Proceedings

ANTI-BIOFILM TECHNOLOGIES

PATHWAYS

TO PRODUCT DEVELOPMENT

Feb. 7-8, 2023

CENTER FOR BIOFILM

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ENGINEERING

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KEYNOTE PRESENTATION

OSEL sterility and infection control regulatory science program: a focus on stakeholder needs

Presenter: **Michael Eppihimer**, Director
Affiliation: Division of Biology, Chemistry and Materials Science, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, US FDA, Silver Spring, MD, USA.

Technological advances in products often move at a greater rate than the methodologies needed to evaluate their risks and benefits. The advancement of regulatory science by the Office of Science and Engineering Laboratories (OSEL) through the development Regulatory Science Tools (RSTs) aims to provide methodologies where current standardized methods do not exist. RSTs enable device developers to focus their resources on maximizing how the product works rather than how well it may be tested. To meet this objective, OSEL has established a defined RST development process incorporating inputs such as submission trends, technology forecasting and stakeholder input on major challenges related to device development and assessment, which are used to identify high priority programmatic areas. Given the importance of medical device associated infections to public health, the Division of Biology, Chemistry and Material has established a Sterilization and Infection Control program to develop RSTs in key areas. Programmatic priorities were identified through robust input from internal and external stakeholders as Alternatives to Ethylene Oxide Sterilization and Device related Infections. Within the Device Related Infections area, efforts in reprocessing to address cleaning and drying of endoscopes, testing methodologies to address biofilms and antimicrobial technologies are being pursued internally and in collaboration with external stakeholders to accelerate RST development with the potential to aid manufacturers in assessing safety and efficacy of products.

SESSION 1: REUSABLE MEDICAL DEVICES

Effect of protein concentration on biofilm formation in polytetrafluoroethylene tubing

Presenter: **Garth James**, Principal Investigator[1], Associate Research Professor[2]
Co-author: **Laura Boegli**, Research Associate[1]
Affiliation: [1]Medical Biofilms Laboratory, Center for Biofilm Engineering, Bozeman, MT, USA.
[2]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Polytetrafluoroethylene (PTFE), also known by the brand Name Teflon™, is used in a wide range of applications ranging from cookware to medical devices. This is due to unique properties including heat resistance, low reactivity, high hydrophobicity, and low friction coefficient. It is the only known surface to which a gecko cannot stick. Biofilm formation in PTFE tubing is a concern for reusable medical devices if cleaning and disinfection procedures are inadequate. In this study, the amount of biofilm formed in PTFE tubing by *Enterococcus faecalis* and *Pseudomonas aeruginosa* under low nutrient conditions was investigated. Tubing pieces were inoculated with approximately 4 log colony-forming units (CFU) per milliliter of bacteria and protein (derived from ATS2015 artificial test soil) was added at concentrations of 0, 0.61, 6.1, and 61 μg/ml. The tubing was then incubated for 1 and 3 days at room temperature and the number of bacteria recovered from the surface was assessed by viable plate count. For experiments with a mixed species inoculum, selective agars were used for each species. The tubing surfaces were also examined by confocal scanning laser microscopy (CSLM) of LIVE/DEAD™ stained samples as well as scanning electron microscopy (SEM).

Not surprisingly, the number of bacteria recovered from the surfaces increased with protein concentration, with a mean ± standard deviation of 0.45±0.86 and 1.78±0.81 CFU/cm2 at 0 μg/ml and 3.63±0.08 and 5.01±0.06 CFU/cm2 at 61 μg/ml for *E. faecalis* and *P. aeruginosa*, respectively, after one day. *E. faecalis* had a greater CFU/cm2 than *P. aeruginosa* after one day. Variability was higher at the lower protein concentrations. For *E. faecalis*, significant increases in CFU/cm2 were not observed when the incubation time was increased to 3 days. However, for *P. aeruginosa* the mean CFU/cm2 increased at all protein concentrations, except 0 μg/ml. Comparison of CFU/cm2 along the top, middle, and bottom of the tubing segments indicated that the bacteria were relatively uniformly distributed along the tubing length.

CSLM analysis revealed low numbers of individual bacterial cells at the lower protein concentrations, and more bacteria, as well as microcolonies, when the protein concentration was 61 μg/ml. Interpretation of SEM images was complicated by the presence of many objects on the surfaces.

Experiments were also conducted where the fluid was drained from the tubing after two hours and then the tubing was incubated for one day. For E. faecalis, the CFU/cm2 was lower for the drained tubing than the filled tubing at all protein concentrations except 0 μg/ml.

When the inoculum contained both *E. faecalis* and *P. aeruginosa*, the latter dominated at all protein concentrations except 0 μg/ml, even when the inoculated population of *E. faecalis*, was 10 times higher than *P. aeruginosa*.

Overall, this study indicated that at low protein concentrations bacteria attached to the PTFE tubing as dispersed individual cells. The number of bacteria on the surface increased as the protein concentration was increased. At the 61 μg/ml protein concentration, some small microcolonies were observed.

Biofilm cleaning performance evaluation in automated endoscope reprocessors

Presenter: **Bruno Haas**, Group Leader
Affiliation: Scientific, Research and Validation Team, STERIS Corporation, Quebec, CA.

