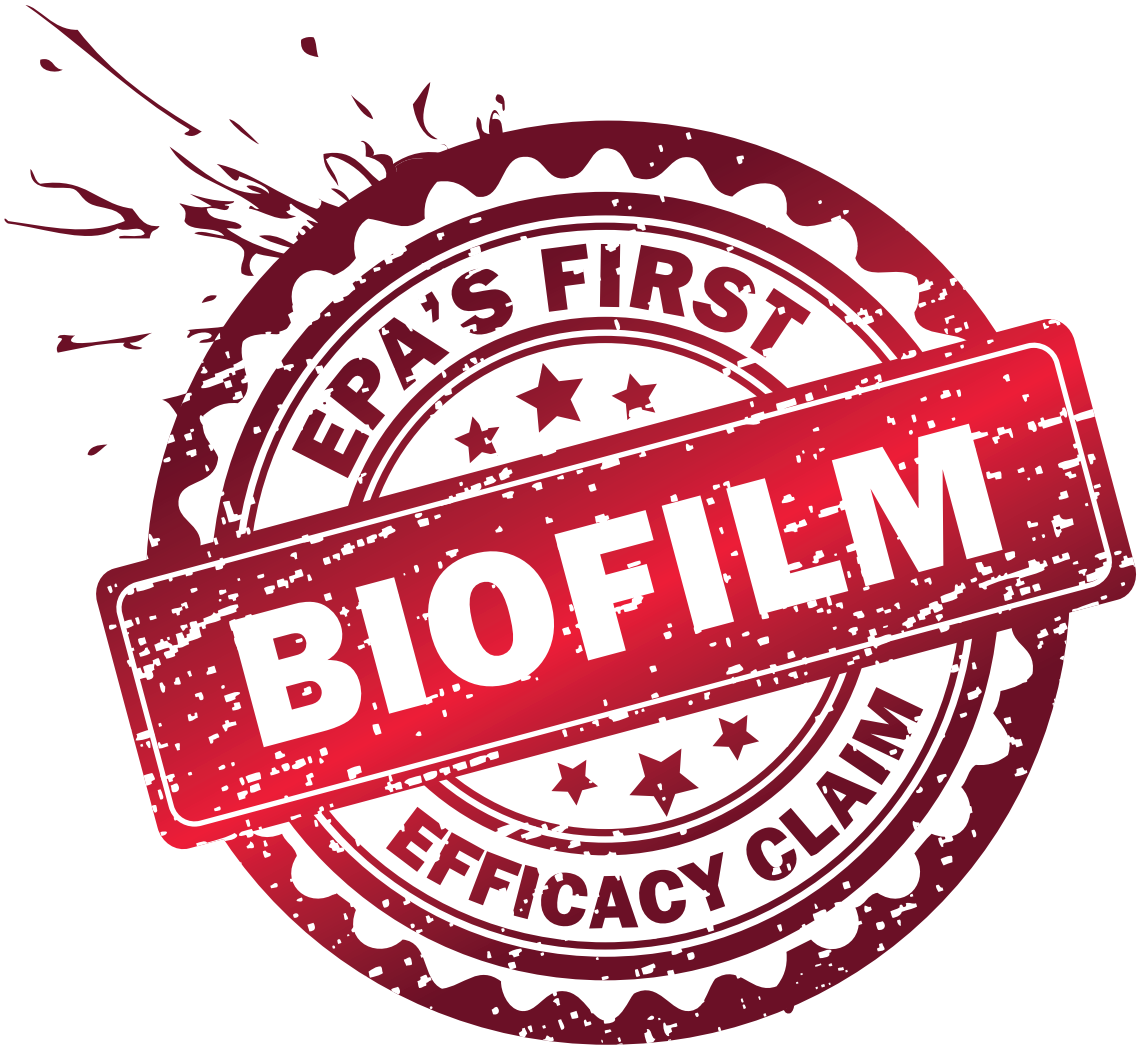
CBE ANNUAL REPORT

Center for Biofilm Engineering at Montana State University • 2019



A year after EPA adopts CBE's standard testing method, STERIS earns the country's first anti-biofilm efficacy claim

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CENTER FOR BIOFILM ENGINEERING

MONTANA STATE UNIVERSITY

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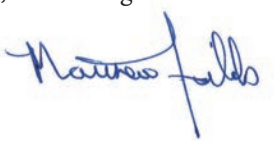
Biofilms Built the World's First Communities

Biofilms have always been about interactive communities. In fact, biofilms could be considered the world's first community builders. Some $3.5x10^9$ years later, scientists and engineers study the many ways biofilms impact the world. I'm thinking about this more and more as the year 2020 nears, when we will celebrate the 30th anniversary of the Center for Biofilm Engineering's founding. In 1990, the CBE became the world's first research center dedicated to biofilm. Today, the biofilm-research community includes scientists and engineers from around the world.

Between 1990 and 2019, the top biofilm research centers – the CBE, Harvard, University of Washington, University of Copenhagen, University of Calgary, and Stanford – have published nearly 180,000 papers on biofilms. And the expansion of knowledge will continue to grow. We look forward to identifying new avenues for collaboration with biofilm researchers around the world, including the Singapore Centre for Environmental Life Sciences and Engineering, the Costerton Biofilm Center in Copenhagen, and the new National Biofilms Innovation Centre comprised of top-notch researchers throughout the United Kingdom. CBE faculty, staff, and students travel to collaborate around the globe, and there has long been an international presence at the two CBE meetings hosted annually. The international biofilm research community network continues to expand, and the CBE was a "primary colonizer" for these interactive communities.

At the CBE, important interactions provide foundations for the CBE ecosystem. Our Industrial Associates are major "nodes" with whom close interactions provide applied context to the fundamentals we teach to our undergraduate and graduate students. These nodes are interconnected with our affiliated faculty from across multiple departments and truly bring interdisciplinary perspectives to our work.

In closing, I want to mention one group in particular, since the 2019-20 academic year is designated as the Year of Undergraduate Research at MSU. These emerging scientists and engineers are critical to our work. That's why throughout the pages of this magazine, you'll find articles that highlight the many ways these emerging scientists and engineers – and one high-profile alumna who worked here as an undergraduate! – enrich our community. So, look for the "Year of Undergraduate Research" logo that identifies these articles that convey so well why since 1990 the CBE has invested in nearly 1,100 undergraduate researchers to date.



Matthew W. Fields
Director, Center for Biofilm Engineering
Professor of Microbiology & Immunology and Research Fellow

IN MEMORIAM

Tim Magnuson, former CBE postdoctoral researcher and friend to many who continue to work here, died Oct. 5, 2019. He was 57.

Industry

Sharpens CBE's Focus

Partnering with Fortune 500 organizations and smaller companies alike helps ensure the CBE focuses on finding real-world solutions to real-world problems.

* Small-Business Member

The International Space Station has a biofilm problem in its wastewater plumbing. Without a solution, the problem could ground spacecraft to be used in future manned-missions to Mars. *Photo courtesy NASA.*

NASA Turns to the CBE

for Brainstorming Session on Biofilms in Space

By Skip Anderson

The International Space Station has a biofilm problem in its wastewater plumbing. While the biofilm that's growing in the water recycling system poses no immediate threat to the astronaut scientists orbiting some 220 miles above earth, it's a problem that needs a solution, nonetheless. Without one, NASA's biofilm problem is sure to follow tomorrow's astronauts on missions to the moon, Mars, and possibly other destinations where maintenance shuttles will be impractical to come by.

