
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana State University

The recent outbreak of the COVID-19 pandemic, caused by the SARS-CoV-2 virus, underscores the need for testing capabilities relevant to novel virus threats. Although the major transmission route for SARS-CoV-2 is believed to be person to person contact via respiratory droplets, contact transmission via surfaces may still play a role. In general, determining the survival time of specific viruses on surfaces and most effective disinfectants are important for helping control the spread of viruses. Ideally, testing should be performed on the actual virus of concern, however, in the case of SARS-CoV-2 this requires BSL-3 facilities. The use of surrogate viruses enables work in BSL-2 facilities to reduce costs and timelines for technology development. The method developed by the CBE is based on the accepted EPA test method for high-level anti-viral disinfectants for nonporous surfaces ASTM E2197–1, the "quantitative carrier test" (QCT) using the canine coronavirus (ATCC VR-2068). This project creates an opportunity for the CBE to enter new testing and research areas relevant to SARS-CoV-2 and other human viruses.

CBE Poster #776

Date: 02/2021
Title: **Non-hysteretic capillary pressure in multiphase flow in porous media: an experimental investigation using 2D porous micromodels**
Authors: **Razin Molla**[1], Nishagar Raventhiran[1], Yaofa Li[1]
Affiliation: [1]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.

Multiphase flow in porous media occurs naturally in many industrial and environmental systems. Understanding the fundamental flow physics in such systems is essential for many real-life applications. Among others, capillary pressure is an important parameter for multiphase flow in porous media, which was traditionally modeled only as a function of saturation and this relationship in turn was found to be hysteretic. Extensive research has been going on for decades to investigate and mitigate the hysteresis in capillary pressure-saturation curves. Recently it has been theoretically shown that a unique relation is possible with the inclusion of a few additional variables such as interfacial area, interfacial curvature and Euler characteristic in the functional form. It is also suggested that such a functional form would work for both equilibrium and non-equilibrium conditions. However, systematic and quantitative experimental investigations and validations of such a functional form are still lacking. To this end, capillary pressure along with saturation and other geometric variables are experimentally quantified for a multiphase flow in 2D micromodels. Fabricated 2D micromodel is a powerful tool to perform aforementioned studies as it offers excellent control over porous structures, great repeatability and excellent optical access. Employing fluorescence microscopy coupled with a high-speed camera, flow configurations as well as its dynamics are captured, which are then analyzed using advanced image processing algorithms. In this poster, techniques for 2D micromodel fabrication and simultaneous measurements of capillary pressure, saturation, interfacial area and

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Euler Characteristic have been delineated, thus providing a general method for 2D micromodel validation of novel theories related to capillary pressure hysteresis. The results will provide new insight into the hysteretic behavior of capillary pressure as well as validations of new functional forms.

CBE Poster #777

Date: 02/2021

Title: **Design and fabrication of a membrane-based sensor for capillary pressure measurement in 2D micromodel**

Authors: **Nishagar Raventhiran**[1], Razin Molla[1], Yaofa Li[1]

Affiliation: [1]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.

Capillary pressure and capillarity are central to the description of multiphase flow in porous media. For decades, practical and theoretical descriptions of multiphase flow in porous media have been inevitably relying on empirical relations between capillary pressure and phase saturation, which have long been recognized to be hysteretic. Extensive studies have been devoted to understanding and mitigating such hysteresis in hope of achieving a unique description of the state of the porous medium flow system. Although a direct in-situ measurement of pore-scale capillary pressure would be extremely valuable, on-chip measurement of pore-scale capillary pressure is still lacking due to a number of experimental challenges. Only very few proposed designs are suitable for measurement in multiphase flow in porous media. To that end, we aim to design and fabricate an on-chip sensor that enables direct capillary pressure quantification within individual pores in 2D porous micromodels. The micromodel used in the current study is fabricated in polydimethylsiloxane (PDMS) using soft lithography with a thin membrane incorporated which deflects when subject to pressure variations in the fluid flow. With this technique, a 2D pressure field can be inferred by means of a pre-calibrated correlation between the membrane deflection in the z-direction and pressure change. A microscope coupled with a high-speed camera is employed to provide optical readout, allowing for possible simultaneous quantification of other flow characteristics, such as velocity fields, phase distribution and interfacial area. By this experiment, we hope to provide a novel method for direct quantification of capillary pressure at the pore scale and this study will lead to an enhanced understanding of pore-scale physics of multiphase flow in porous media.

