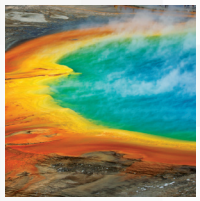
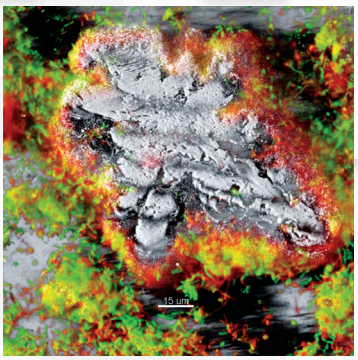
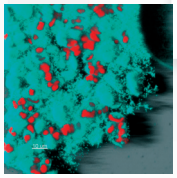
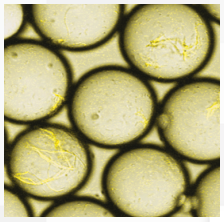
CENTER FOR BIOFILM ENGINEERING

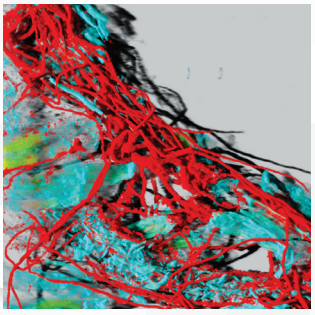
2016 ANNUAL REPORT

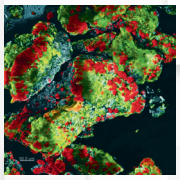


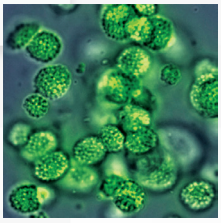


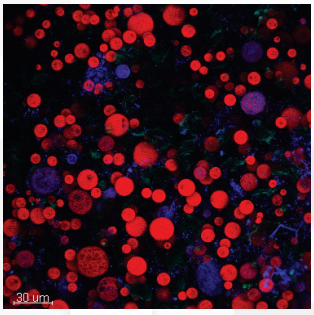


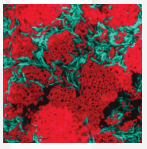


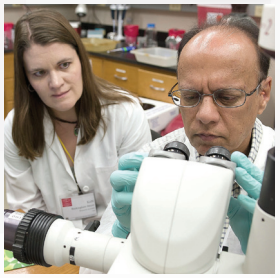


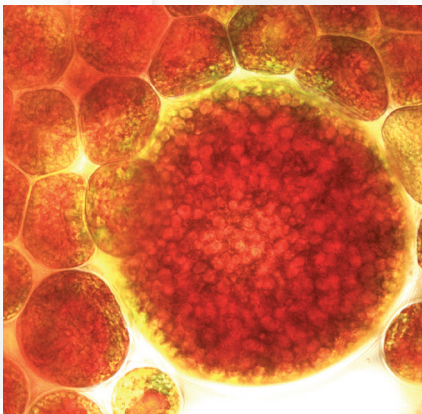












MONTANA STATE UNIVERSITY

College of ENGINEERING

On the front cover: From the top, down — Neerja Zambare, PhD student; Karlene Hoo, MSU Dean of Graduate Studies and Tatsuya Akiyama, PhD student; Al Parker, CBE biostatistician; Muneeb Rathore, PhD student; Kelli Buckingham-Meyer, research assistant III; Maya Singh, industrial representative

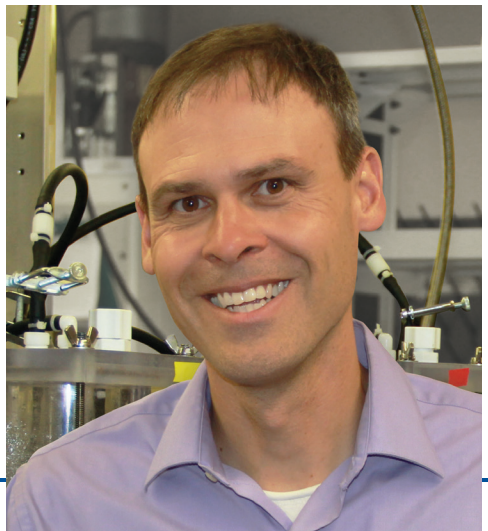
A message from our director

As I look back on my first year as CBE director, I am amazed and humbled by the achievements of the CBE faculty, researchers, staff, and students. I am fortunate and thankful to be part of such a talented family. The CBE continues to strive to be a center of excellence for research, education, and outreach; where students work with faculty and researchers in an interdisciplinary environment addressing questions important to both academia and industry. Biofilms impact both applied and fundamental aspects of biology and engineering, and require multi-disciplinary approaches in both research and education. In the following pages, you'll see examples of how we build teams across disciplines to better understand biofilms and formulate solutions to biofilm related problems. Just as biofilms are an evolutionary adaption to changing environments where cooperation and differentiation were likely forged and selected, the CBE brings together faculty, staff and students with diverse skill sets to advance our understanding of these diverse communities.

"Biofilm research continues to expand its exciting and impactful contributions to our common knowledge base."
- Matthew Fields

Microorganisms are predicted to comprise the largest amount of biomass on Earth. They play a vital role in ecosystem function and represent immense and undiscovered biochemical capacities. Microorganisms also play a major role in global biogeochemical processes for the cycling of nutrients that are beneficial and detrimental in almost every environment on the planet including human and human-made environments. The majority of this microbiological diversity and biomass resides as attached growth at phase boundaries—biofilm. Most microbial environments are physically dynamic habitats where fluxes in water, nutrients, temperature, pH, and osmolarity challenge organisms to survive and thrive. Biofilms can retain water, sorb nutrients, and protect against salinity, osmolarity, pH, nutrient availability, redox potential, and predation. These survival mechanisms are demonstrated in the stories throughout this year's annual report.