In the same week as the 50th anniversary of man first walking on the moon, NASA engineers convened in Bozeman to brainstorm potential solutions with scientists from the Center for Biofilm Engineering and attendees of its Montana Biofilm Meeting shortly after the annual meeting wrapped up July 18.

"We plan to use these ideas to design concepts that we'll develop into a trade study," says Layne Carter, manager of the water subsystem on the International Space Station. "If it goes as planned, we'll do a technology demonstration on the ISS. Will that be in two years? Three years? I do not know. But it will be as fast as we can do it."

The wastewater on the ISS is comprised of two primary waste streams: crew urine and humidity condensate from respiration and perspiration, according to information provided by NASA. None of these liquids have a biocide to inhibit microbial growth. As a result, biofilm forms in the wastewater plumbing.

Carter and other NASA personnel briefed the group on the problem and what the solution needs to accomplish. He also stated an obvious — but important — caveat: while the long-term solution for future spaceships will be installed on earth, the solution that will be tested on the ISS needs to be able to be implemented in microgravity.

Ideas discussed in the freeform meeting included biocides, antimicrobial coatings, redesigned storage receptacles, and using the sun's ultraviolet rays as a disinfectant.

"I'm thrilled by the feedback we've gotten from the people in this group," Carter says. "I'm ecstatic about that."

"The STERIS biofilm claim represents the culmination of 18 years of research at the CBE, starting with the development and validation of a method for growing biofilm in the CDC biofilm reactor,"

— Darla Goeres, Principal Investigator in the CBE's Standardized Biofilms Methods Laboratory

CBE Industrial Associate STERIS Earns

First EPA Biofilm Claim

By Skip Anderson

An Industrial Associate of the Center for Biofilm Engineering received the first biofilm claim to be authorized under U.S. EPA regulations. Ohio-based STERIS Corporation earned the claim for its ProKlenz® ONE alkaline detergent in 2018. The CBE was part of the team that developed the standard testing method that the U.S. EPA requires products meet or exceed for the company to use the biofilm claim in the marketing of the product.

"The STERIS biofilm claim represents the culmination of 18 years of research at the CBE, starting with the development and validation of a method for growing biofilm in the CDC biofilm reactor," says Darla Goeres, the principal investigator in the CBE's Standardized Biofilms Methods Laboratory. "The biofilm claim is an accomplishment for STERIS, and it represents what can be accomplished when academia, regulatory agencies, industry, and standard-setting organizations collaborate."

Goeres was instrumental in developing the method adopted by the EPA in 2017.

"It's fitting that the first biofilm claim went to a CBE Industrial Associate," says Paul Sturman, research professor of environmental engineering and coordinator for the CBE's Industrial Associates program. "Our researchers work closely with STERIS and our other Industrial Associates, as well as with regulatory agencies, to ensure their decisions are based on sound science. As the awareness of the challenges and costs caused by biofilms continues to grow, this is a competitive advantage to our Industrial Associates in the marketplace."

STERIS is a leading provider of infection prevention and other procedural products and services. The company, which has 70 offices on five continents, focuses primarily on healthcare, pharmaceutical, and medical device customers. STERIS announced via a press release that it had obtained the first biofilm claim.

"Microbial contamination, specifically biofilms, can be a significant challenge for our customers," Michael Gietl, senior product manager for STERIS Life Sciences, said in the December 2018 press release. "Years of research and data collection gave us the confidence to pursue this performance claim, and we've demonstrated the product's ability to remove one of the most challenging soils in the pharmaceutical and biopharmaceutical industries." ■

SEE STERIS CORPORATION'S PRESS RELEASE FOR MORE INFORMATION.
HTTP://BIT.LY/CBE_STERIS

CBE by the Numbers

FY2019

52
TESTING PROJECTS

34
SPONSORS

$687,530
GENERATED

CBE Ramps Up
Outreach Tools

By Skip Anderson

The Center for Biofilm Engineering has recently upgraded its outreach initiatives, especially when it comes to supporting and expanding its innovative Industrial Associates program.

"We heard some suggestions during our annual Montana Biofilm Meeting that made a lot of sense to [CBE director Matthew Fields] and me," says Paul Sturman, coordinator of the Industrial Associates program. "We're always looking for ways to connect with our IAs, so we've launched several communication tools to facilitate that."

The newly launched tools include a webinar series titled "5 in 25" in which a CBE researcher delivers five key points in 25 minutes, followed by a Q&A session. The live, invitation-only webinars are available at no charge exclusively to Industrial Associates.

"Our first webinar, conducted by Darla Goeres in September, exceeded our expectations," Sturman says. "Several of our Industrial Associates each had more than 10 people attend. Dr. Goeres' presentation was as insightful as it was succinct, and the questions posed by attendees demonstrated a level of engagement that speaks to the strength of the partnerships with our IAs."

Goeres, principal investigator of the CBE's Standardized Biofilm Methods Laboratory, titled her talk, "Viable Plate Counts Can't Do It Alone."

Other new tools, including a set of 15 slides available exclusively to IAs for download, are designed to help designated representatives educate their colleagues and clients about biofilms. The CBE also recently published a new training video that focuses on the single-tube method for determining disinfectant efficacy against biofilm grown on coupons. The CBE is also placing a renewed emphasis on its Twitter stream [@Center4Biofilm]. Other tools already under development prior to the meeting include a 6-minute documentary that explains broadly what biofilms are, some of the risks they carry, and opportunities they present. The CBE also recently made available a "one-pager" that provides elevator-speech information about biofilms' costly impact on several key sectors, including medical, energy, water, and infrastructure.

For more information, contact Paul Sturman at paul_s@montana.edu or 406-994-2102.

INDUSTRY VISITORS

Takashi Abe, Jason Ashraf, Takashi Kinebuchi, Mason Nagasaki from Olympus

Denis Bendejacq, Richard Jacubinas, Gilda Lizarraga, Sasha Zavgorodnya from Solvay

Sailaja Chandrapati from 3M

Mike Marotz, Todd Roper from Carboceramics

Jillian Vocke, Tyler Zanon from Medline

CBE VISITS TO INDUSTRY

Matthew Fields, Argonne National Laboratory, Chicago, IL,

Paul Sturman, L'Oreal, Newark, NJ

Paul Sturman, PureLine Systems, Chicago, IL

Jim Wilking, STERIS Corporation, St. Louis, MO

FACULTY VISITORS

Nicolas Forquet, National Institute for Environmental and Agricultural Science and Research, Lyon, France

Bastiaan Krom, University of Amsterdam, The Netherlands

Ayrat Ziganshin, Kazan Federal University, Kazan, Russia

Elvira Ziganshina, Kazan Federal University, Kazan, Russia

The CBE also hosted Holger Class, Johannes Hommel, Nikos Karadimitriou, Joseph Piotrowski, Holger Steeb, and Felix Weinhardt from the University of Stuttgart, Germany, for a weeklong biofilm and biomineralization workshop.

STUDENT VISITORS

Mathieu Devos, Masters Student, University of Antwerp, Belgium

Emily Gan, High School Student, Jericho, NY

Rachel Kleiman, Masters Student, University of North Carolina, Chapel Hill, NC

Elizabeth Lee, High School Student, Bozeman, MT

Albert Enrique Tafur Rangel, PhD Student, Universidad de los Andes, Bogotá, Colombia

Leading the Way in Biofilm Research

The CBE is the world's first, largest, and best-known biofilm research center.