Automated Endoscope Reprocessors (AER) are devices designed to automatically clean and disinfect endoscopes. They ensure a reproducible and monitored process that can, under certain conditions, replace manual cleaning. Indeed, manual cleaning of endoscopes is complex and prone to errors that can lead to biofilm formation within the channels. Such biofilm is known to be responsible for outbreaks in healthcare facilities. The American National Standard ANSI/AAMI ST91:2021 describes processing of endoscopes in healthcare facilities. Throughout the standard, an emphasis is made around biofilm prevention. However, neither the American National Standard Institute nor the US FDA endorse biofilm methods to assess AER performance testing for biofilm cleaning in endoscopes. The International Standards Organization (ISO) 15883-4 standard describes tests to validate endoscope washer-disinfectors, including AERs. This standard describes the use of a biofilm test soil to demonstrate performance of AER self disinfection cycle, as well as biofilm cleaning in endoscope channels. The model allows biofilm growth inside polytetrafluoroethylene (PTFE) channels to create test samples that will later be embedded in endoscope channels surrogate devices. The test allows assessment of biofilm reduction during the cleaning phases of the AER process. This talk will present an overview of the challenges in endoscope reprocessing, the reality of regulatory expectations regarding biofilm cleaning in endoscopes, and a description of the methodology to obtain biofilm test soil samples and how they are used in order to validate biofilm cleaning performances of endoscopes channels in AERs according to ISO 15883 standards.

Current state of flexible endoscope reprocessing: Why is build-up biofilm a concern?

Presenter: **Michelle Alfa**, FCCM, President
Affiliation: AlfaMed Consulting Inc, Winnipeg, MB, CA.

Outbreaks associated with multi-antibiotic resistant Gram negative bacteria have been reported for a number of different flexible endoscope models and makes. A 2022 post-market clinical study in the USA reported that in non-outbreak situations 4.1% of duodenoscopes may be contaminated with High Concern (HC) organisms derived from the gastrointestinal tract, skin, environment, or water. Build-up Biofilm (BBF) in flexible endoscopes (*Fig. 1*) is different from traditional continuously hydrated biofilm because it includes cyclical exposure to patient-derived organic material and microorganisms, cleaning, High Level Disinfection (HLD) and storage. The key "weak links" that currently exist in endoscope reprocessing that facilitate BBF will be reviewed. These include lack of adequate friction in current manual and automated cleaning methods for narrow channels, contamination of final rinse water, residual moisture during storage, micro-cracks in flexible polymers that may sequester bacteria and the inner lumen junctions and diameter changes that affect cleaning efficacy. Environmental Gram positive spore forming organisms are frequently ignored and their role in BBF will be discussed. In addition, the need for appropriate sampling and culturing of endoscope channels to facilitate detection of bacteria in the viable but non-culturable state is a crucial consideration. The key objective is to identify areas where new, novel advancements are needed.

SEE NEXT PAGE FOR FIGURE 1

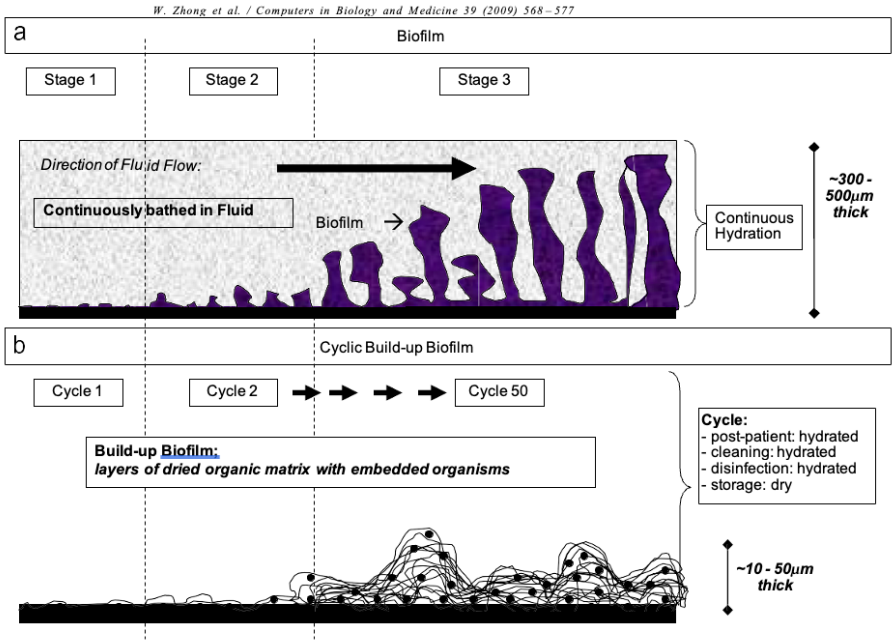

Fig. 1. Biofilm and cyclic build-up biofilm on medical devices.

Analyzing biofilm reduction for cleaning of medical device surfaces

Presenter: **Katie Segars**, Assistant Director
Author: **Jon Weeks**, Research Microbiologist
Affiliation: Division of Biology, Chemistry and Materials Science, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, US FDA, Silver Spring, MD, USA.

Healthcare-associated infections (HCAIs) attributable to medical devices not only threaten the patients' health and life but also bring additional economic burden to the patients and healthcare system. Some conditions which can relate to infections may include inadequate cleaning, disinfection and sterilization of reusable medical devices. In particular, biofilm formation on medical devices can occur because of insufficient reprocessing. Without adequate reprocessing soils can be left behind after cleaning of the device, resulting in insufficient disinfection. These can lead to microbial proliferation on and biofouling of reusable medical devices, increasing the potential for patient infection. Through stakeholder engagement we have identified a research gap related to quantifiable demonstration of biofilm cleaning. Additional related concerns include a lack of reliable biofilm production methods, absence of markers specific to biofilms and unknown acceptable residual levels. Based on these concerns, we plan to develop reproducible methods of biofilm development, markers for assess biofilm reduction, and quantification of these markers. We intend for these to be used during reprocessing validation testing to evaluate reduction or removal of biofilm. We believe that this science-based approach and collaboration with academic researchers and manufacturers will increase the efficacy of reprocessing procedures.