Biofilm research continues to expand its exciting and impactful contributions to our common knowledge base. The investigation of microbial biofilms has far-reaching implications for society's challenges with water, food, energy, environmental health, and human health. Our mission at the CBE is to help advance the understanding and study of biofilms to all stakeholder communities—academic, industrial, and regulatory.



One measure of the CBE's commitment to providing students with a meaningful research experience is the number of peer-reviewed papers with undergraduate and graduate authors.

49 publications **39 journals**

Student Authors

Aristotelous AC, **Klapper I,** Grabovsky Y, **Pabst B, Pitts B, Stewart PS,** "Diffusive transport through a model host-biofilm system," *Phys Rev E Stat Nonlin Soft Matter Phys.* 2015 Aug; 92(2): 022703.

Beck A, Hunt KA, Bernstein HC, Carlson RP, "Interpreting and designing microbial communities for bioprocess applications, from components to interactions to emergent properties (book chapter)," *Biotechnologies for Biofuel Production and Optimization,* 1st edition. Eckert CA, Trinh CT (eds.) Elsevier 2016; pp. 407–432.

Bell TA, Prithiviraj B, Wahlen BD, **Fields MW, Peyton BM,** "A lipid-accumulating alga maintains growth in outdoor, alkaliphilic raceway pond with mixed microbial communities," *Front Microbiol.* 2016 Jan 7; 6:1480.

Connolly J, Gerlach R, "Microbially induced carbonate precipitation in the subsurface: Fundamental reaction and transport processes (book chapter)," *Handbook of Porous Media.* 3rd Edition. K Vafai (ed.), CRC press Taylor and Francis Group, 2015; pp. 891–922.

Figueroa LO, Schwarz B, Richards AM, "Structural characterization of amphiphilic siderophores produced by a soda lake isolate, Halomonas sp. SL01, reveals cysteine-, phenylalanine- and proline-containing head groups," *Extremophiles* Nov 2015; 19(6):1183–1192.

Figueroa LO, Pitts B, Uchida M, **Richards AM,** "Vesicle self-assembly of amphiphilic siderophores produced by bacterial isolates from Soap Lake, Washington," *Can. J. Chem.* 2016; 94(1):35-43.

Kirkland CM, Hiebert R, Phillips A, Grunewald E, Walsh DO, **Seymour JD, Codd SL,** "Biofilm detection in a model well-bore environment using low-field magnetic resonance," *Groundwater Monitoring & Remediation* 2015 Fall; 35(4) 36–44.

Kirkland CM, Herrling MP, Hiebert R, Bender AT, Grunewald E, Walsh DO, **Codd SL,** "In situ detection of subsurface biofilm using low-field NMR: A field study," *Environ Sci Technol.* 2015 Sep 15; 49(18):11045–52.

Schultz L, Connolly J, Lauchnor EL, Hobbs T, Gerlach R, "Struvite stone formation by ureolytic biofilm infections (book chapter)," *The Role of Bacteria in Urology.* Lange D, Chew B, (Eds.): Springer, 2015; pp. 41–49.

Smith HJ, Foreman CM, Akiyama T, Franklin MJ, Devitt NP, Ramaraj T, "Genome sequence of *Janthinobacterium* sp. CG23_2, a violacein-producing isolate from an Antarctic supraglacial stream," *Genome Announc.* 2016 Jan-Feb; 4(1).

VanKempen-Fryling RJ, Stein OR, Camper AK, "Presence and persistence of wastewater pathogen *Escherichia coli* O157:H7 in hydroponic reactors of treatment wetland species," *Water Sci Technol.* 2015; 72(1):135–40.

Notable publications

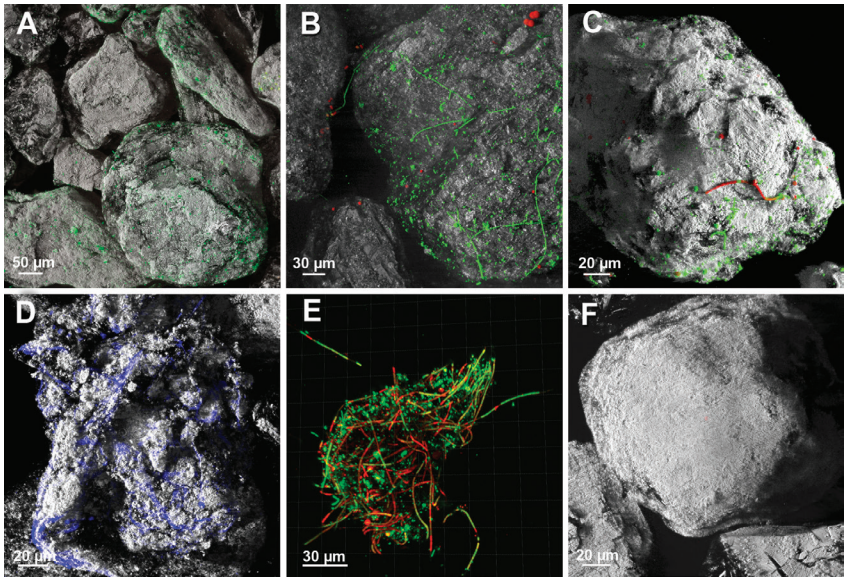
Nature Scientific Reports

Barnhart EP, McClure MA, Johnson K, Cleveland S, **Hunt KA, Fields MW,** "Potential role of Acetyl-CoA Synthetase (acs) and Malate Dehydrogenase (mae) in the evolution of the acetate switch in bacteria and archaea," *Sci Rep.,* 2015 Aug 3; 5:12498.