CBE Primary Areas of Research

- Biofilm Control Strategies
- Energy Solutions
- Environmental Technologies
- Health/Medical Biofilms
- Industrial Systems and Processes
- Standardized Methods
- Water Systems

Analyze This

CBE Biostatistician Al Parker Helps Researchers, Industrial Associates Find Meaning in the Data

By Skip Anderson

Albert Parker, PhD, joined the Center for Biofilm Engineering as its biostatistician in 2008. His is a highly specialized skill set that many bioscience-based research centers in academia and industries alike don't have. Instead, they often rely on analyses performed by statisticians with no formal training in the biosciences. This can limit the effectiveness of the analyses and risks shortchanging the researchers in the process. "A statistician can interpret the data, but they might not be able to speak the language of the researchers," Parker says.

You earned a master's degree in statistics from Montana State in 2004, a year *after* earning your PhD in mathematics. That's a bit unusual. How is it that the PhD came first?

By the time I graduated with my PhD in mathematics, I had taken so many stats courses that I only needed a few more to get an MS in statistics. And I'm glad I did. For my PhD, my collaborators and I studied how sensory neurons in crickets encode external wind stimuli. As I analyzed the neural "spike trains" and related them to different wind patterns, I not only learned the basics of probability and statistics, but I was advancing the science. The statistics allowed us to contextualize the data in a quantitative and meaningful way.

Where at the CBE might those skills apply?

Statisticians are experts at assessing variability. Anybody can guess how many jellybeans are in a jar. But a statistician will put error bars — a measure of the variability of the data — around the estimate, for example, by gathering data from other jars with jellybeans. In a biofilm, we might want to know how many "bugs" are in there. Anyone could make a guess at that. But statisticians are going to put error bars on their estimate, which is backed by data and provides scientific confidence in the result.

One focus of my work is image analysis. There are lots of available software packages that will guess how the bugs are spatially arranged in a particular biofilm sample. But, unfortunately, many of these software packages do not provide error bars to go along with estimates of different biofilm characteristics. This makes the estimate less statistically valid. I have a background in computer coding. So, I worked with several researchers at the CBE and developed an image-analysis program that estimates quantities like the intensity threshold, biofilm thickness, and biofilm volume, and also provides error bars that can be used to convey probability statements about how confident we are in the estimates of these biofilm characteristics. That program extended our toolbox for image analysis of biofilms. We published a paper on this in 2018 in the *Journal of the American Statistical Association* on this work titled, "Polynomial Accelerated Solutions to a Large Gaussian Model for Imaging Biofilms: In Theory and Finite Precision." [See the URL and QR code at end of the interview.]

Experimental design is another facet of what I do. I encourage the students and faculty at the CBE to consult me before they conduct their experiments.

"Anybody can guess how many jellybeans are in a jar. But a statistician will put error bars — a measure of the variability of the data — around the estimate, for example, by gathering data from other jars with jellybeans."

— Al Parker, Assistant Research Professor of Mathematical Sciences and CBE Biostatistician

Sometimes researchers work too hard and spend more time and money than is necessary to get the data they need — they might do 20 replicates when they could have generated relevant data with many fewer replicates. I can help researchers design their experiments in a way that maximizes their efficiency while delivering scientifically sound results.

Photo by Sepp Jannotta.

You also work with the regulatory aspects of the CBE.

Yes. The CBE has had an active contract with the U.S. Environmental Protection Agency for more than 20 years now to assess and develop methods that test the efficacy of antimicrobials on hard surfaces. In fact, in 2016 the CBE team won the EPA's top science award for the innovative way that we put error bars on our method assessments. We published a paper on this in 2014 in the *Journal of the Association of Official Analytical Chemists International* titled, "A Statistical Model for Assessing Performance Standards for Quantitative and Semi-quantitative Disinfectant Test Methods." [See the URL and QR code at end of the interview.] The award didn't go to climate-change scientists or agricultural pesticide work or other high-profile work — it recognized CBE's hard-surface disinfectant work.

Which of the CBE labs do you work with?

I work with most of the labs we have here at the CBE, as well as other labs across MSU's campus. Many times I work with graduate students to assist in analyzing their data so they can analyze future data sets themselves.

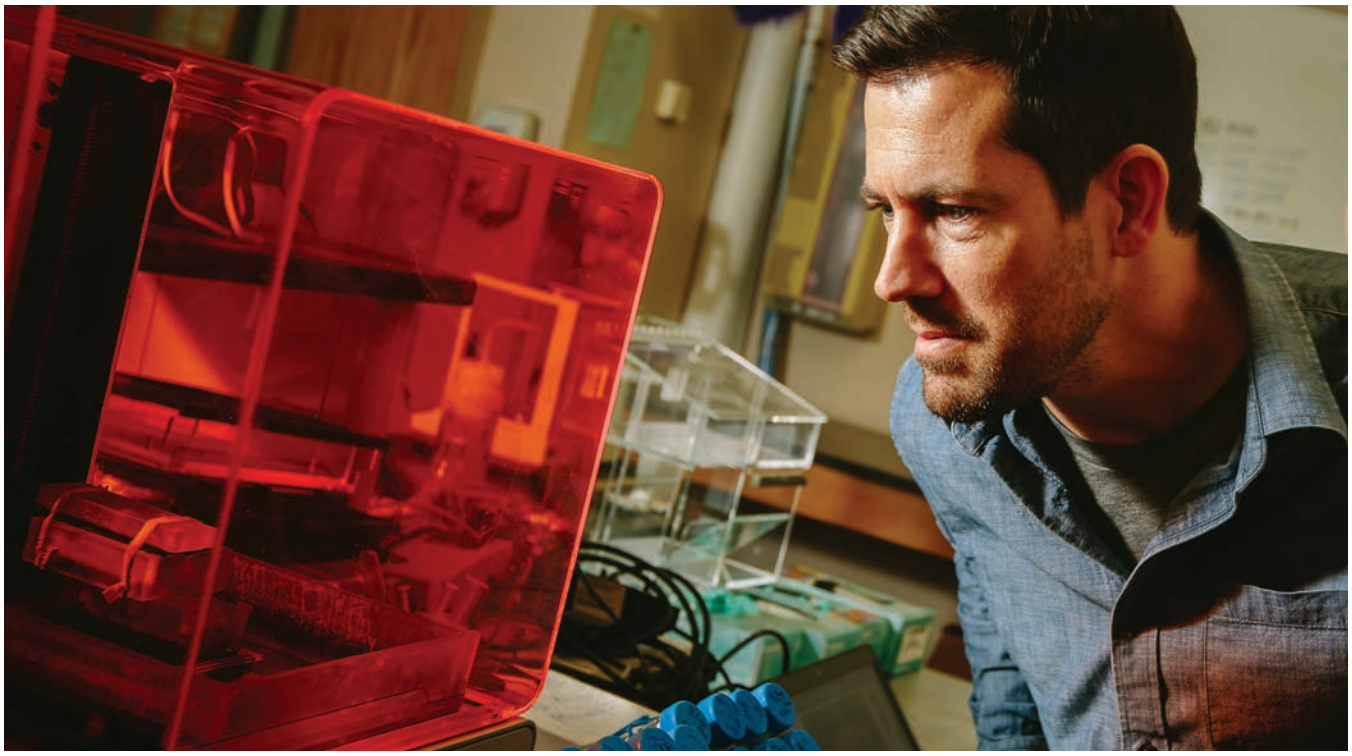
I also work extensively with our Industrial Associates and potential member companies. I provide study designs and analyses for clinical trials, animal studies, and in vitro laboratory testing of our members' products. This is a value-added benefit for company's participation in our Industrial Associate program. Our faculty provide site visits for our Industrial Associates that many times includes a workshop for their statisticians who might not have a background in the biosciences. Those workshops give them insight into how they could — and should — improve their experimental designs and statistical analyses; how to put error bars on it.