CBE Poster #778

Date: 01/2021

Title: **Improving the microbiome of high pH-high alkalinity algal cultures**

Authors: **Huyen Bui**[1], Isaac Miller[1], Calvin Cicha[2], Blake Wiedenheft[2], Matthew Fields[1,2], Robin Gerlach[1], Sridhar Viamajala[3]

Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA. [2]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA. [3]Department of Chemical Engineering, The University of Toledo, Toledo, OH, USA.

We isolated and characterized a green alga, *Chlorella sorokiniana* SLA-04, capable of growing at high pH (~10.2) and high alkalinity (>50mEq). High pH/high alkalinity algal cultivation has the potential to drastically reduce the cost and increase the range of possible locations for industrial scale algal biomass production for biofuel and high value product generation. The high pH and high alkalinity conditions provide non-limiting concentrations of inorganic carbon for photosynthesis and the effective scavenging of atmospheric CO_2, thereby allowing for high productivity algal growth (pilot biomass productivity >16 g/m^2/day) in the absence of concentrated CO_2 sources. Additionally, alkaliphilic strains thrive under high pH-high alkalinity conditions inhibitory to many competing mesophilic microalgae, bacteria, archaea, viruses and predatory zooplankton. We have begun characterizing the microbial community in these algal cultures using microscopic and phylogenetic approaches, which has allowed us to identify potentially beneficial and detrimental interactions of algae and other microorganisms. Using state-of-the-art DNA sequencing technologies, we were able to detect bacterial phyla as well as potential grazers (amoeba and ciliates) in high pH-high alkalinity SLA-04 cultures. We are also in the process of characterizing the physiology of strain SLA-04 and its interactions with associated microorganisms including 19 bacterial strains isolated from indoor and outdoor cultures of SLA-04. We recently obtained an axenic culture of SLA-04 and other high pH-

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adapted algae through repeated antibiotic treatments and are sequencing their genomes. Algal-prokaryotic interactions are being characterized using metagenomic and metatranscriptomic sequencing in combination with activity-based and metabolomic analyses (BONCAT, NanoSIMS, and Raman confocal microspectroscopy). These data are providing the foundation for developing metabolic network models, which will guide both microbiome and algal genome engineering approaches (using e.g. CRISPR-Cas9-based approaches) for overall cultivation improvement. Our goal is to understand and exploit the synergistic effects of algae-microbiome interactions for maximum benefit in high pH/high alkalinity cultivations and provide a framework for controlling inter-organismal interactions in other algal cultures important for biofuel and bioproduct generation.

CBE Poster #779

Date: 02/2021
Title: **Impedance spectroscopy sensor platform to detect biofilm in industrial settings**
Authors: **Matthew McGlennen**[1,2], Markus Dieser[1,3], Christine Foreman[1,3] and Stephan Warnat[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Mechanical and Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation

Electrochemical impedance spectroscopy (EIS) is a powerful technique for characterizing bulk and interfacial properties in aqueous, solid and gas systems. The technique is based on applying an oscillating voltage at a single-frequency to a device under test (DUT) and measuring the complex electrical current. Varying the frequency and calculating the complex resistance/impedance allows modeling of the DUT using electrical equivalent circuits. Changes to the recorded spectra indicate *in situ* biofilm formation and an increase of microbial concentrations in the media. We have developed microfabricated EIS sensors that are small (~ 9 x 26 mm), low cost and amendable to use in a variety of environments, providing exciting opportunities for spatially resolved, real-time monitoring of biofilm in industrial settings. This poster presents preliminary results of the use of these sensors in metalworking fluids, where microbial contamination is a significant factor in their degradation, causing biofouling and corrosion of equipment, the imperilment of product quality, and posing occupational safety risks.