Nature npj Biofilms and Microbiomes

Connolly JM, Jackson B, Rothman AP, Klapper I, Gerlach R, "Estimation of a biofilm-specific reaction rate: Kinetics of bacterial urea hydrolysis in a biofilm," *npj Biofilms and Microbiomes* 2015; 1: 15014.

Smith HJ, Schmit A, Foster R, Littmann S, Kuypers MMM, **Foreman CM,** "Biofilms on glacial surfaces: Hotspots for biological activity," *npj Biofilms and Microbiomes* 2016 June; (2):16008. In this study, we show via confocal microscopy that microbial communities on glacial surfaces in Antarctica persist in biofilms, and that ~ 35% of the cryoconite sediment surfaces were covered by biofilm. The figure below shows cryoconite sediment with associated microbial communities.



Color key: (a) Green=SYBR Green stained microbes, grey=reflection of the sediment. (b,c) Red =auto-fluorescencing cells, green =SYBR Green stained microbes, grey=reflection of the sediment. (d) Blue =Calcofluor White stained EPS, grey=reflection of the sediment. (e) Red=Propidium Iodide (membrane compromised cells) Green =Syto9 stain (live cells). (f) Control image of combusted cryoconite sediment following described staining protocol, grey=reflection of the sediment.

Primary areas of applied research:

- Biofilm control strategies
- Energy solutions
- Environmental technologies
- Health/medical biofilms
- Industrial systems and processes
- Standardized methods
- Water systems

Inside the LAB with Darla Goeres


photo by Kelly Gorham

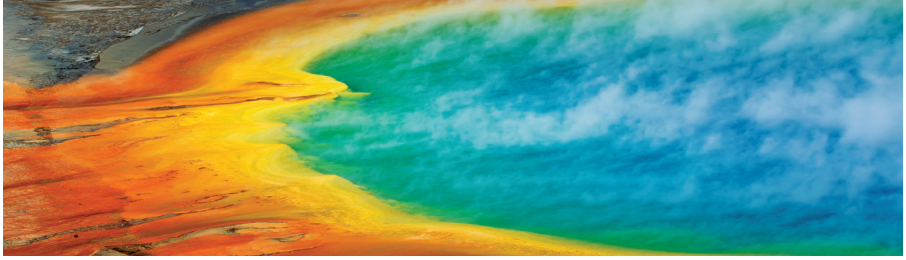
- **What is the focus of your lab?**
  The Standardized Biofilm Methods Laboratory (SBML) is responsible for the development & validation of standard methods for growing, treating, harvesting & analyzing biofilm bacteria.

- **What is the make up of your lab?**
  The SBML is composed of an interdisciplinary team of engineers, microbiologists & a biostatistician. We also employ undergraduate researchers & have one student working towards a master's degree in chemical engineering.

- **Tell us about your new Standardized Biofilm Methods Laboratory training videos.**
  The training videos are an exciting new approach for facilitating the technology transfer of biofilm methods to a larger audience. For many people, videos provide a more tangible example of how to accomplish a highly technical task. My goal is that the videos will go a long way towards reducing researcher-to-researcher & laboratory-to-laboratory variability.

- **What has been the focus of your lab over the last year?**
  Urinary catheters & beer draught lines. Separate projects, of course! As well as continued investigations in the ASTM Single Tube Method & providing statistical support to the EPA.

- **What projects are you looking forward to this coming year?**
  *I am excited about work being done on a reactor system designed to model cooling towers & a method for growing a **Legionella** biofilm in the CDC biofilm reactor.*



Investigating heat-loving microbes for biotech applications

Excerpts taken from an article by Skip Anderson for the MSU News Service

In 2016, Brent Peyton, professor in MSU's Department of Chemical and Biological Engineering, and a team of MSU researchers were awarded a prestigious $1 million grant from the W.M. Keck Foundation to expand the understanding of how microorganisms thrive in hostile, high-pH hot springs. Peyton and his team are using the Keck grant to learn more about the heat-loving archaea found in Yellowstone's hot springs. Microbes that survive in extreme conditions have potential for applications in medicine, energy, and materials but require a great amount of fundamental research. "They live in such extreme conditions—some in boiling acids others in hot caustic solutions—it's amazing that they can even survive," said Peyton. "There has been work done on the microbes in acidic environments, but archaea at the other end of the scale, in hot caustic environments, have had relatively little attention. The biodiversity in these caustic environments is virtually unexplored. It is sometimes called 'microbial dark matter.'"


photo by Kelly Gorham

Understanding how these alkaliphilic, or caustic-loving, archaea use enzymes to convert woody materials into their food—a process known as chemosynthesis—may open unimaginable opportunities. "For these organisms to take a stick or leaf that falls into their boiling, caustic environment and turn it into sugar – their food source – is incredible. It's a trick that Mother Nature learned and she is not going to share that secret easily," Peyton said.

To assist with the fundamental research, the award supports a lab-based class for twelve undergraduate students called "Extreme Microbiology of Yellowstone," which launched in fall 2016. During the course, students learn how extreme microorganisms contribute to the cycling of nutrients in geothermal systems, and the engineering and use of extreme microorganisms in a variety of biotechnology applications. They visit these unique ecosystems on a field sampling trip, and through sample collection, laboratory work, and analysis develop culturing strategies needed to grow unique extremophilic organisms in high pH ecosystems.