TO READ "POLYNOMIAL ACCELERATED SOLUTIONS TO A LARGE GAUSSIAN MODEL FOR IMAGING BIOFILMS: IN THEORY AND FINITE PRECISION," VISIT WWW.BIT.LY/CBE-PARKERPAPER-JASA OR SCAN THE QR CODE.

TO READ "A STATISTICAL MODEL FOR ASSESSING PERFORMANCE STANDARDS FOR QUANTITATIVE AND SEMI-QUANTITATIVE DISINFECTANT TEST METHODS," VISIT WWW.BIT.LY/CBE-PARKERPAPER-JAOACI OR SCAN THE QR CODE.

Researchers Turn to 3-D Printing

to Push Knowledge of Microbial Communities



James Wilking, CBE researcher and assistant professor of chemical and biological engineering (pictured) and Matthew Fields, CBE director, are utilizing 3-D printing technologies to enhance biofilm research. *Photo by Adrian Sanchez-Gonzalez.*

By Marshall Swearingen

As enthusiasm grows for 3-D printing, hailed by hobbyists and high tech industry as a new frontier in the creation of custom products, researchers at Montana State University are using the technology for another purpose: studying bacteria.

Backed by a $679,000 grant from the research wing of the U.S. Army, the research could lead to new insights into how microbial communities assemble to create antibiotic-resistant infections on hip implants or remove pollution from groundwater, among other things.

"It's exciting," says project co-leader James Wilking, researcher at the Center for Biofilm Engineering and assistant professor of chemical and biological engineering. "We're pushing the limits of this technology."

The project is riding a wave of recent advances in 3-D printers that use lasers to congeal water-based liquids. The results, according to Wilking, are objects that have the consistency of Jell-O and resolution of one micron — roughly 50 times smaller than the width of a human hair.

Until recently, such precise 3-D printers were largely confined to the automotive and aerospace industries because of costs exceeding $1 million. But prices have dropped dramatically in the past decade, making the tools more available for science, Wilking says.

His team, which is co-led by Matthew Fields, director of the Center for Biofilm Engineering, will use the tool to construct tiny grids of bacteria and study the microbes' interactions.

All forms of life, whether termites in an earthen mound or grasses on the prairie, assemble to perform basic

"We're pushing the limits of this technology."

— James Wilking, CBE Researcher and Assistant Professor of Chemical and Biological Engineering

functions, Fields says. And it's no different for biofilms, which are microbial communities that organize themselves in thin, slimy mats. Studying biofilms is a challenge because those structures are often spontaneous, ever-changing, and are often situated in living systems – including the human body.

Wilking "is making tools that allow us to control the structure, and we can see how that affects the function," says Fields, who has studied a wide range of biofilms for decades as a professor in the Department of Microbiology and Immunology in MSU's College of Agriculture and College of Letters and Science. The use of 3-D printers has the potential to generate major breakthroughs in scientists' understanding of how biofilms work, he says.

Scott McCalla, assistant professor in the Department of Mathematical Sciences in the College of Letters and Science, will use sophisticated mathematical modeling to study the patterns that the bacteria form as they grow within the constructed matrix, potentially leading to further insights about the microbes' behavior. Advanced 3-D mathematical models are a relatively new field, he says.

Wilking said he has watched eagerly for years as the pieces have come together to make the current project possible. The Army grant is the biggest confirmation yet that the research concept's time has come, he says.

The grant is from the Army Research Office. Robert Kokoska, microbiology program manager at the ARO, says the Army is interested in the potential of the technology to generate fuels, sense harmful contaminants and possibly convert them into less harmful substances, and convert food waste into electricity or fuel. ■

CBE by the Numbers

FY2019

NEW RESEARCH GRANTS
$2,543,630

42
PUBLICATIONS

2 **IMAGES PUBLISHED ON JOURNAL COVERS**

36
JOURNALS

40 MSU FACULTY AFFILIATED WITH THE CBE

Elliott Barnhart
Assistant Research Professor
Center for Biofilm Engineering

Roberta Amendola
Assistant Professor
Mechanical & Industrial Engineering

Jennifer Brown
Associate Professor
Chemical & Biological Engineering

Anne Camper
Professor
Civil Engineering

Ross Carlson
Professor
Chemical & Biological Engineering

Connie Chang
Assistant Professor
Chemical & Biological Engineering

Sarah Codd
Professor
Mechanical & Industrial Engineering

Kevin Cook
Associate Professor
Mechanical & Industrial Engineering

Al Cunningham
Professor, Emeritus
Civil Engineering

Markus Dieser
Assistant Research Professor
Chemical & Biological Engineering

Erika Espinosa-Ortiz
Assistant Research Professor
Chemical & Biological Engineering

Matthew Fields
Professor
Microbiology & Immunology

Christine Foreman
Associate Professor
Chemical & Biological Engineering

Michael Franklin
Professor
Microbiology & Immunology

Robin Gerlach
Professor
Chemical & Biological Engineering

Darla Goeres
Associate Research Professor
Chemical & Biological Engineering

Martin Hamilton
Professor, Emeritus
Mathematical Sciences

Roland Hatzenpichler
Assistant Professor
Chemistry & Biochemistry

Jeffrey Heys
Professor
Chemical & Biological Engineering

Garth James
Associate Research Professor
Chemical & Biological Engineering

Kelly Kirker
Assistant Research Professor
Chemical & Biological Engineering

Catherine Kirkland
Assistant Professor
Chemical & Biological Engineering

Ellen Lauchnor
Assistant Professor
Civil Engineering

Zbigniew Lewandowski
Professor
Civil Engineering

Luke McKay
Assistant Research Professor
Land Resources and Environ. Sciences

Albert Parker
Assistant Research Professor
Mathematical Sciences

Brent Peyton
Professor
Chemical & Biological Engineering

Adrienne Phillips
Assistant Professor
Civil Engineering

Elinor Pulcini
Assistant Research Professor
Chemical & Biological Engineering

Abbie Richards
Associate Professor
Chemical & Biological Engineering

Cecily Ryan
Assistant Professor
Mechanical & Industrial Engineering

Joseph Seymour
Professor
Chemical & Biological Engineering

Dana Skorupa
Assistant Research Professor
Chemical & Biological Engineering

Heidi Smith
Assistant Research Professor
Microbiology & Immunology

Otto Stein
Professor
Civil Engineering

Phil Stewart
Professor
Chemical & Biological Engineering

Paul Sturman
Associate Research Professor
Civil Engineering

Stephan Warnat
Assistant Professor
Mechanical & Industrial Engineering

James Wilking
Assistant Professor
Chemical & Biological Engineering

Tianyu Zhang
Associate Professor
Mathematical Sciences

*FY2019

Christine Foreman and members of the Cotton Glacier Field Team collect water samples from a supraglacial stream in Antarctica. Microbes on the melting glacier provide insights into climate change and its impacts. Foreman is a CBE-affiliated associate professor of chemical and biological engineering. *Photo courtesy Christine Foreman.*

Foreman, et al, Cite Microbes' Importance to

Fighting Climate Change

By Marshall Swearingen

A CBE researcher joined scientific colleagues around the world in calling for greater attention to microbes when studying and addressing global climate change.