PANEL DISCUSSION

Assessing cleaning and disinfecting procedures for reusable medical devices to improve patient safety

Moderator: **Darla Goeres** (CBE)
Panelists: **Michelle Alfa** (AlfaMed Consulting), **Bruno Haas** (STERIS), **Garth James** (CBE), **Laura Wahlen**, (Baxter Healthcare), **Katie Segars** (US FDA)

The medical community is reliant upon reusable medical devices. For instance, medical scopes are necessary clinical tools for imaging, diagnosing, and in some instances, required for treatment procedures, yet these scopes are not compatible with heat sterilization. Nor are the heater/cooler units used in open heart surgery. Therefore, these devices require very intricate cleaning and disinfection/sanitization procedures, yet patient safety remains a critical issue. In this panel, we

will continue to explore the questions surrounding how to ascertain whether or not cleaning and disinfection/sanitization procedures effectively reduce cross-contamination for reusable medical devices and therefore provide for patient safety.

SESSION 2: BIOREMEDIATION

In situ bioremediation of selenium and nitrate for full scale treatment of mine waste

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

Active in-situ microbial reduction of nitrate and soluble selenate to selenite and elemental selenium (less mobile) was induced by subsurface methanol injections and can stabilize selenium (Se) in mined waste rock. Biogeochemical processes require careful balancing of oxidants (oxygen and nitrate) and reductants (methanol). Pulsed nutrient injection strategies were used in the field in attempts to minimize near-well biofouling. Molecular biology and biological engineering methods have been used to characterize the microbial ecology and metabolic capacity of waste rock to treat mine-affected water for mining operations in the Elk Valley, located in southern British Columbia, Canada.

Laboratory scale batch and column studies with native microbes demonstrated the capacity to reduce nitrate and Se in saturated waste rock and showed that oxygen and nitrate inhibition of Se reduction was overcome via carbon addition. Biofilm grown on waste rock in saturated aerobic column tests was capable of 50% to 99% nitrate reduction followed by 40% to 95% Se removal; Se was sequestered in the biofilm predominantly as elemental selenium. Denitrification and Se reduction was most rapid and efficient under suboxic conditions, and as high as 99% removal.

These results were scaled up to a pilot test and ultimately to a full scale in-situ saturated rock fill bioremediation system treating over 20 million L/d. In-situ biofilm coupons were deployed to track the microbial community structure using 16S rRNA gene sequencing. Applying the tools of molecular biology, bioengineering, geochemistry, and principles of microbial ecology to the understanding of biomineralization/bioprecipitation has been effective for management of nitrate and Se in mining settings.

Pharmaceutical effects on a novel wastewater treatment biotechnology

Presenter: **Kylie Bodle**[1,2], PhD Student
Co-authors: Madeline Pernat[1,2], Rebecca Mueller[1,3], Heidi Smith[1,2], Catherine Kirkland[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Civil Engineering, Montana State University, Bozeman, MT, USA.
[3]USDA NRS Agricultural Research Service, Albany, CA, USA.

Pharmaceutical compounds are increasingly detected in environmental matrices around the globe. The presence of these compounds can be traced, in large part, to their release from wastewater treatment plants. Conventional activated sludge treatment processes are generally not designed to remove pharmaceutical compounds from wastewater; thus, any removal is incidental at best and often ineffective. Numerous pharmaceuticals have toxic effects on plants, wildlife, and microbiota at part per billion concentrations. Additionally, pharmaceuticals can harm wastewater-treating bacteria, potentially resulting in decreased treatment efficacy as these compounds accumulate.

Aerobic granular sludge (AGS) is a promising emerging biotechnology for wastewater treatment that also shows promise for pharmaceutical removal. AGS consists of highly diverse communities of wastewater bacteria that self-aggregate into dense, spherical biofilms several millimeters in diameter. The high concentration and variety of extracellular polymeric substances (EPS) in AGS confer protection from various toxins, such as pharmaceuticals, and may provide a sorptive medium for pharmaceutical removal. However, the body of literature available on granule-driven pharmaceutical treatment is limited, and therefore more information is needed on how granules respond to a wide range of pharmaceuticals.

This study investigated how a mixture of three model pharmaceuticals impacted granule morphology and microbial community and function. Erythromycin (ERY, antibiotic), diclofenac (DCF, anti-inflammatory), and gemfibrozil (GEM,

lipid regulator) are commonly found in wastewater and the greater environment, but few studies exist on the impacts of these compounds on AGS. As such, a 150 μg/L mixture of the forementioned pharmaceuticals was dosed into an AGS-sequencing batch reactor for 80 days, and wastewater treatment efficacy and pharmaceutical fate were also monitored. The performance and characteristics of AGS in a control reactor were also evaluated and compared with those of the test reactor.

In brief, pharmaceutical removal occurred temporarily due to a combination of adsorption and biodegradation and caused a significant reduction of the lipid barrier in exposed granules (*Figure 1*). Phosphate and total nitrogen removal also decreased and did not recover. The relative abundance and activity of key wastewater-treating microbial communities declined in the test reactor, which likely explains poor treatment performance. Metatranscriptomics analyses are planned and may further understanding of pharmaceutical impacts. For example, GEM exposure may be the reason for decreased lipid barriers: GEM stimulates human production of lipoprotein lipase, an extracellular enzyme that degrades triglycerides. Multiple bacterial genera, such as *Pseudomonas* and *Bacillus*, also produce lipases; therefore, it is possible that lipase production by these bacteria may have been stimulated by GEM.