Peyton is working with a multidisciplinary team of faculty from across MSU's campus, which includes: Brian Bothner, chemistry and biochemistry; Eric Boyd, microbiology and immunology; Valerie Copié, chemistry and biochemistry; Bill Inskeep, land resources and environmental sciences; and CBE affiliated faculty members: Matthew Fields, microbiology and immunology; and Robin Gerlach and Ross Carlson, both in chemical and biological engineering.

The W.M. Keck Foundation is one of the largest private foundations in the US, supporting fundamental and applied research in science, engineering and medicine as well as educational initiatives.

The photo on the left shows **Kori Klingelsmith**, undergraduate student in microbiology & immunology, using a sampling pole to collect microbial mat from a hot spring in the remote Heart Lake Geyser Basin region of Yellowstone National Park.

photo by Dana Skorupa

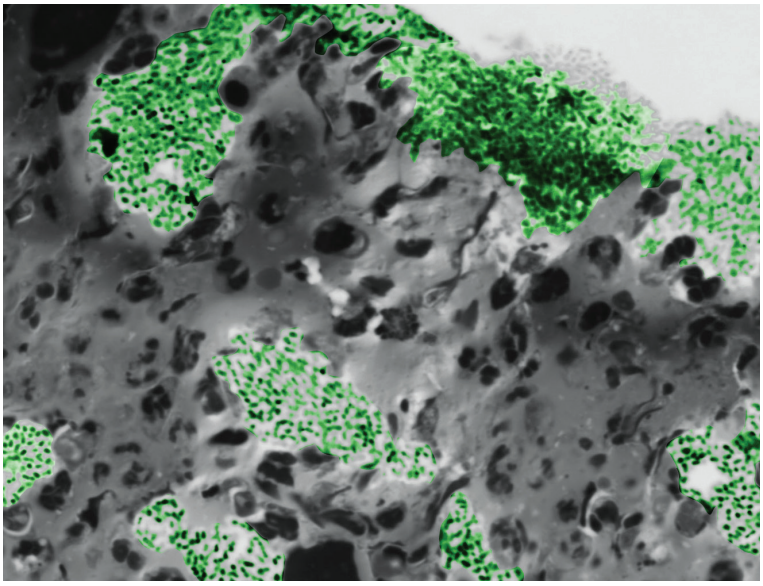
Medical Biofilm Laboratory

CHRONIC WOUNDS

The CBE Medical Biofilm Laboratory (MBL) continues to investigate the role of biofilms in the pathophysiology of chronic wounds. Garth James, manager of the MBL, and a multidisciplinary team of researchers recently published a paper on oxygen depletion in wounds in the March/April 2016 issue of *Wound Repair and Regeneration*. "Microsensor and transcriptomic signatures of oxygen depletion in biofilms associated with chronic wounds," looks at oxygen consumption by biofilms and responding immune cells as a contributing factor to delayed healing in chronic wounds. This paper contributes to the understanding of the role of biofilms in wound chronicity, an important public health issue.

"Microsensor and transcriptomic signatures of oxygen depletion in biofilms associated with chronic wounds," James GA, Ge Zhao A, Usui M, Underwood RA, Nguyen H, Beyenal H, deLancey Pulcini E, Agostinho Hunt A, Bernstein HC, Fleckman P, Olerud J, Williamson KS, Franklin MJ, Stewart PS *Wound Repair and Regeneration* 2016 Mar; 24(2):373–83.

Further reading from *Wound Repair and Regeneration:* "Biofilms in chronic wounds," James GA, Swogger E, Wolcott R, deLancey Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS *Wound Repair and Regeneration* 2008; 16:37–44.



Light microscopy image of *Pseudomonas aeruginosa* biofilm clusters in the scab from a diabetic mouse model of delayed wound healing. Zhao et al., *Wound Rep Reg* (2010) 18: 467–477.

$2.3 million New biofilm grants in the 2016 fiscal year

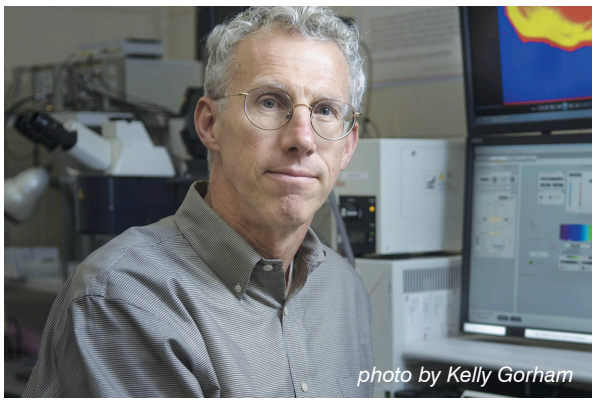


photo by Kelly Gorham

Phil Stewart Co-PI on $3 million award for nanotechnology research

The National Science Foundation awarded $3 million to MSU for the creation of a new nanotechnology center. Phil Stewart, professor in chemical and biological engineering, is a Co-PI on the grant, which is part of an $81 million, national effort to boost American research and development in a fast-growing and promising field that requires very specialized equipment and expertise. Nanoscience and nanotechnology—the study and application of extremely small things—are pushing the envelope in the full array of science and engineering fields.

photo by Kelly Gorham

Jim Wilking earns NSF CAREER Award

Jim Wilking's biofilm work has brought him a prestigious NSF CAREER award, the National Science Foundation's top award for young scientists. The Young Investigator CAREER Award provides $503,396 over five years, which he will use to study the physical and material properties of biofilms. He will also use the award to develop teaching modules for after-school programs across Montana.