Christine Foreman, associate professor of chemical and biological engineering, is one of 33 co-authors of an article titled "Scientists' warning to humanity: microorganisms and climate change," in the journal *Nature Reviews Microbiology*.

"Microbes are integral to the habitability of our planet," Foreman says. "A better understanding of global microbial diversity and activity is needed to appreciate future responses to our changing environment."

The article cites nearly 300 other published papers in summarizing how microbes underpin life on Earth, making them central to how the planet's biological systems will respond to human-caused climate change. Despite that important role, microbes are an "unseen majority" in need of greater scientific study, the authors state.

For example, half of all conversion of carbon dioxide into oxygen by photosynthesis is performed by cyanobacteria and other phytoplankton in the ocean, even though they constitute only 1 percent of the Earth's plant biomass, according to the authors. Some studies suggest that marine phytoplankton are in decline as a result of climate change.

By impacting microbes, climate change will also impact humans by way of changes in agriculture and infectious disease, according to the paper. For instance, increasing temperatures are expected to disrupt microbial communities that support soil fertility while expanding the habitable ranges of disease-carrying organisms such as mosquitoes and ticks.

Foreman's previous research on the relationship between microbes and climate change includes a 2017 paper in *Nature Geoscience* about microbes in glacial meltwater in Antarctica. The paper challenged the prevailing theory that the microbes consume primarily ancient organic carbon that was once deposited on glacial surfaces and incorporated into ice as glaciers formed by demonstrating that a large proportion of the carbon instead comes from photosynthetic bacteria. The researchers found that the photosynthetic bacteria produced roughly four times more carbon than was taken up by the microbes, resulting in an excess of organic carbon being flushed downstream.

The consensus statement paper also summarizes ways microbes could be used to stem climate change. For instance, microbes could be combined with municipal wastewater and plant matter to produce cellulosic biofuel, a substitute for gasoline.

"Hopefully this paper will help spur action that can help mitigate climate change," Foreman says. ■

CBE Researcher Helps Doctors Understand,

Treat Chronic Wounds

By Marshall Swearingen

Like physicians around the world, diabetic foot ulcers confounded Dr. Randy Wolcott. When patients would come into his Texas clinic with the small and seemingly harmless sores, the wounds would often resist traditional antibiotics and refuse to heal. In many cases, the infection would spread to the bone and become life-threatening.

"Someone who comes in with a diabetic foot ulcer is more likely to die than someone who comes in with a heart attack," Wolcott says, adding that today's rising incidence of the wounds is a "crisis."

Around 2001, Wolcott attended a conference where someone mentioned that the ulcers appeared to involve biofilm. Then he called the Center for Biofilm Engineering at Montana State University, one of the few places in the world that specializes in biofilm research.

As a result of research at the CBE, "our healing outcomes have increased dramatically," says Wolcott, who shared the latest details of this story at the annual Montana Biofilm Meeting in Bozeman in July. The research "has changed not just my practice, it has changed wound care," he says.

The turning point came in 2008, when Center for Biofilm Engineering researchers and Wolcott's clinic published the results of a study.

"What we found was that the majority of these chronic wounds had biofilm in them," says CBE researcher Garth James, the study's lead author.

The finding explained why the ulcers resisted antibiotics. Biofilms are uniquely resistant because they form layers with some bacteria acting as a shield for others.

The 2008 paper "basically created what we now call biofilm-based wound care," Wolcott says. It gave him the evidence he needed to explore new treatment methods. One, called debridement, involves removing larger areas of tissue from around the wound, a more drastic approach that pays off in long-term recovery. The practice was recommended by an international expert panel that Wolcott and James served on in 2017.

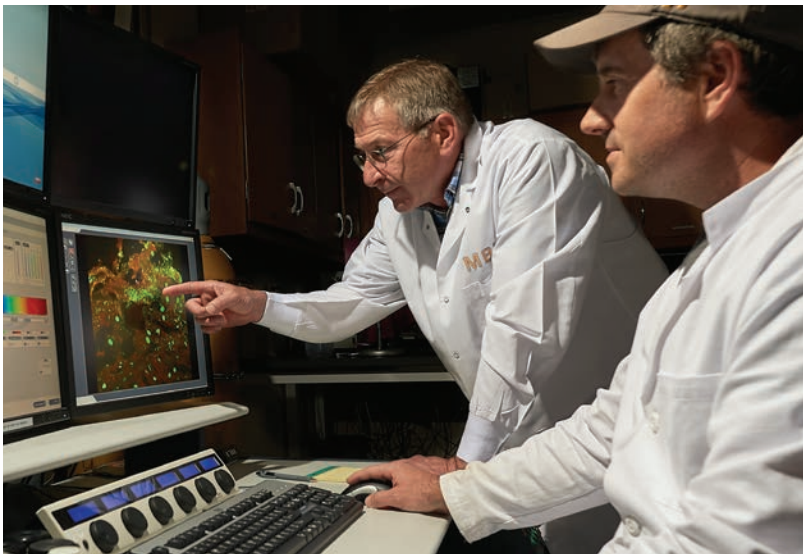
Chronic wound research in James' Medical Biofilms Lab at the CBE has also led to the development of medical products specifically designed to treat biofilm infections, including antimicrobial ointments that are more effective at penetrating and treating biofilms.

"The wound-care companies were impressed with the work that was going on here," says Paul Sturman, who coordinates the center's work with Industrial Associates. "They sought us out as a resource."

> Surgeons amputate 296 limbs with diabetic ulcers per day in the U.S. Biofilm is the culprit.

According to Wolcott, "there's a lot of work yet to be done" to improve treatment of chronic wounds. According to the American Diabetes Association, diabetic ulcers lead to 108,000 amputations in the U.S. each year.

"But now we know what the barrier is," Wolcott says. "And that's biofilm." ■



Garth James (left), principal investigator in the CBE's Medical Biofilms Lab, says biofilms are the reason chronic wounds such as diabetic foot ulcers and bed sores, fail to heal. *Photo by Kelly Gorham.*

Algae Researchers are

Driving Biofuels Toward Economic Practicality

Experiments being conducted by the CBE's Algal Research Group could lead to less reliance on fossil fuels. *Photo by Kelly Gorham.*

By Marshall Swearingen

In an effort to improve the feasibility of a renewable energy source, MSU researchers are exploring a potential breakthrough in producing biofuel from algae.

Backed by a $3 million grant from the U.S. Department of Energy, the research team, which includes scientists from University of Toledo and University of North Carolina, is in the early stages of a three-year project aimed at developing a biofuel process that could bypass a limitation that has long hampered the industry, according to Robin Gerlach, researcher at the Center for Biofilm Engineering and professor of chemical and biological engineering.

"This could transform the algae biofuel industry," says Gerlach, one of the project's principal investigators.

Algae contain oily substances that can be extracted and refined into biodiesel. Typically, algae are cultivated in large water tanks where carbon dioxide is injected to stimulate the growth of the photosynthesizing organisms.