CBE Poster #780

Date: 01/2021
Title: **Detachment of algal biofilms in a biofilm reactor with a novel harvesting mechanism**
Authors: **Muneeb S. Rathore**[1,2], Brent M. Peyton[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Church & Dwight, Fulbright Foreign Scholars Program

Algal biofilm reactors (ABRs) have some possible advantages for biomass production due to the concentrated nature of the resulting biomass potentially requiring less energy for water removal via settling as compared to suspended culture growth. Current biofilm systems often have many moving belts, disks or scrapers for biomass harvesting with some requiring complex mechanical systems for proper operation. Biomass productivity for *Chlorella vulgaris* was investigated in a novel ABR that uses reverse-flow aeration as a harvesting mechanism for biofilm detachment. Effect of different medium compositions was also examined. Calcium (Ca2+) is well known to stabilize biofilms by crosslinking extracellular polysaccharide (EPS) cross linkers (Cooksey 1981). The effects of Ca2+ and sodium bicarbonate on biomass and lipid productivity (Gardner, Cooksey et al. 2012) were evaluated in the ABR. Addition of Ca2+ and bicarbonate resulted in the fastest growth of *C. vulgaris* biofilms. Areal biomass productivities, measured as ash free dry weight (AFDW), ranged from 0.278 ± 0.013 to 0.700 ± 0.159 g m-2 day-1 and were the highest in treatments with additional calcium and bicarbonate. Supplementing the media with only bicarbonate resulted in an AFDW productivity of 0.39 g m-2 day-1. Harvesting with aeration resulted in an average biomass detachment of 69.04 ± 13.9 %. End point lipid content was determined after harvesting the biomass at steady state areal cell density. Lipid production in the biofilm was limited to membrane lipids (7-10 % wt/wt fatty acid methyl esters (FAME) and bicarbonate had no effect on lipid productivity.

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SESSION 1: Medical Technologies

High-throughput microplate approach to study bacterial adhesion to topographic features on medical devices such as breast implants

Presenter: **K. Scott Phillips**[1], Regulatory Research Scientist
Co-authors: Dacheng Ren[2], Sang Won Lee[2]
Affiliation: [1]Center for Device & Radiological Health, US FDA, Silver Spring, MD, USA.
[2]Syracuse University, Syracuse, NY, USA.

In recent years, the emergence of breast implant associated anaplastic large cell lymphoma (BIA-ALCL) linked with textured breast implants has raised questions about how medical device surface topography contributes to the benefit/risk profile of devices. Given the importance of bacterial biofilms in medical device-associated infections, there has been substantial research on how bacteria interact with 3D surface topographies and how to design surface topography as a strategy to create antifouling and contact killing materials. In this talk, I will review existing work and principals of bacterial adhesion that can be applied to the BIA-ALCL problem. Then I will discuss a high-throughput microplate-based approach that we developed to study simulated bacterial interactions with a library of systematically designed simulated breast implant topographies. The textures were designed to create surfaces with similar roughness but different pattern characteristics. We discovered that rather than surface roughness, a correlated aspect of the surface—edge effects—was driving bacterial adhesion in our in vitro study. This was confirmed through static and dynamic experiments, bulk microplate measurements, plating and culturing, and time-lapse confocal microscopy. The results underscore the importance of careful mechanistic study of bacterial biofilm formation and point to the need for rational development of medical device surface topography.

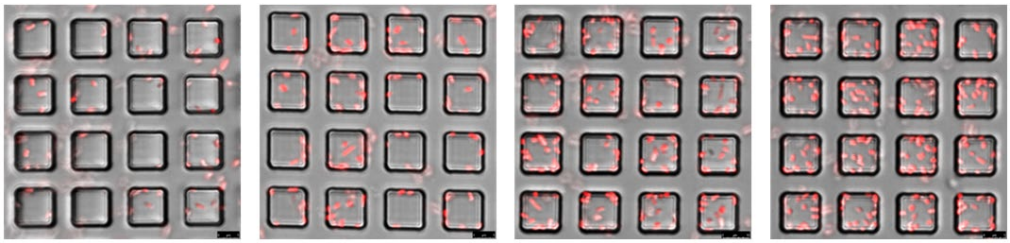

Figure 1. Confocal microscopic images of S10D5 patterns (top and bottom focal point) with the attached cells at increasing attachment times: 30, 90, 150, and 240 min. Scale bar = 5 μm.