Student Spotlight

Using MRI for early detection of biofouling

Jeffrey Simkins, a Montana State University doctoral student in the Department of Chemical and Biological Engineering in the College of Engineering, was awarded a fellowship in the National Science Foundations East Asia and Pacific Summer Institutes Program (EAPSI). The fellowship is designed to foster future international scientific collaborations.

Simkins traveled to Australia to work with Drs. Michael Johns and Einar Fridjonsson at the University of Western Australia in Perth. His research focused on whether MRI technology can be used to determine oxygen levels in reverse osmosis membranes.

"Reverse osmosis membranes are going to become more important in the next century," Simkins said, "as water scarcity increasingly becomes an issue." He explained that while the membranes work well, they are susceptible to microbial growth — a process called biofouling — which clogs the membrane, eventually breaking it down. Since replacing the membranes is expensive, Simkins is looking at MRI technology as a way to identify biofouling of the membranes in the early stages.

Simkins earned his undergraduate degree in biology from Bucknell University in Pennsylvania. He returned to Bozeman in 2013 to begin graduate school at MSU. Currently researching under Magnetic Resonance Lab Co-director Joseph Seymour and Chemical and Biological Engineering Professor Phil Stewart, Simkins is looking to pioneer a new technique using magnetic resonance imaging technology to measure oxygen distribution in clinical biofilm.

Excerpts taken from an article by Denise Hoepfner, MSU News Service

Diving into Bioremediation

Excerpts taken from an article by Denise Hoepfner, MSU News Service

Eric Troyer, a 2016 graduate of MSU's Department of Chemical and Biological Engineering in the College of Engineering, has been awarded a National Science Foundation Graduate Research Program Fellowship to study the role of microorganisms in remediating harmful chemicals from groundwater. Troyer is one of 2,000 graduate students chosen from nearly 17,000 applicants to receive the award, which gives recipients at least $34,000 a year for three years to attend graduate school and conduct their research.

photo by Kelly Gorham

"It also gives me more control of what I'm doing and makes it easier on the person I will be working for because they don't have to cover those expenses," he said.

Troyer, of Hardin, Montana, will pursue his graduate studies in fall 2016 at the University of California, Berkeley, in the lab of Lisa Alvarez-Cohen, Fred and Clair Sauer Professor of Environmental Engineering.

At Berkeley, Troyer will research ways to use microorganisms to remediate trichloroethylene, more commonly known as TCE, from groundwater. "Beginning with its widespread production in the 1920s, TCE was used in industrial settings as a degreaser for metal parts, as an anesthetic and as a dry cleaning solvent, among other uses," Troyer said. It was later found to cause cancer and is now a known contaminant in some groundwater aquifers.

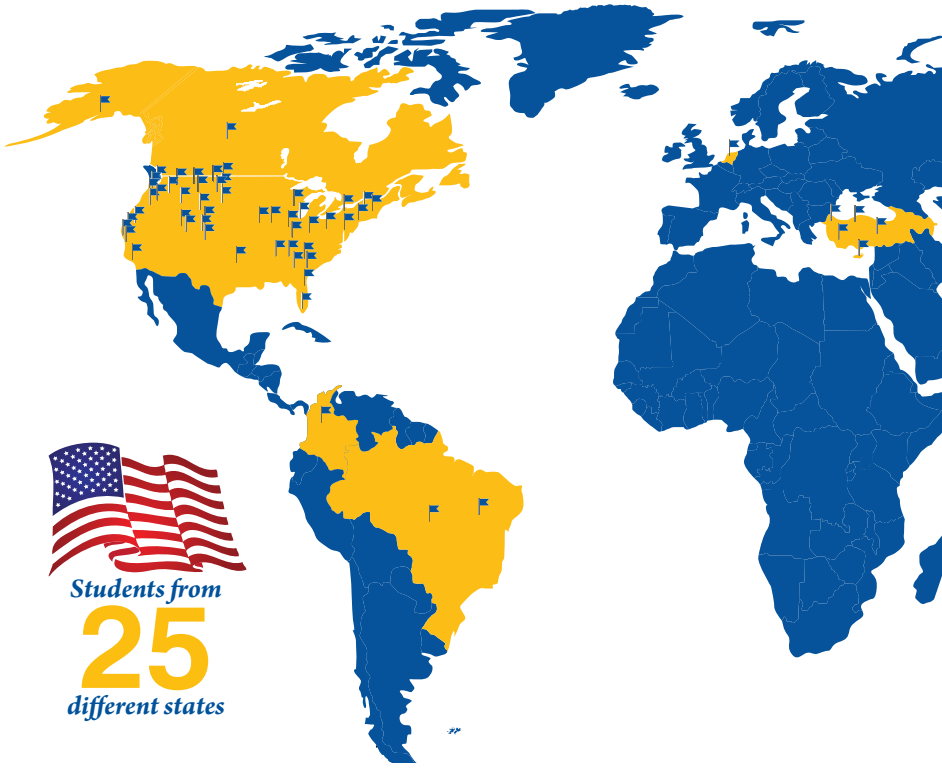
"Research into using microorganisms in this way—a technology called bioremediation—has been underway in Alvarez-Cohen's lab for some time," Troyer said, "particularly using a microbe group called *Dehaloccoccoides*, which has shown promise in breaking down TCE into ethene, a non-toxic organic chemical compound." However, a side-effect of remediation is that it can change the chemistry in groundwater and soil, potentially leading to the release of other harmful compounds.