Interest in algae biofuel peaked during the 1970s energy shortages and has since ebbed and flowed in response to oil prices. But the costs associated with supplying the algae with supplemental carbon dioxide from sources such as coal-fired power plants have discouraged commercial production, according to Gerlach. Now, the researchers think that a recently discovered strain of algae could be cultivated using only the ambient carbon dioxide of the atmosphere.

"This is a major societal challenge, and we're motivated to contribute to a solution."

Matthew W. Fields, CBE Director
and Professor of Microbiology and Immunology

"We're really excited about this," says Brent Peyton, CBE researcher, professor of chemical and biological engineering, and director of MSU's Thermal Biology Institute.

The algae, called SLA-04, has been shown to metabolize ambient carbon dioxide very efficiently, Peyton says.

"In the past we've found some algae and tried them out (with making biofuel)," Peyton says. "Now we're using state-of-the-art tools to move the technology forward. This is really quite advanced for a project on algae biofuel."

For instance, the team will sequence the algae's DNA and then use a process called "metabolic mapping" to identify which genes correspond with the algae's biochemical strategies under a variety of conditions.

"Ultimately, we want to optimize those strategies for biofuel production," says Ross Carlson, CBE researcher and professor of chemical and biological engineering. For example, the results could be used to fine-tune the amount of carbonate mineral added to the cultivation tanks.

Using the metabolic models, Blake Wiedenheft, associate professor of microbiology and immunology, will explore the use of the genome editing technique called CRISPR for enhancing the algae's ability to produce the oils desired for biofuel.

"I feel fortunate to be part of such an accomplished research team," Wiedenheft says, adding that the project could also provide insights into algae biology because much remains unknown about the genome of the aquatic organisms.

Project member Matthew Fields, CBE director and professor of microbiology and immunology, says he has long been familiar with the economic headwinds facing the algae biofuel industry.

"If we can find a way that is more cost effective, we could have a big impact," he said. "This is a major societal challenge, and we're motivated to contribute to a solution."

MSU's portion of the funding is $1.3 million.

YOUR YEAR OF UNDERGRADUATE RESEARCH MONTANA STATE UNIVERSITY

High school intern seeks answers to limitations of algae as a biofuel

In February, Emily Gan, 17, was reading a publication dedicated to biofuels. When she wanted to learn more about a particular strain of algae used in research at the Center for Biofilm Engineering, she called up the lead author of the study cited in the industry publication.

"She immediately struck me as a highly motivated young lady who had done a lot of background research to find proper laboratory support for her passion of studying algae for water remediation and biofuel production," says Robin Gerlach, professor of chemical and biological engineering and one of the principal investigators in the CBE's Algal Research Group.

Her initiative paid off. Gan, who is expected to graduate from Jericho (N.Y.) Senior High School in 2020, spent six weeks this summer working as an intern in the CBE Algal Research Group. After spending two weeks learning the ropes of her new surroundings, Gan was able to launch her own experiment designed to solve a dilemma limiting the viability of large-scale algae-based biofuels.

"Algae require light to thrive, but light penetration is limited once you get to a certain cell density," Gan says. "But if you can get the bacteria that live in the algae to glow, it could solve that problem."

Previous attempts to use bioluminescence as a light source for algae have not produced a large-scale commercial solution. Gan theorizes that may be because the bacteria genetically altered to glow in previous experiments weren't native to the algae. Her experiment would genetically modify native bacteria with a gene that would cause them to glow, thus providing light to the algal cells hidden from external light sources. Gan did the genetic work this summer at the CBE under the mentorship of Huyen Bui, post-doctoral algal research scientist, and hopes to complete the experiment this fall in New York.

"She is super-smart. But what impressed me most is her persistence, reliability, and hard work," Gerlach says. "This has allowed her to very quickly integrate herself into the algal research group and the CBE. Seeing a young lady putting all this together by herself is most, most impressive."

– Skip Anderson

YEAR OF UNDERGRADUATE RESEARCH MONTANA STATE UNIVERSITY

An MSU Homecoming

Before she was a policy advisor to the Obama administration, Jayne Morrow was an undergraduate researcher at the CBE. Now she's connecting MSU to challenges facing Montanans.

Former CBE undergraduate researcher Jayne Morrow joined MSU in 2019 as the assistant vice president of research, economic development, and graduate education. *Photo by Skip Anderson.*

By Skip Anderson

When Jayne Morrow, PhD, joined Montana State University as the assistant vice president of research, economic development, and graduate education in early 2019, she brought with her a balanced blend of work as a bench scientist, as an environmental engineer for the National Institute of Standards and Technology, and as a White House science policy advisor. And long before she helped the Obama administration keep the Ebola outbreak in Africa from becoming an Ebola outbreak in the United States, she was a freshman at Montana State, uncertain whether she had the mettle for a career as an engineer. Then she saw that the Center for Biofilm Engineering was looking for a work-study student to help out in its labs. Peering into a microscope as a second-semester freshman, she saw that the microbes on her slide were communicating and bioluminescent. The movement of those faintly glowing microbes on her slide meant that her experiment was a success, which gave her confidence the boost she needed to see herself as an emerging engineer. Little did she know that she would one day credit these experiences as an undergraduate researcher at the CBE with launching her career that would lead her to serving the leaders at the highest levels of the federal government and back to Montana State.

"I was a good student in high school," Morrow says. "But I really had to buckle down and work in college. One of the most important things I learned at MSU was discovering that I am a visual learner who has to tinker with things to understand the underlying theory."

What she tinkered with at the start of her time at the CBE was what everybody tinkers with the first time they're working in a lab.

"I was mostly pouring petri dishes, autoclaving glassware, and washing test tubes for [then-CBE PhD student] Rob Sharp," Morrow says. "He would leave me a to-do list on his bench each day. By the summer, the list became more complex and he started trusting me with more and more things such as sampling reactors."

She would earn an NSF fellowship that enabled her to take on a heavier workload and gain valuable experience in other CBE labs.

"I started my own research project with [Sharp]

Clockwise from Top Left: Jayne Morrow (left) worked at the CBE for four years as an undergraduate researcher while studying civil engineering at Montana State University. President Barack Obama presented Morrow the Presidential Early Career in Science and Engineer award during a White House ceremony in 2011. Jayne and Aaron, her husband, have two children, Anna and Liam. Aaron is also an MSU alumnus. *Photos courtesy Jayne Morrow.*

and Al Cunningham [now a professor emeritus of civil engineering]," Morrow says. "Al and my PhD advisor [at UConn] are like academic fathers to me."

Immersing herself in her work at the CBE, she not only found established scientists who would become mentors, but she also learned about how she best processes information.

"I quickly realized the power of science, and learning how to read a scientific journal and a manuscript was empowering to me," Morrow says. "And realizing that I could build on that knowledge was enlightening. To think that this girl growing up in Montana could be a part of this big, grand field of science and contribute to the knowledge of the world was amazing. Working with Betsey Pitts [then a research scientist and manager of the CBE Microscopy Facility] and Darla Goeres, now the principal investigator in the Standardized Biofilm Methods Laboratory, my eyes opened to the beautiful world you could only see through a microscope. And Nick Zelver [who was the CBE industrial coordinator at the time] really taught me a lot about the way research connects with industry."

While she found success early and often in the CBE labs, Morrow struggled in some of her classes.

"My grades alone at MSU wouldn't have shown people my potential as a researcher," Morrow says. "My grades were much higher in graduate school."

Seeking help from a peer in the class thermodynamics would prove fortuitous for them both.

"I was not good at thermodynamics, and Aaron Morrow was known as the thermo-guy in our class," she says.