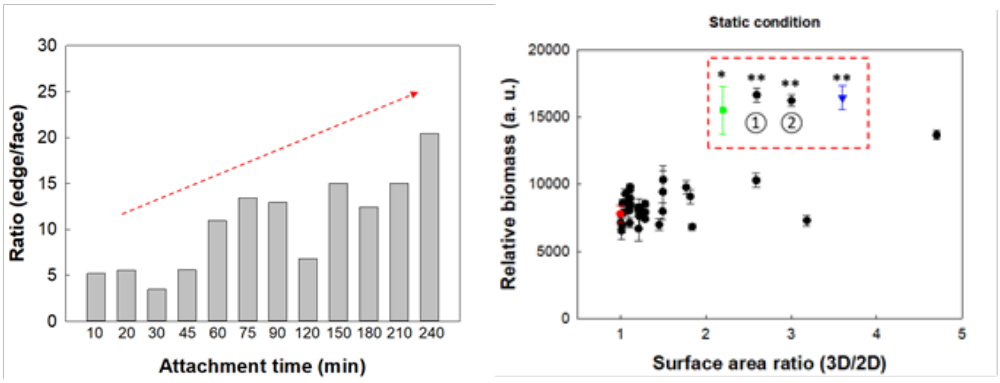

Figure 2. Left: Ratio of number of cells on edge of features to face of features for S10D5. Right: Relative biomass of cells attached to simulated breast implant patterns in microplates, plotted by 3D/2D surface area ratio. The points in the dotted red square are outliers and include two commercial textured implants and two of the patterns (S10D5 and S10D2).

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Dynamic adaptive response of *Pseudomonas aeruginosa* to clindamycin/rifampicin-impregnated catheters

Presenter: **Kidon Sung**, Staff Fellow
Affiliation: Division of Microbiology, National Center for Toxicological Research, US FDA, Jefferson, AR, USA.

Pseudomonas aeruginosa is the most common Gram-negative pathogen causing nosocomial pneumonia; it is often multidrug-resistant, a good biofilm-producer and has the potential for tainting antimicrobial-impregnated medical devices. Despite the widespread use of antimicrobial-impregnated catheters, little is known about their effects on antibiotics in *P. aeruginosa*. In this study, we investigated adaptive resistance potential of *P. aeruginosa* strain PAO1 in response to continuous antibiotic exposure from clindamycin/rifampicin-impregnated catheters (CR-IC). During exposure for 144 hr to clindamycin and rifampicin released from CR-IC, strain PAO1 formed biofilms on catheters featuring elongated and swollen cells. There were 545 and 372 differentially expressed proteins identified in the planktonic and the biofilm cells, respectively, by an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Both Cluster of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) simultaneous analyses showed that the planktonic cells responded to the released antibiotics more actively than the biofilm cells, with metabolism and ribosomal biosynthesis-associated proteins being significantly over-expressed. Not only were certain groups of virulence proteins, including the outer membrane-associated (flagella, type IV pili, type III secretion system) and extracellular (pyoverdine) virulence factors, up-regulated, but also the phenotypic invasion capability to HeLa cells was increased, raising concern about the potential risk for CR-IC. Continuous exposure of CR-IC to *P. aeruginosa* induced over-expression of antibiotic resistances proteins, including porin, efflux pump, translation and transcription proteins even though the changes did not affect the phenotypic minimum inhibitory concentration (MIC).