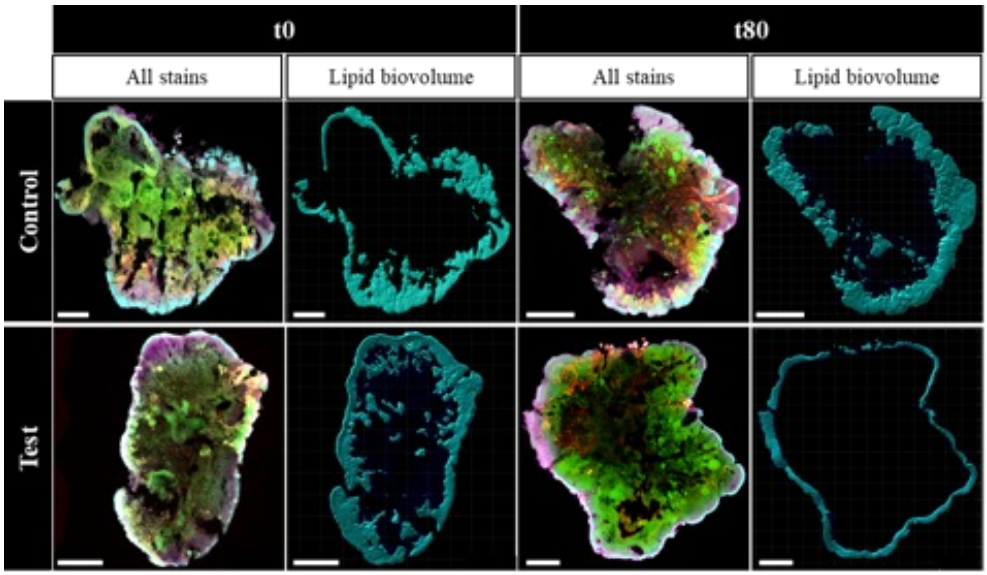

Fig. 1. Lipid barriers declined in pharmaceutical-exposed ("test") granules over time. Results of EPS staining are shown: Green indicates proteins (FITC); cyan, lipids (nile red); red, alpha-polysaccharides (concanavalin A); and pink, beta-polysaccharides (calcofluor white). Multiple EPS components overlay each other resulting in blended color schemes in stained images. Lipid biovolume images were created with Imaris software and show the 3D-distribution of lipids in a 30 µm-thick granule section. At day 0, lipids were present in test and control granules at both the granule boundary and deeper into the granule center; however, by day 80, lipids in test granules only existed in a thin boundary at the outer edge of the granule. All scale bars are 200 µm.

Bioremediation – State of practice – A 'sampler' for biofilm devotees

Presenter: Jim Cummings
Affiliation: US EPA, Washington, DC, USA.

Abstract forthcoming.

Biofilm considerations: Bioremediation of contaminated groundwater

Presenter: **Birthe Veno Kjellerup**, Associate Professor

Affiliation: Department of Civil & Environmental Engineering, University of Maryland, College Park, MD, USA.

Access to clean drinking water is an important human right, which is threatened many places in the US and in countries around the world. Trichloroethene (TCE) is a common and toxic groundwater contaminant impacting millions of people thus development of cheap and accessible materials to sustainably remediate contaminated groundwater is important. In this study, we discuss considerations for how biofilm-based bioremediation can be implemented for in situ clean-up. We investigate if upcycled organic waste products can promote biofilm formation and also enhance TCE bioremediation in a contaminated groundwater site. In the field, a trench was installed that was made up by organic waste products from biosolids, limestone, and biochar. Field monitoring of water quality parameters was compared with microbial community changes obtained by analyzing soil samples collected from the site before and nine months after installation. The post-treatment results showed that the TCE concentration had decreased significantly from up-gradient the trench to below the detection limit down-gradient with simultaneous identification of TCE biodegradation products showing that dechlorination occurred in the trench. These findings corresponded with the presence of bacteria present in biofilm in the amended trench on this site.

Expedited organo-halide destruction via biostimulation without augmentation supported by introduction of abiotic electron donor

Presenter: **Kent Armstrong**, President[1]
Co-authors: Kent C. Armstrong[1], K. Rapp[2], H. Anderson[1], M.W. Fields[3]
Affiliation: [1]TerraStryke Products LLC, Andover, NH, USA.
[2]Pinnacle Engineering, Inc., Minneapolis, MN, USA.
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Background: This paper presents a concept suggesting the biogeochemical tools to remediate a given site biologically are present and regardless of the type of remedial input (amendment or augmentation) the results are ultimately dependent upon the carrying capacity of the treatment zone (ecosystem) to distribute microbial biomass and support microbial growth and activity.

Strategy: Sites where baseline conditions are supportive of microbial growth, communication and biofilm formation prior to introduction of an input (additive) realize positive results with the input of an additive whereas, site that are not supportive at baseline conditions, regardless of input, often fail to realize performance expectations. Are the results reportedly achieved serendipitously? Unfortunately, due to logistic, and financial limitations, sediment/soil/groundwater-associated microbial components are not properly tracked over space and time.