He and Jayne would eventually marry and have two children together.

Aaron helped his future wife through a class she found troublesome. But she credits her mentors at the CBE – specifically Cunningham, Pitts, Sharp, Goeres, and Zelver – with nurturing a newfound confidence that would propel her to earning a BS in civil engineering from Montana State (1998), an MS (2001) and a PhD (2005) in environmental engineering degree with a focus on molecular and microbiology from the University of Connecticut.

Morrow studied in a new graduate program focused on growing skills in Environmental Biotechnology under microbial ecologist Barth Smets at UConn and Domenico Grasso, now chancellor at the University of Michigan-Dearborn. Like the CBE, the interdisciplinary program combined engineering with emerging techniques in molecular biology.

"Domenico studied how nonliving particles moved,

> "It is an opportunity for Montana to lead when Montanans are experiencing a problem that hasn't yet shown up at a national level."
>
> — Jayne Morrow
> Assistant Vice President of Research,
> Economic Development, and Graduate Education

It's possible that Jayne Morrow is the first CBE alumna to take her family bowling in the White House. *Photo courtesy Jayne Morrow.*

and surface interaction," she says. "I was still stuck with that original question from staring under the microscope years earlier and wanted to know how microbes moved and why they sometimes stuck to things. Most microbes should be repelled electrostatically from things like glass and sand used in drinking water filters, but they don't – they stick. So that's what I worked on for my PhD."

Jayne and Aaron live in Chinook, Montana, with their children Anna (15) and Liam (12). Chinook is definitely rural and decidedly small – its geographic footprint is a half square mile. These qualities are, in part, why Morrow splits her time working remotely and in her office in Montana Hall each month.

"It's beneficial to the university to have ears and a voice in our rural communities, as we are looking for ways to strengthen our bridges and continue to grow meaningful research programs for Montana," says Morrow, whose responsibilities include aligning the expertise of MSU faculty with opportunities to solve problems in rural Montana, the nation, and the world. "Community engagement and outreach happens through our Extension service and our other campuses at Great Falls, Billings, and Northern. It sometimes falls to faculty members to understand and translate their work at a local level in Montana. Sometimes it's a natural fit, especially when they're working with stakeholders in these communities. But the outreach is not always natural, and I strive to help make those connections even stronger while also establishing new ones."

In her new position, Morrow is leveraging her experience in federal government both as a scientist, engineer, and a policy advisor.

"I understand the systems on the federal level really well, and how good ideas turn into multi-agency, cross-sector industry initiatives," Morrow says, noting that this insight can be useful in guiding the development of our research and outreach program strengths, and targeting federal funding. "Working across government agencies taught me how to start a conversation, find the innovative ideas, and foster a vision we can all get excited about and grow."

The idea is to draw attention to issues that federal agencies and industry could collaborate with MSU researchers to deliver solutions.

"It is an opportunity for Montana to lead when Montanans are experiencing a problem that hasn't yet shown up at a national level. That might mean starting a dialogue on new technology areas that will lead to economic vitality and increased value extraction for Montana in the future like what we are starting to see with some precision technologies and manufacturing processes in agriculture. We have the capability at MSU to shine a light on issues and showcase the great ideas growing out of our communities all across the state. In my role, I get to work with faculty and Montanans to demonstrate where additional funding and partnership would enable MSU faculty and Extension to step into a needed space and strategically grow our research programs."

Morrow cited increasing suicide awareness across the state as well as the need for costly upgrades to water-treatment systems to help engineers interpret the chemistry data as concerns for rural communities that align with MSU programs.

Morrow works closely with Jason Carter, who joined MSU as vice president of research, graduate education, and economic development in September.

"The CBE has a great reputation nationally," Carter says. "It does not surprise me that Jayne has done so well with such a great discovery-based experience. We are pleased to have her back with our office to help us scale research at Montana State." ■

Educating

the Next Generation of Scientists and Engineers

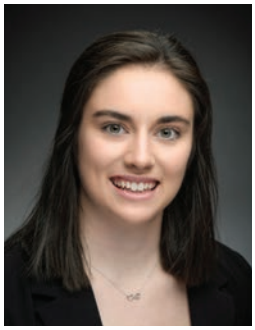
CBE students receive hands-on training in experimental design, research, and presentations while working with world-renowned faculty.

YEAR OF UNDERGRADUATE RESEARCH
MONTANA STATE UNIVERSITY

To Wash, or Not to Wash

That is the Question of 'Chickensplash'

By Skip Anderson



Caitlin Carmody, a junior mechanical engineering student, hails from Butte, Montana. In 2018, she joined James Wilking's Soft Materials Laboratory housed at the CBE and began working on a continuing project colloquially referred to as "Chickensplash." By virtue of the work itself, laypersons have an opinion. And the findings have garnered media attention (and almost assuredly sparked more than a few household debates): Should you wash raw chicken before cooking it? Wilking credits Harold McGee, visiting lecturer on science and cooking at Harvard University, author of several related books, and contributing columnist for *The New York Times*, with popularizing the question. "When Harold found out Cati was working on the topic, he asked to present some of her results at Harvard in the course, Science & Cooking," Wilking says.

How did the Chickensplash experiment come about?
Several years ago, the FDA issued a recommendation for people not to wash raw chicken. But that recommendation was based on research that only indicated how far water splashing off chicken in a sink could travel to countertops and other nearby surfaces — nobody had tested whether those water droplets carried microbes with them. Jim Wilking was really curious about that, and Ben Grodner, an undergraduate chemical and biological engineering student, began the experiments. I was hired in 2018 to transition into Ben's position when he graduated. So, I picked up where he left off.

What surprised you about this work?
When I would tell people what I was researching, they thought it was funny. But once I explained it, I noticed that nearly everybody had an opinion about whether or not to wash raw chicken.

There's a definitely a relatability aspect to that work.
Right. We can sometimes forget that there's a social aspect to science. These particular experiments showed conclusively that bacteria *does* get transferred by washing chicken under running water, and the CDC has statistics on how often people get sick from salmonella traced to chicken. But, that's not necessarily going to change a chicken-washer's mind.

How do you overcome people's entrenched habits?
That's a bit beyond the focus of these experiments. I think one goal for us is to provide data on splash contamination, so that if people still prefer to wash chicken, they can be aware of the risks and take steps to wash chicken safely. Anecdotally, my mom has always washed chicken and taught me to do the same. And I still wash chicken, because it feels gross not too. But I'm also aware that bacteria are transferred wherever water splashes in my kitchen. So, I take steps to prevent it from causing harm.

What steps do you take when handling raw chicken?

When water from the faucet impacts the chicken, it creates chickensplash, and that can carry bacteria such as salmonella more than 2 feet from the sink. So, I never have food, plates, or utensils anywhere near the sink when I'm handling raw chicken. And rather than rinse the chicken under running water, I fill a bowl with unheated tap water in the sink and dip the chicken. When I'm done, I sanitize all surfaces within a splashzone of three feet or so, and wash my hands with soap and hot water.

What have you learned so far that no one else knew before?

We have confirmed that bacteria are transferred in the chickensplash water droplets. Before our experiments, we didn't know for sure that bacteria could sheer off the chicken's surface and travel with the water. I was also surprised at where the chickensplash went. We thought that angling a splash surface away from an area would minimize splashing in that direction. But what we found was that the water launches off the angled surface in a backward direction. I don't think any of us expected that.