The shield hypothesis of biofilm chronic infections

Presenter: **Philip S. Stewart**, Regents Professor of Chemical & Biological Engineering
Co-authors: Brian Pettygrove, PhD candidate, Microbiology & Immunology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Hypothesis: The host reaction to implanted materials and to localized infection results in formation of a physical barrier that shields the infectious biofilm from immune cell access and thereby contributes to chronicity. Medical devices when implanted typically induce a process of fibrotic encapsulation termed the foreign body response. This response deposits a mechanically tenacious film of host-derived polymers around the device, creating a capsule. This presentation explores the possibility that the capsule creates a protected niche where a biofilm infection can evade early clearance and then persist. Data from the literature show an average rate of capsule thickness development around a biomaterial *in vivo* of 28 ± 25 microns per day, potentially rapid enough to afford protection to contaminating bacteria within 24 hours. Capsule deposition is faster in the presence of introduced microorganisms. Similar processes of fibrin sheath formation in biomaterials within vasculature and neutrophil extracellular trap (NET) formation at sites of localized infection could also contribute to creation of a physical shield. Host-deposited materials may provide a barrier that both shields the biofilm from immune cell clearance and protects the host from dissemination of the localized infection. The likely connection between host responses to biomaterials and subsequent biofilm infection has been underexplored. A corollary of this hypothesis is that therapies that interdict capsule formation around biomaterials will prevent biofilm infection on medical devices.

Testing standards for medical implants with an antimicrobial activity

Presenter: **John Rose**, Principal Scientist
Affiliation: Smith & Nephew Orthopedics, Memphis, TN, USA.

To aid the development of implantable medical devices with an antimicrobial effect, it is desirable to have well understood standard test methods with which to evaluate technologies and devices during development. Currently there are few, if any, standard test methods which are specific to the clinical environment faced by medical devices with an antimicrobial effect. In this presentation I will outline the work of an ASTM group which has started to address this needed area. The goal is to optimize preclinical tests to the extent that they are more predictive of clinical performance, reduce animal use, and allow for improved clinical trial design. This will be done by developing tests that consider the potential clinical benefit of the product in mind. Some of the steps in considering new test methods are outlined in the Figure 1.

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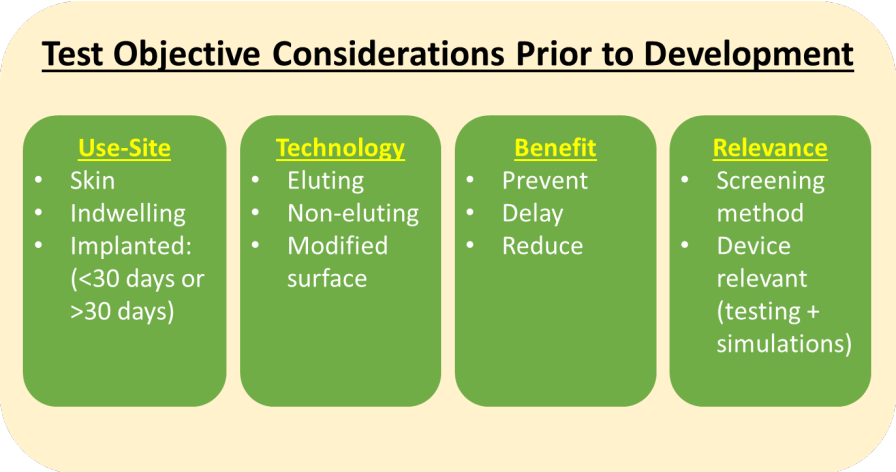

Figure 1. *Chart shows some of the considerations that will be important in developing a range of test methods that could be relevant to the development of new medical devices with an antimicrobial effect.*

Effects of a cadexomer iodine wound gel on viability, oxygen penetration and pH in mature *in vitro* *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms

Presenter: **Garth James**[1], Associate Research Professor
Co-authors: Paul Renick[2], Laura Boegli[1], Erika Espinosa-Ortiz[1], Ellen Lauchnor[1], Daniel Fitzgerald[3], Emma Woodmansey[3],Eric Roche[2]; and Philip Stewart[1].
Affiliations: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Smith & Nephew, Fort Worth, Texas, USA.
[3]Smith & Nephew, Hull, United Kingdom.