Conversely, sites where the nutritive capacity is depleted (scavenged by anthropogenic introduction of excessive amounts of PHCs/cVOCs), input(s) often don't work because the subsurface system did not have the carrying capacity to allow microbial growth and/or activity. In these cases, the industry conclusion is that the additive failed.

Process: To evaluate the effect of abiotic inputs to a biostimulated microcosm a 28-day study was performed. Microcosm 1 used the biostimulant ERDENHANCED as sole electron donor. Microcosm 2 contained approximately 8% by weight Hogänäs Sri5 metal in the ERDENHANCED to serve as an electron donating 'pump' (input). Both microcosms augmented with dehalorespiring consortium KB-1.

An accompanying on-site proof-of-concept study was performed to determine if results of the microcosm study were transferable to the field. Two monitoring wells were amended with respective microcosm additive formulations: MW-23A with ERDENHANCED as sole electron donor, MW-24A the combined formulation. Baseline TCE in MW-23A was 28,900 ug/L with Parent-Parent Daughter Ratio (P: PD) of 56%; In MW-24A baseline TCE was 176,000 ug/L with P:PD of 89%. Contaminants in saturated highly fractured bedrock sandstone. Groundwater was monitored every 6-weeks for 18-months.

Results: The laboratory study documented that the combined biotic/abiotic microcosm enhance the supportive capacity of the ecosystem by 1) expedited expression of autoinducing signaling molecule AI-2 (communication), 2) the presence of protein (biofilm), and 3) complete Tetrachlorethylene (PCE) biotransformation.

Six-weeks after the initial amending process TCE decreased >99% at both locations. TCE remained BDL at MW-24A throughout the evaluation. TCE at MW-23 increased slightly month 4-5; however, realized >99.99% reduction TCE at month 18 and a 3 order of magnitude increase Ethene month 10. Overall MW-23A realized >99.99% reduction TCE, 78.6% reduction in cis-DCE, 98.4% reduction in VC, all from peak bioavailability.

MW-24A realized >99.9% reductions in TCE, cis-DCE and VC with an increase in Ethene at month 6. The field evaluation confirmed that results of the microcosm evaluation were transferrable to non-augmented field conditions and addition of abiotic electron donor enhanced bioremediation and microbial activity.

Conclusion: Biofilms constitute the predominant mode of terrestrial growth. Biofilm microcolonies align themselves to optimize metabolic cooperation and efficiencies. We suggest the nutritive capacity of the ecosystem and its ability to support microbial growth, distribution, communication, and biofilm formation is paramount to realize sustainable and maximum microbial performance, regardless of who is there and how many are there to start.

SESSION 3: EMERGING SURFACE DISINFECTION METHODS

An overview of EPA's guidance and methods for adding residual efficacy claims

Presenter: **Rebecca Pines**, Branch Chief
Affiliation: Pesticide Programs; Biological and Economic Analysis Division; Microbiology Laboratory Branch, US EPA, Fort Meade, MD, USA.

Throughout the first year of the COVID-19 pandemic, external stakeholders frequently approached the United States Environmental Protection Agency (EPA) with proposals regarding a public health need for products with residual efficacy (*i.e.*, ongoing antimicrobial effect beyond the initial time of application, ranging from days to weeks to months). Currently, most EPA-registered liquid-based antimicrobial products are intended to treat hard, non-porous surfaces at the time of application, but do not provide efficacy beyond the time of application. Recurring requests from stakeholders and the public for products that are continuously active and can provide efficacy in between regular cleaning and disinfection led EPA to issue interim guidance and test methods for public comment in October 2020, thereby providing a pathway for companies to add claims of residual efficacy to their products' labels. Per agency guidance, products with residual efficacy claims fall into two major categories: (1) disinfectants that also provide residual efficacy (residual disinfectants), and (2) supplemental residual antimicrobial products (*e.g.*, coatings, paints, solid surfaces) that do not meet EPA's standards for disinfectants but are intended to be used as a supplement to standard List N disinfectants (products that meet EPA's criteria for use against SARS-CoV-2 (COVID-19) on surfaces). After receiving stakeholder feedback, revisions were made to the guidance document and the test methods for supplemental residual antimicrobial products. In October 2022, EPA issued revised guidance and test methods.

Residual disinfectants are evaluated using EPA's Interim Residual Self-Sanitization Protocol to continuously reduce bacteria and viruses in ≤10 minutes of exposure for up to 24 hours. To support residual bactericidal and virucidal disinfectant claims, products should achieve a ≥5-log reduction for bacteria and a ≥3-log reduction for viruses when compared to the parallel abrasion and re-inoculation controls. Supplemental residual antimicrobial products are evaluated using either EPA's Test Method for Evaluating the Efficacy of Antimicrobial Surface Coatings (EPA MLB Standard Operating Procedure (SOP) MB-40) or the EPA's Method for the Evaluation of Antimicrobial Activity of Hard, Non-porous Copper-Containing Surface Products (EPA MLB SOP MB-41) to demonstrate continuous reduction of bacteria and viruses within 1-2 hours of exposure when used as part of a comprehensive infection control program for weeks to years, depending on the product type. To support supplemental residual bactericidal or virucidal claims, these supplemental residual antimicrobial products should achieve a ≥3-log reduction for bacteria and viruses when compared to the parallel abrasion/chemical treatment controls.