"With only about three percent of the water on Earth considered drinkable, it's very important to protect what drinking water we have," Troyer said. "We need to make sure our clean water is protected and develop technologies that, if water were limited, we could use to clean as much water as needed for the ever-increasing population."

"Some of those technologies could include ways to reuse the wastewater from our drinking water for other purposes or converting it back to drinking water quality," Troyer said.

Coming to MSU, Troyer said he didn't have a plan to go into research, or a clear picture of what a chemical and biological engineering major would entail.

"I thought it sounded interesting," he said.



Graduate and undergraduate students work under the guidance of the CBE's multidisciplinary faculty in centrally located laboratories on the MSU campus to solve problems associated with biofilms in medical, industrial, & environmental contexts.

"Then, when I took a microbiology class and learned about the research opportunities, I knew I wanted to get into research."

Troyer said the opportunities MSU provides for undergraduate research helped him develop research skills, find confidence in his abilities and contributed to a better understanding of his coursework.

"You're able to get in the lab and immediately start doing your own projects," he said. "The advisers are there to help, but the work you do is your own, which is an advantage you get at Montana State. At some bigger institutions, as an undergrad you'd probably just work underneath a graduate student."

Troyer's research began at the CBE with studying biomineralization, a biological process in which living organisms produce hardened minerals. Specifically, Troyer studied the organisms that make up biocement, calcium carbonate formed by bacteria found in the soil. Biocement can potentially be used to increase the stability of buildings and other structures and in the conservation and restoration of historical projects, Troyer said.

At MSU, Troyer was advised by CBE affiliated faculty members Robin Gerlach, professor in chemical and biological engineering, and Adrienne Phillips and Ellen Lauchnor, both assistant professors in civil engineering.

Equality in Education

Departments

Undergraduate Students
7 departments

- Chemical & Biological Eng.
- Chemistry & Biochemistry
- Civil & Environmental Eng.
- Ecology
- Land Resources & Environ. Sci.
- Microbiology & Immunology
- Modern Languages & Literature

Graduate Students
8 departments

- Chemical & Biological Eng.
- Civil & Environmental Eng.
- Health & Human Development
- Land Resources & Environ. Sci.
- Material Sciences
- Mathematical Sciences
- Microbiology & Immunology
- Nursing

Faculty Spotlight

Teaching a perfect fit

When Abigail Richards landed at the Center for Biofilm Engineering in Montana State University's College of Engineering, she was sure it was a temporary move.

It was 2002, and she had recently finished her masters in chemical engineering from Washington State University. Though she was a visiting scholar, Richards said she felt right at home from the beginning. For one thing, the microbes she was studying thrived in an extreme environment, much like those discovered by MSU researchers in pools of highly acidic boiling water found in Yellowstone National Park.

MSU was a good fit. So good, that when a junior faculty position opened up in the Department of Chemical and Biological Engineering, Richards jumped.

The College of Engineering responded in kind, bringing her on as a tenure-track faculty member in 2007 and tapping the then 31-year-old Richards to teach the rigorous program's entry-level courses and introduce students to the world of chemical engineering.

In the nine years since, Richards has helped transform the Department of Chemical and Biological Engineering's introductory level courses during a time of unprecedented growth in enrollment. When she started teaching, Richards said the freshmen-level course typically enrolled 40-some students. This past fall, Richards taught the introductory class to more than 170 students who had identified chemical and biological engineering as their desired major.

"The energy and devotion she brings to improving the student experience is a leading example of how a professor can be more than a person delivering a lecture," said Anne Camper, Montana University System Regents Professor, professor in the MSU Department of Civil Engineering, and associate dean for faculty and administration in the College of Engineering.

Excerpts taken from an article by Sepp Jannotta, ***Mountains & Minds Magazine***

Science & Industry

COME TOGETHER

MSU team reaches milestone toward commercialization of fracture sealing

A recent field-scale demonstration marked a significant milestone toward commercialization of a technology developed by Montana State University (MSU) to mitigate fluid migration through unwanted leakage pathways near wells. The potential for upward leakage of fluids (CO_2, methane, and other fluids) near compromised wells, especially oil and gas wells, can pose a major environmental risk. When these fluids leak from the subsurface they may migrate to the surface or into functional aquifers above, leading to potential atmospheric release of greenhouse gases or damage to drinking water aquifers.

In response to concerns about this environmental risk, MSU's Center for Biofilm Engineering established a major research program centered on the use of microbial biofilms to hydrolyze urea, resulting in the precipitation of crystalline calcium carbonate (calcite)—a process referred to as microbially induced calcite precipitation (MICP). The effective use of ureolytic, biofilm-forming bacteria (i.e., *Sporosarcina pasteurii*) has been demonstrated to seal fractures in sandstone as well as seal microfractures occurring in well cement. MICP technology has the potential to reduce near-wellbore permeability, coat cement to reduce CO_2-related degradation, and lower the risk of unwanted migration of CO_2, methane or other fluids.