Cadexomer iodine has shown clinical effectiveness for reducing wound bioburden and improving healing. It also has shown effectiveness against biofilms using both *in vitro* and *in vivo* models. This *in vitro* study evaluated the impact of a cadexomer iodine gel (CIG) on bacterial viability within mature *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms compared to a cadexomer gel (CG) without iodine. The biofilms were grown on collagen-coated surfaces in a drip flow reactor and viability was assessed using plate counts and confocal scanning laser microscopy of LIVE/DEAD stained biofilms. The influence of these gels, along with other wound care gels, on the oxygen concentration and pH within the biofilms was then assessed to further elucidate potential mechanisms for the anti-biofilm action of the gels. The mean log reductions (MLR) for CIG and CG ranged from 4.0-5.63 and 0.75-2.68, respectively, and CIG had a significantly greater MLR than CG ($p<0.001$). CSLM and LIVE/DEAD staining also indicated that CIG had more of a bactericidal effect than CG. CSLM also revealed that both CIG and CG cadexomer beads embedded within the biofilm and for CIG bacterial viability was reduced in the vicinity of the beads. All of the wound gels tested decreased oxygen gradients within the biofilms, however this was most pronounced for CIG and CG. This may have been due to both the reduction of bacterial viability and physical disruption of the biofilm. CIG and CG also drastically reduced the pH within the biofilms, which may have contributed to bactericidal effects. Overall, the results of this study indicated that CIG likely impacts biofilms through multiple interrelated mechanisms including reducing pH, increasing oxygen penetration, physical disruption, and iodine release into the biofilm.

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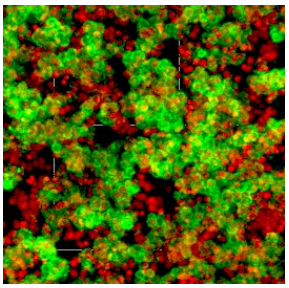
SESSION 2: Surface Technologies

The secret life of DNA: A tale of DNA's many roles in bacterial biofilms

Presenter: **Rikke Louise Meyer**, Associate Professor
Affiliation: Bioscience and Interdisciplinary Nanoscience Centre, Aarhus University, Aarhus, Denmark.



DNA was first recognized as recognized as a biofilm matrix component two decades ago. Since then an increasing body of evidence has shown that DNA is a ubiquitous molecule that is important for initiation of biofilms by bacterial species from across distant phylogenetic groups. But it seems that DNA can do much more than being a sticky polymer. It turns out that DNA contributes in many ways to bacterial attachment, biofilm cohesiveness, protection against biocides, and even metabolic activity of bacteria in biofilms. In this presentation, I will give an overview of our current understanding of DNA's many roles in bacterial attachment and biofilm formation, and how this knowledge might be applied in biofilm control.

Figure 1. *Confocal laser scanning image of* Staphylococcus epidermidis *biofilm showing bacteria in red and extracellular DNA in green.*

The power of nature's enzymes—Tailoring green solutions for biofilm control

Presenter: **Lorena Gonzalez-Palmen**, Senior Scientist
Affiliation: Novozymes, Copenhagen, Denmark.

Novozymes is the world leader in biological solutions and multiple applications, ranging from laundry to food, which are today at the core of our business. Inspired by nature, we discover and develop unique enzymes and microbes and upscale them to industrial products that serve our customers' needs. Biofilms are interesting from a business point of view both by being ubiquitous and by being connected to problems in many industries. From an enzyme biotech perspective, biofilms are multi-substrate systems. Despite their complex and dynamic nature, it seems possible to identify access-points where enzymes can be used to degrade/disrupt the biofilm matrix. This will allow industries to replace harsh chemicals and antibiotics by biological solutions for biofilm control. In Novozymes, we continuously build the biofilm-acting enzymes toolbox, covering a broad diversity to access different targets on the biofilm matrix and stay at the front of innovation, discovering unique solutions. A showcase for the waste-water management will be presented.

An update on antimicrobial product initiatives at the EPA Microbiology Laboratory

Presenter: **Steve Tomasino**, Senior Scientist
Affiliation: Office of Pesticide Programs, US EPA, Fort Meade, MD, USA.