ABSTRACTS CONTINUE ON FOLLOWING PAGE

Multiple multi-lab evaluations of the quantitative method for determining the efficacy of antimicrobial substances against bacteria dried onto hard non-porous surfaces

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

Since 1953, the use dilution method (UDM) has been the primary regulatory pathway in the US for registering liquid antimicrobials with public health claims. The original version of the UDM, standardized with AOAC International, was qualitative, testing antimicrobial efficacy against bacteria dried onto a surface based only on positive and negative outcomes with no concurrent positive controls. In 2009 the UDM was revised to require concurrent controls from which colony forming units (CFU) are enumerated, transforming the UDM to a semi-quantitative test method. A fully quantitative dried surface test method (QM) has also been developed and is being considered as an alternate regulatory pathway, in addition to the UDM, for registering liquid antimicrobials with public health claims in the US, for which CFUs are enumerated from both control carriers and carriers treated with an antimicrobial. Initial versions of the QM were standardized with AOAC International and ASTM international in the 1990's. The regulatory version of the QM has been updated several times over the past decade. Remarkably, after each revision, the reproducibility of the revised QM has been assessed by a multi-laboratory study. This talk will review the reproducibility results from the multiple multi-lab studies of the QM over the last 30 years and compare to the reproducibility of the semi-quantitative UDM.

Industry perspectives on the quantitative method to measure disinfection efficacy: Progress to improve reproducibility and parameters for assessing suitability as a regulatory standard

Presenter: **John Hilgren**, Vice President
Co-authors: The Efficacy Working Group consortium*
Affiliation: Regulatory Affairs, North America Region, Ecolab Inc., St. Paul, MN, USA.

Reproducibility is critically important to the scientific method, and scientists are increasingly concerned that lack of emphasis on reproducibility is creating predicaments that erode public confidence in science. For example, a reproducibility concern with the US standard to measure disinfection efficacy led to a government report claiming that the US EPA lacked assurance that disinfectant products worked. The question of disinfectant efficacy is further complicated by differing regulatory standards used across the globe for these products.

Progress has been made to improve regulatory standards for disinfectants. For example, an international workshop on efficacy test methods resulted in a scientific review that gave rise to a specification for technical elements to include in a future international method. The US EPA leveraged this work to develop the Quantitative Method.

In 2018, an industry consortium began research to understand the suitability of the QM as an alternative to the current US standard – the AOAC Use Dilution Method (UDM). Initial consortium testing focused on method equivalency. Before the equivalency research was complete, the consortium's testing revealed a reproducibility problem with the QM.

This presentation will cover follow-up research to explore laboratory versus analyst factors impacting reproducibility of the QM as well as suggestions for the practical application of a quantitative decision process developed by the Center for Biofilm Engineering for objectively determining whether a disinfectant efficacy method is reproducible.

**The Efficacy Working Group is a consortium consisting of Arxada LLC, The Clorox Company, Ecolab Inc., Procter & Gamble, Pilot Chemical, Reckitt, and Stepan Company. The EWG is administered by Patrick Quinn of The Accord Group and Rhonda Jones of Scientific & Regulatory Consultants, Inc.*

ABSTRACTS CONTINUE ON FOLLOWING PAGE

The use of antimicrobial pesticides to address Legionella in cooling towers

Presenter: **Anastasia Swearingen**, Senior Director
Affiliation: Chemical Products and Technology, American Chemistry Council, Washington, DC, USA.

Abstract forthcoming.

New method for *Legionella pneumophila* claims in cooling tower water

Presenter: **Lisa S. Smith**, Team Leader
Co-authors: Rebecca Pines, Michele Cottrill
Affiliation: Microbiology Laboratory Branch, US Environmental Protection Agency, US EPA, Fort Meade, MD, USA.

There is a need for antimicrobial products with label claims for *Legionella pneumophila* in cooling tower water due to the public health implications of Legionella in cooling tower water and requirements imposed by New York State that cooling towers be monitored for *L. pneumophila* and then treated with antimicrobials if necessary. Currently there are no EPA-registered biocides for use in cooling towers with label claims for *L. pneumophila* or other public health pathogens. Over the past few years, EPA has worked with the Centers for Biocide Chemistry and other relevant stakeholders to develop a draft method for assessing the efficacy of antimicrobial products against *L. pneumophila* in simulated cooling tower water (LSCTW). This quantitative suspension-based test method incorporates challenging test conditions such as water hardness and interferences (soil load and additives) to mimic the complex environment of cooling tower water.

To support the development of this new method, a three phase multi-laboratory collaborative study was conducted. In Phase 1, one concentration of reagent-grade sodium hypochlorite was evaluated at three contact times (5, 15, and 60 minutes) to generate a range of log reductions. Analysis of the data showed that the method was statistically significantly responsive to the increasing contact times (5 to 15 to 60 minutes). In Phases 2 and 3, a revised method was used to evaluate two biocides with only non-public health uses (e.g., slime control) at a variety of concentrations and contact times; further statistical analysis of the revised method is anticipated at the conclusion of Phase 3 (in progress).

Good riddance – The importance of biofilm removal and the need for standardized methods

Presenter: **Bruce Urtz**, Manager
Affiliation: Microbiology, Sterilex, Hunt Valley, MD, USA.