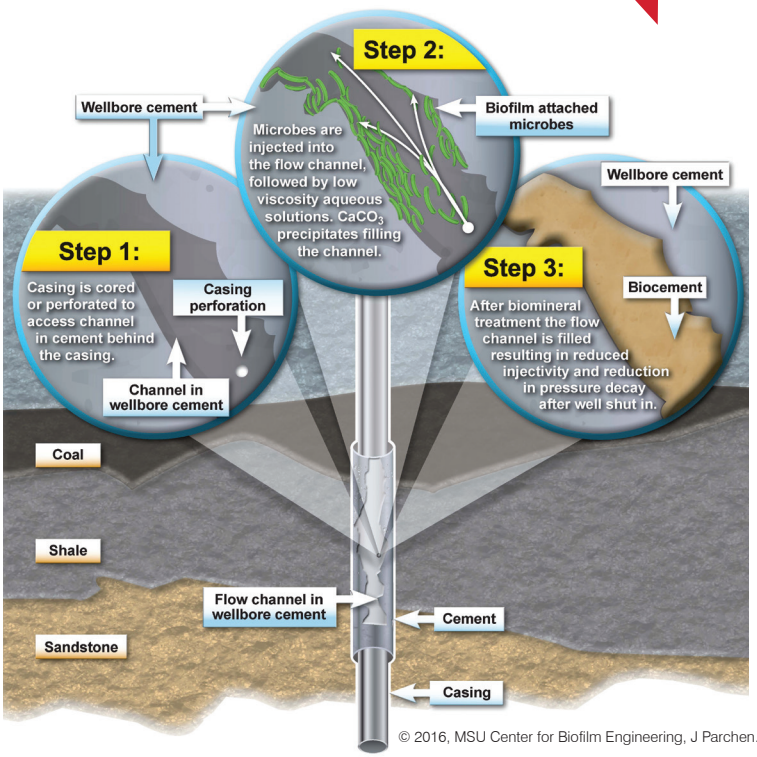
In April 2016, the researchers from MSU - Drs. **Al Cunningham, Robin Gerlach, and Adrienne Phillips** - and their industrial collaborators conducted a field demonstration at a well at the Southern Company Gorgas Power Plant in Walker County, Alabama. The goal was to demonstrate the validity of the MICP technology to seal small fractures and channels in the cement surrounding the wellbore. A vertical channel in the wellbore cement was identified using an ultrasonic imaging tool. Such a channel would potentially allow fluid movement from a deep permeable formation into a shallower zone. To perform the MICP treatment, a hole was drilled through the casing, and MICP fluids were injected into the channel. The injection pressure steadily increased during MICP application until no more fluid could be injected at pressures below the estimated formation fracturing pressure. After MICP sealing, an ultrasonic imaging device was used to indicate a significant reduction in the void space surrounding the MICP treatment zone. Additionally, the project goal to improve wellbore integrity as measured by a Mechanical Integrity Test (MIT) for wells was met with less than 10% pressure decay after shut-in over 15 minutes.

The reaction from the team's industrial collaborators has been positive. "This is significant as it is one of the first successful attempts to affect a channel in a cemented well," stated Jim Kirksey, contractor for Schlumberger Technology Corporation and a collaborator on the project.

"We are very excited about the commercial potential of this innovative technology," said Randy Hiebert, Vice President of Montana Emergent Technologies, Inc., a small business based in Butte, Montana and a collaborator on the project. "Following the success of the Gorgas project, we've had serious conversations with several oil companies about using MICP for wellbore sealing. Two companies in particular, Noble Energy (Colorado) and Star of Texas Mineral Resources, have expressed very strong interest for employing the technology in their wells and have offered the use of their wells for a field demonstration."

"The [MICP] technique was effective in filling a cement channel in the annulus between casing and the formation. It is my opinion that this test was successful and merits testing to further validate this bio-remediation technology as a promising technique to broaden our ability to restore wellbore integrity," said Wayne Rowe, Carbon Services Operations Manager for Schlumberger Technology Corporation.

> **"We are very excited about the commercial potential of this innovative technology"**
>
> - Randy Hiebert
> Vice President of Montana Emergent Technologies, Inc.



The figure above illustrates that channels in wellbore cement can be repaired when certain attached microbes (biofilm) promote the precipitation of calcium carbonate.

The CBE engages with industry across a wide spectrum of markets and application areas.

2015-16 Industrial Associates

3M
Accuratus Testing Labs*
American Chemet*
BASF /Ciba Specialty Chemicals
Baxter Healthcare
BP
Church & Dwight
Colgate-Palmolive
Dow Corning
Dow Microbial Control
Ecolab
ICU Medical
Acelity
Lonza
Masco Corporation
NASA
NCH Corporation
Next Science*
Novozymes
PPG Industries
Procter & Gamble
Sani-Marc, Inc.
SANUWAVE Health
Sealed Air Corporation
Sterilex*
STERIS
The Clorox Company
The Sherwin-Williams Co.
W.L. Gore & Associates
ZimmerBiomet

*** denotes small business member**
Bold denotes new members in 2015-16

Industry focus:

- *specialty chemicals*
- *consumer products*
- *medical/healthcare*
- *oil/energy production*
- *testing services*

INDUSTRIAL MEMBER BENEFIT

Regulatory interactions

For many companies, registering products with a biofilm claim from a regulatory agency is a new market opportunity. The guidelines companies will be required to meet in order to achieve these claims are quickly evolving. Because dialogue is crucial, every winter the CBE hosts a Regulatory Pathways meeting in Washington DC as a forum for our industry members and regulators to discuss the science behind these guidelines. The CBE invites key decision makers from the EPA and FDA to present at the DC meetings, including a panel discussion. In addition, CBE researchers are at the forefront of biofilm methods development, providing critical biofilm and statistical expertise to the regulatory community.