EPA is responsible for regulating antimicrobial products (e.g. hospital disinfectants), including those with virucidal claims. The registration process is conducted by the Office of Pesticide Programs (OPP) Antimicrobials Division (AD). The registrant (manufacturer) of an antimicrobial product with a claim to control a public health pathogen is required to submit efficacy data for review and approval. The Microbiology Laboratory Branch (MLB), under the Biological and Economic Analysis Division of OPP, supports the AD through the development and standardization of methods for evaluating the efficacy of antimicrobial products, and when requested by AD, conducts efficacy assays to verify product performance. MLB has been instrumental in advancing the science of antimicrobial product testing, leading multi-laboratory collaborative studies, and providing technical expertise to standard-setting organizations and various agency stakeholder groups. This presentation will provide an update on MLB's contribution to the agency's response to SARS-CoV-2 and method development initiatives pertaining to residual antimicrobial coatings, soft/porous surface claims, a quantitative method for testing bacteria on hard non-porous surfaces, and Legionella pneumophila in cooling tower water.

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Statistical techniques for analyzing presence and absence data from microbes: MPN and TCID50

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

For highly effective antimicrobials that kill or remove all microbes from a surface, it is common to validate efficacy via a presence/absence assay. The most rudimentary low-tech assay simply dumps a sample treated with the antimicrobial into a nutrient rich medium to allow any surviving organisms to grow until the medium becomes cloudy to the naked eye. Clearly, this approach cannot be applied to articles composed of expensive electronics like re-usable endoscopes or larger re-usable medical devices like ventilators or heart-lung machines. In these cases, presence/absence may be assessed with chemical, molecular, or microscopic signals. Regardless of the technology used to generate the presence/absence data, it is remarkable that the number of microbes that survive the treatment can be estimated using the venerable most probable number (MPN) statistical technique (McCrady 1915). FDA recommends using the MPN for assessing microbes in food. EPA recommends use of the MPN to assess highly effective antimicrobial treatments on hard non-porous surfaces via the use-dilution method. In another application, it is common to collect presence/absence information about viruses (via the death of host cells) over multiple dilutions to assess viral loads in a sample, for example after application of a virucide. Interestingly, the measure of viral load that historically is preferred by virologists is the Tissue Culture Infective Dose (TCID50) (Spearman 1908; Karber 1931) which is the mean dilution at which half (50%) of the samples or wells are positive for the virus. Importantly, TCID50 does not directly provide an estimate of the number or abundance of viruses in the sample. So the question arises, given virologists' preference to describe viral loads indirectly with TCID50, and the fact that the MPN directly estimates viral abundances, how do the two quantities relate when applied to presence/absence data? Under some conditions, statistical theory suggests that TCID50 is proportional to the abundance of viruses, and hence proportional to MPN. However, the predicted proportionality constant differs depending on the specific conditions (e.g. the efficacy of the treatment). The relationship between MPN and TCID50 is investigated empirically in a data set generated by virucides applied to the feline calicivirus.

Pathways to innovation: Update on the CBE regulatory science program

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

The Center for Biofilm Engineering has launched a new regulatory science program with the intent to promote innovation by creating a culture that bridges a regulatory science mission and novel technology solutions to real world problems. The goal is for the CBE to be at the nexus of innovation and regulatory science with regards to the fate and transport of biofilm in the body, environment and engineered systems. The regulatory science program is built upon the pillars of education, research, and technology transfer with standard methods serving as the key communication and decision-making tool. During this interactive presentation we will step back and identify new trends and technology aimed towards reshaping biofilm research that will promote the advancement of innovation in the marketplace. Discussion points will include technology that complements data obtained using the viable plate count method for determining the number of viable microbes averaged over a defined surface area. We will explore what information is being lost when only viable plate counts are used, and what new tools exist that that will enable us to understand and creatively manipulate or utilize the complexity of a biofilm community.