This presentation will explore the importance of biofilm removal, the pros and cons of various lab-based methods for assessing biofilm removal, and the need for standardized testing methods. While achieving some level of biofilm kill is useful, laboratory and field studies indicate that biofilm regrowth can occur, sometimes in as little as 24h. Regrowth may also result in a change in the microbial population making it more tolerant to future antimicrobial treatments. Furthermore, a biofilm matrix devoid of viable cells can still be problematic impacting various parameters like heat exchange and membrane permeability. For these reasons and more, biofilm removal is often critical in providing a successful microbial control outcome. As in other industries, biofilms are particularly problematic for food processors due to the resistance of biofilms to antimicrobial treatment and their potential to serve as a reservoir for the microbial contamination of food. As a result, food processors are aware of the importance of biofilm removal as a component of their cleaning and sanitization programs. In order to develop products and/or conditions capable of biofilm removal for this and other industries, standardized methods are needed. This is easier said than done. Decisions need to be made about which organism(s) to use, conditions for biofilm formation and treatment, and how to measure removal. Furthermore, what constitutes removal, 90%, 99%? While no method will ever truly mimic the "real world," the same can be said for any lab-based method (e.g., disinfection testing). Therefore, as has been done with other antimicrobial claims and associated test methods, there is a need for academic, industry and government laboratories to work together to develop a standardized method(s) so products can be evaluated and compared on a level playing field.

SESSION 4: GLOBAL PERSPECTIVES ON BIOFILM CLAIMS

Antibiofilm surfaces in the built environment: Current methods and considerations for method development

Presenter: **James Redfern**[1], Senior Lecturer
Co-authors: Alexander J Cunliffe[1], Darla Goeres[2], Nuno Azevedo[3], Joanna Verran[1]
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This presentation will consider data and potential future work for standardization resulting from a review of literature where authors are claiming a hard, non-porous material is "antibiofilm." Currently, there is no standard of testing a hard material in the built environment that claims antibiofilm properties (where the surface itself providing the action as opposed to application of disinfectants etc), and to help develop one, identification and analysis of existing methods and approaches used in the literature is required. Using a systematic approach, a search containing the key words "antibiofilm OR anti-biofilm AND coating OR surface OR material OR paint OR interface" was carried out using the SCOPUS database, resulting in 3,411 papers. These were filtered to ensure the paper was describing a hard material (but excluding those associated with the human body/medical devices), drastically reducing the number. The remaining papers were assessed to see if authors provided robust detail in their methodology, and consider: (i) if they define what they mean by a biofilm, (ii) if they define their pass criteria for antibiofilm activity, (iii) methodological approach including quantification of biofilm, use of controls and assessment of bacteria remaining on the surface after quantification or biofilm regrowth. Data provides an overview of current approaches in the literature, often mixed and missing information that would enable a third party to confidently agree a material is antibiofilm. Recommendations for future standardization are made.

Prevention and treatment of biofilms–Update on European method development and claims

Presenter: **Florian H.H. Brill**, Managing Partner
Affiliation: Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Hamburg, DE.

The talk will provide information about the legal and methodology situation in the European Union regarding biofilm claims. There are options for biocidal products regulated under the Biocidal Products Regulation (BPR) and active medical devices under the Medical Device regulation (MDR) where biofilm claims can be granted. In both regulatory frameworks preventive and curative biofilm claims are possible.

However, there is a lack of regulatory-wise accepted practice-like standard methods but the BPR e.g. mentions ASTM methods like ASTM E2196 and ASTM E2562 as acceptable. Some examples for biofilm claims and the underlaying methods used on currently marketed products in Europe will be shown and discussed.

A new, international biofilm task force under the CEN TC 216, Working Group 5 was formed recently which is in close cooperation with the UK-based group. Consequently, an outlook about the current and future developments of methods will be included in the talk.

The UK's standpoint on biofilm claims and methods in the medical devices sector

Presenter: **Paulina D. Rakowska**, Manager
Affiliation: Research and Innovation Development, National Biofilms Innovation Centre, UK.

The National Biofilms Innovation Centre aims to harness the UK's academic and industrial strengths in biofilms and to build a biofilm network focused on Research, Innovation, and Training across the UK and internationally. A critical unmet need for innovation across industry sectors is the infrastructure and support needed to demonstrate alignment to relevant standards and the associated analytical competencies. NBIC's national and international academic-industry road-mapping has consistently identified the establishment of global standards in biofilms as a priority need.

NBIC is actively addressing this need by establishing networks and collaborations and engaging in a variety of initiatives and research projects. We will provide an overview of our progress to date through road-mapping exercises, working with

standards bodies and direct stakeholder consultations, aimed at driving progress in achieving biofilm standardization.

Recently, NBIC in partnership with the Center for Biofilm Engineering, organized a Workshop on Biofilm Regulations and Standardization in Medical Devices and Pharma sectors, which took place in Birmingham, UK, on April 29, 2022. The meeting brought together over 40 representatives from industry, academia, metrology and standardization and regulatory bodies to map the current landscape, the needs, trends and expectations in biofilm standardization within the UK and to establish industry and regulatory participation in a forward working group. One clear point emerged from the discussions: There is a strong need for a comprehensive review of standards, methods and practices that are already in use by the community. This review will provide a base for gap analysis and identification of the most needed by the community standards. Following the workshop's outcome, NBIC commissioned a review of methods and standards to support biofilm-related claims in medical devices sector, the findings of which will be presented.