Work you did as a sophomore has been cited in a course at Harvard. Did you expect as much when you applied to the CBE?

Ha! No, not at all. Definitely not. ■

CBE *Awards*

FACULTY AWARD RECIPIENTS

SARAH CODD
Distinguished Professor Award
Norm Asbjornson College of Engineering
Montana State University

ERIKA ESPINOSA-ORTIZ
Outstanding Researcher Award
Center for Biofilm Engineering
Montana State University

ROBIN GERLACH
Outstanding Faculty Award
Center for Biofilm Engineering
Montana State University

Lloyd Berg Faculty Mentorship Award
Norm Asbjornson College of Engineering
Montana State University

DARLA GOERES
Excellence in Outreach Award
Norm Asbjornson College of Engineering
Montana State University

JOE SEYMOUR
Provost's Award for Graduate Research/ Creative Mentoring
Montana State University

STUDENT AWARD RECIPIENTS

REHA ABBASI
PhD Student
Judges' Prize Three Minute Thesis competition
Norm Asbjornson College of Engineering
Montana State University

MATT JACKSON
PhD Student
Raymond E. and Erin S. Schultz Emerging Fellows Award
Norm Asbjornson College of Engineering
Montana State University

BRIAN PETTYGROVE
PhD Student
W.G. Characklis Award
Center for Biofilm Engineering
Montana State University

Student Travel Award
American Society for Microbiology Biofilms Conference

GEORGE PLATT
MS Student
John Neuman Lab Citizen Award
Center for Biofilm Engineering
Montana State University

SHAWNA PRATT
PhD student
First Place, Graduate Student Competition
Microbes at Biomedical Interfaces session
American Institute for Chemical Engineering's annual meeting

NEERJA ZAMBARE
PhD Student
W.G. Characklis Award
Center for Biofilm Engineering
Montana State University

GEOFF ZATH
PhD Student
Raymond E. and Erin S. Schultz Emerging Fellows Award
Norm Asbjornson College of Engineering
Montana State University

Two CBE PhD Students Collaborate on

Polymer Research

By Skip Anderson

Two PhD students working in separate labs at the Center for Biofilm Engineering traveled to Germany last spring to gain insights into nontoxic substances for potential use in their research projects involving living microbes.

Reha Abbasi, a chemical engineering student who works for Dr. Jim Wilking, and Humberto Sanchez, a chemical engineering student working in Dr. Connie Chang's Lab, collaborated for nearly three weeks with researchers at the Leibniz Institute of Polymer Research in Dresden, located in eastern Germany near the Czech Republic border.

Sanchez traveled to the Leibniz Institute to understand better the chemical processes required to make hydrogel microparticles, substances that can be used to grow living cells. He accomplishes this using microfluidic techniques, a specialty of the lab run by CBE principal investigator Connie Chang, assistant professor of chemical and biological engineering.

"We hope that tissue cells can be grown on these microparticles so we can better control how they are infected with various viruses," Sanchez says.

Microcarriers, such as these particles, are used in bioprocesses such as vaccine production because the cells naturally adhere to particular surfaces.

"This work might lead to a platform where therapeutics intended to knock out viruses can be studied more thoroughly," Sanchez says, noting that his German collaborators will supply him the materials necessary to create a hydrogel particularly well suited for his research.

Abbasi is interested in polymers, a substance he uses to create 3D-printed biofilms in the lab of CBE principal investigator James Wilking, an assistant professor of chemical and biological engineering. Abbasi accomplishes this by adding living microbes to the polymer-based resin that a 3D printer uses to create a material suitable for studying the microbes. His collaborators at the Leibniz Institute create polymers more specialized than what's available commercially.

"The polymer we use in the Wilking Lab is nontoxic to the microbes we're studying, but there are practical limitations to it," Abbasi says. "The researchers at the Leibniz Institute are experts at understanding the chemistry of the polymers, how they work, and creating new polymers with different properties."

Reha Abbasi (above) and Humberto Sanchez, each a PhD student working in CBE laboratories, traveled to the Leibniz Polymer Institute in Dresden, Germany, in 2019 to collaborate on polymers and hydrogels respectively. *Photos by Adrian Sanchez-Gonzalez.*

Properties, Abbasi says, such as being less brittle, more elastic, or structurally stronger.

"The CBE and the Leibniz Polymer Institute reached a collaboration agreement that will last for many years," he says.

Abbasi's and Sanchez's work in Germany was part of a Leibniz Institute project titled "3D for 3M – Additive Manufacturing for Medicine, Microsystems, and Intelligent Materials." The German Ministry of Education and Research is providing public funds for the project. The principal investigators at the Leibniz Institute are Julian Thiele and Stefan Schwurack. 3M is a member of the CBE's Industrial Associate program in which corporate entities partner with the CBE on applied-science research projects.

PhD student's image lands on cover of prestigious journal

CBE PhD student Jeffrey Simkins, from the Department of Chemical and Biological Engineering, earned the cover image and feature article in the August 2018 issue of the *Journal of Magnetic Resonance*. Accoding to Simkins, the article details a study that pioneers a new, MRI-based technique for the measurement of oxygen distribution in biofilms which exploits the sensitivity of fluorocarbons to local oxygen concentration. Oxygen gradients are a critical determinant of biofilm behavior, regardless of whether the context is medical, industrial, or environmental. But they are notoriously difficult to measure using conventional approaches. The MRI method is noninvasive and allows for measurement of oxygen distribution in multiple dimensions simultaneously, and these unique advantages allow novel insights to be made about bacterial oxygen usage under different conditions.


Simkins

– Kristen Griffin

YEAR OF UNDERGRADUATE RESEARCH
MONTANA STATE UNIVERSITY

CBE undergrads receive honors, spotlight on research


Anderson


Naser

Bekah Anderson, an Honors College undergraduate student in chemical and biological engineering, won the Outstanding Poster Award at the Western Region Honors Conference held on MSU's campus March 29-31. Bekah presented "Characterization of microplastics in precipitation," at the annual conference that promotes the advancement of undergraduate honors education. Bekah also received an Honorable Mention at the National Council of Undergraduate Research Posters on the Hill event and participated at the NCUR National Conference held April 10–13 in Atlanta, Georgia.

Nada Naser, undergraduate scholar in chemical and biological engineering, also participated in the NCUR National Conference earlier this month. Nada was invited to deliver a platform presentation of her research "Visualization of microbially induced calcium carbonate precipitation on the single-cell scale using droplet-based microfluidics."

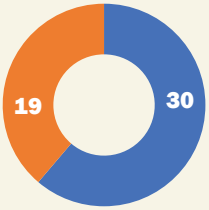
Montana State University will host the NCUR National Conference March 26–28, 2020. The event is expected to bring 4,000 students from around the world to campus.

– Kristen Griffin

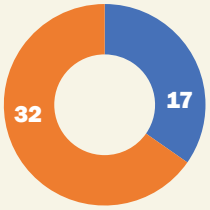
1,142
Students Trained
since 1990

FY2019 Student Snapshot

49 GRADUATE STUDENTS



49 UNDERGRADS



8 Departments Represented by Students

- Cell Biology & Neuroscience
- Chemical & Biological Engineering
- Chemistry & Biochemistry
- Civil Engineering
- Mathematical Sciences
- Mechanical & Industrial Engineering
- Microbiology & Immunology
- Plant Science & Plant Pathology

CENTER FOR BIOFILM ENGINEERING

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