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*All times are Mountain Standard Time

Draft AGENDA

Monday, February 1—WORKSHOP & POSTER SESSION		
Time	**Title**	**Presenter**
9:30–10:30 a.m.	A statistical assessment of standard methods: Case studies of ASTM, CDC, STM & MBEC biofilm methods	Al Parker, Biostatistician, CBE; Associate Research Professor, Mathematical Sciences, MSU
10:30–10:45 a.m.	CBE online biofilm resources	Diane Walker, Research Engineer, CBE
11:30 a.m.–1:30 p.m.	**Virtual Poster Session**	

Tuesday, February 2		
Time	**Title**	**Presenter**
9:15–9:25	Opening remarks	Matthew Fields, CBE Director; Professor, Microbiology & Immunology, MSU Paul Sturman, CBE Industrial Coord.
Session 1: Medical Technologies		
9:25–9:30 a.m.	Session Introduction	Garth James, PI, Medical Biofilms Laboratory, CBE; Associate Research Professor, Chemical & Biological Eng., MSU
9:30–10:00 a.m.	High-throughput microplate approach to study bacterial adhesion to topographic features on medical devices such as breast implants	Scott Phillips, Regulatory Research Scientist, Center for Device & Radiological Health US FDA
10:00–10:30 a.m.	Dynamic adaptive response of *Pseudomonas aeruginosa* to clindamycin/rifampicin-impregnated catheters	Kidon Sung, Staff Fellow, Div. of Microbiology, National Center for Toxicological Research, US FDA
10:30–11:00 a.m.	The shield hypothesis of biofilm chronic infections	Phil Stewart, Regents Professor, Chemical & Biological Engineering, MSU, CBE
11:00–11:30 a.m.	**Break**	
11:30 a.m.–12:00 p.m.	Testing standards for medical implants with an antimicrobial activity	John Rose, Principal Scientist Smith & Nephew
12:00—12:30 p.m.	Effects of a cadexomer iodine wound gel on viability, oxygen penetration and pH in mature in-vitro *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms	Garth James
12:30–1:00 p.m.	**Break**	

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1:00–2:00 p.m.	Panel Discussion *What are the components that a test method must contain to be considered relevant for FDA data submission?*	Moderator: Garth James, CBE Co-moderator: Phil Stewart, CBE Petra Kohler Riedi, 3M Scott Phillips, US FDA John Rose, Smith & Nephew Sousan Sheldon, Medical Devices Consultants, LLC

Wednesday, February 3		
Time	**Title**	**Presenter**
9:15–9:25	Opening remarks	Matthew Fields Paul Sturman
Session 2: Surface Technologies		
9:25–9:30 a.m.	Session Introduction	Darla Goeres, PI, Standardized Biofilm Methods Laboratory; Research Professor of Regulatory Science, CBE
9:30–10:00 a.m.	The secret life of DNA: A tale of DNA's many roles in bacterial biofilms	Rikke Louise Meyer, Assoc. Professor, Bioscience and Interdisciplinary Nanoscience Centre, Aarhus University, Aarhus, Denmark
10:00–10:30 a.m.	The power of nature's enzymes—Tailoring green solutions for biofilm control	Lorena Gonzalez-Palmen, Senior Scientist, Novozymes
10:30–11:00 a.m.	An update on antimicrobial product initiatives at the EPA Microbiology Laboratory	Steve Tomasino, Senior Scientist, Office of Pesticide Programs, US EPA
11:00–11:30 a.m.	**Break**	
11:30 a.m.–12:00 p.m.	Statistical techniques for analyzing presence and absence data from microbes: MPN and TCID50	Al Parker
12:00—12:30 p.m.	Pathways to innovation: Update on the CBE regulatory science program	Darla Goeres
12:30–1:00 p.m.	**Break**	
1:00–2:00 p.m.	Panel Discussion *Biofilm assessment technologies beyond the viable plate count*	Moderator: Darla Goeres, CBE Co-moderator: Al Parker, CBE Tajah Blackburn, US EPA Lise Duran, Sterilex Tony Rook, Sherwin-Williams Heidi Smith, CBE Steve Tomasino, US EPA

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