AGENDA STARTS ON NEXT PAGE

Center for Biofilm Engineering
a National Science Foundation Engineering Research Center

MONTANA STATE UNIVERSITY

CBE ENGINEERING

February 7-8, 2023

ANTI-BIOFILM TECHNOLOGIES:

Pathways to Product Development

Hilton Arlington National Landing Hotel, Arlington, VA

2/13/2023 4:40 PM

Final AGENDA

Tuesday February 7

7:30–8:00
Registration & Coffee
Commonwealth Foyer

Meeting: Richmond/Roanoke

8:00–8:15
Opening Remarks
Matthew Fields
Director, CBE; Professor, Microbiology & Cell Biology, MSU
Paul Sturman, Industrial Coordinator, CBE

Keynote Presentation

8:15–9:00
OSEL Sterility and Infection Control Regulatory Science Program: A focus on stakeholder needs
Michael Eppihimer, Director, Division of Biology, Chemistry and Material Sciences, OSEL, CDRH, US FDA

SESSION 1: Reusable Medical Devices

9:00–9:30
Effect of protein concentration on biofilm formation in polytetrafluoroethylene tubing
Garth James, PI, Medical Biofilms Laboratory, CBE; Associate Research Professor, Chemical & Biological Engineering, MSU

9:30–10:00
Biofilm cleaning performance evaluation in automated endoscope reprocessors
Bruno Haas, Group Leader, Scientific, Research and Validation Team, STERIS

10:00–10:30 Break

10:30–11:00
Current state of flexible endoscope reprocessing: Why is build-up biofilm a concern?
Michelle Alfa, President, AlfaMed Consulting, Ltd.

11:00–11:30
Analyzing biofilm reduction for cleaning of medical device surfaces
Katie Segars, Assistant Director, Division of Biology, Chemistry, and Materials Science, OSEL, CDRH, US FDA

Panel Discussion

11:30–12:30
Assessing cleaning and disinfecting procedures for reusable medical devices to improve patient safety
Michelle Alfa
Bruno Haas
Garth James
Katie Segars
Laura Wahlen, Baxter Healthcare
Moderator: Darla Goeres, CBE

12:30–1:30 Lunch Will/York

SESSION 2: Bioremediation

1:30–2:10
Session Introduction

In situ bioremediation of selenium and nitrate for full scale treatment of mine waste
Brent Peyton, Professor, Chemical & Biological Engineering, MSU, CBE

2:10–2:40
Pharmaceutical effects on a novel wastewater treatment biotechnology
Kylie Bodle, PhD Student, Civil Eng., MSU, CBE

2:40–3:10 Break

3:10–3:40
Bioremediation—State of Practice—A 'sampler' for biofilm devotees
Jim Cummings, Program Analyst, US EPA

3:40–4:10
Biofilm considerations: Bioremediation of contaminated groundwater
Birthe Veno Kjellerup, Associate Professor, Civil & Environmental Eng., University of Maryland

4:10–4:40
Expedited organo-halide destruction via biostimulation without augmentation supported by introduction of abiotic electron donor
Kent Armstrong, President, TerraStryke Products LLC

5:00–6:30 Networking Reception Crystal Ballroom

Wednesday February 8

7:45–8:20
Registration & Coffee
Commonwealth Foyer

SESSION 3: Emerging Surface Disinfection Methods

8:20–8:30
Session Introduction
Al Parker, Biostatistician, CBE; Associate Research Professor, Mathematical Sciences, MSU

8:30–9:00
An overview of EPA's guidance and methods for adding residual efficacy claims
Rebecca Pines, Branch Chief, Microbiology Laboratory Branch, Office of Pesticide Programs, US EPA

9:00–9:30
Multiple multi-lab evaluations of the Quantitative Method for determining the efficacy of antimicrobial substances against bacteria dried onto hard non-porous surfaces
Al Parker

9:30–10:00
Industry perspectives on the Quantitative Method to measure disinfection efficacy: Progress to improve reproducibility and parameters for assessing suitability as a regulatory standard
John Hilgren, Director, Regulatory Affairs, Ecolab

10:00–10:30 Break

10:30–11:00
Use of antimicrobial pesticides to address Legionella in cooling towers
Anastasia Swearingen, Senior Director, Chemical Products and Technology, American Chemistry Council

11:00–11:30
New method for Legionella pneumophila claims in cooling tower water
Lisa Smith, Team Leader, Microbiology Laboratory Branch, Office of Pesticide Programs, US EPA

11:30–12:00
Good riddance—The importance of biofilm removal and the need for standardized methods
Bruce Urtz, Microbiology Manager, Sterilex

12:00–1:00 Lunch Will/York

SESSION 4: Global Perspectives on Biofilm Claims

1:00-1:10
Session Introduction
Darla Goeres, Research Professor of Regulatory Science, CBE

1:10–1:40
Antibiofilm surfaces in the built environment: Current methods and considerations for method development
James Redfern, Senior Lecturer, Natural Sciences, Manchester Metro University

1:40–2:10
Prevention and treatment of biofilms–Update on European method development and claims
Florian Brill, Managing Director, Dr. Brill + Partner, GmbH

2:10–2:40
The UK's standpoint on biofilm claims and methods in the medical devices sector
Paulina Rakowska, Research and Innovation Development Manager, National Biofilms Innovation Centre (NBIC)

Save the Date!
2023 Montana Biofilm Meeting
July 11-13
Bozeman, MT

Food and Coffee

within a 5-minute walk from the Hilton

1. **Starbucks** — Coffee, $
2. **Subway** — Sandwich Shop, $
3. **Chipotle** — Mexican Fast Food, $
4. **Mezeh Grill** — Mediterranean, $
5. **Chick-fil-A** — Fast Food, $
6. **Buffalo Wild Wings** — Sports Bar, $$
7. **The Portofino** — Italian, $$
8. **Kabob Palace** — Afghani, $$
9. **Ted's Montana Grill** — American, $$
10. **Ruth's Chris** — Steak House, $$$$

Click the image or scan the QR Code for interactive map of the area with these locations marked