CBE and Industry: Intentional interaction for productivity

- Full-day methods and imaging workshops
- Biannual meeting
- Regulatory agency interactions
- Networking
- Research and testing projects
- Faculty visits and member consulting

INDUSTRIAL MEMBER BENEFIT

Montana Biofilm S&T meetings

Each summer CBE members convene for the Montana Biofilm Science and Technology Meeting – the Montana Biofilm Meeting (MBM), an exposition of what's new in CBE research and a perspective on what's happening around the world in biofilm science. The MBM is a great way to keep up on the science as well as interact with other industry and government representatives and CBE researchers. Meetings are open only to CBE members and invited guests.

3rd Annual Regulatory Pathways Meeting
Feb. 10, 2016 • Washington, D.C.

Montana Biofilm S&T Meetings
July 14-16, 2015 • Bozeman, MT

Testing Projects

$730,000
57 projects **32 sponsors**

Access complete academic year 2015-2016 information in the 2016 Appendix at www.biofilm.montana.edu/resources/annual_reports

Al Parker earns top EPA award

Al Parker, CBE bio-statistician, was awarded an EPA 2015 Scientific and Technological Achievement Award, Level 1. This award is given to top contributors to the advancement of science and technology through their publications in peer-reviewed articles and books. Parker was part of a research team that included Steve Tomasino, EPA senior scientist, Martin Hamilton, MSU-CBE professor emeritus in statistics, and Gordon Hamilton, statistical analyst with Big Sky Statistical Analysts. The authors submitted two papers that focused on using statistical tools to evaluate test methods for killing bacteria on surfaces. Their work was published in the Journal of *AOAC International*. AOAC INTERNATIONAL is a globally recognized agency in standards development.

Darla Goeres awarded Burroughs Wellcome Innovation in Regulatory Science Award

MSU-CBE faculty member Darla Goeres, associate research professor of chemical and biological engineering, was awarded a Burroughs Wellcome Innovation in Regulatory Science Award. Goeres will receive $500,000 over five years to develop new methodology to assess the prevention of biofilm formation on medical devices. She is one of five recipients of the award.

LIGHTS, CAMERA, Action

Center for Biofilm Engineering launches methods training videos

The Center for Biofilm Engineering, in collaboration with Montana State University's Department of Visual Media and BioSurface Technologies, is excited to announce the first series of Standardized Biofilm Methods training videos. Continuing the technology transfer initiative that began with the CBE's Knowledge Sharing Articles, which describe the statistical calculations associated with standardized methods development, the Standardized Biofilm Methods Training Videos are designed to assist researchers in the set-up and operation of the biofilm reactors referenced in the ASTM Standard Methods and the BioSurface Technologies Operators Manual.

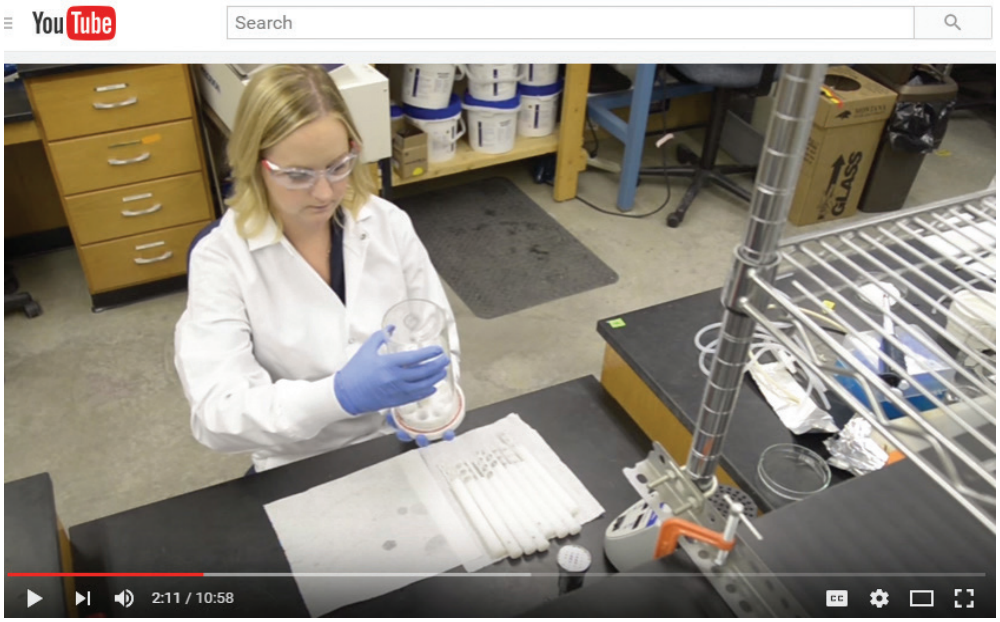
ASTM International is one of the largest international standards development and delivery systems in the world. ASTM standards are accepted and used worldwide in research and development, product testing, quality systems and commercial transactions. Darla Goeres, principal investigator of the CBE Standardized Biofilm Methods Laboratory (SBML), has served on ASTM committee E35, which tests pesticides, antimicrobials, and alternative control agents for over sixteen years and is currently the committee's chairperson.

BioSurface Technologies is a Montana-based company that manufactures reactors that grow, measure, and evaluate biofilms and biofilm processes to aid product development and process control, and to increase understanding of fundamental biofilm processes.

"Watching Kelli Buckingham-Meyer and Lindsey Lorenz demonstrate the steps explained in the ASTM method provides insight to the small operational details that are challenging to capture in a written standard method. Our goal is for these videos to provide a more effective mechanism for educating researchers about the best practices to use with these reactors which ideally will help minimize researcher-to-researcher and laboratory-to-laboratory variability," explained Goeres.

The video quality is the result of a collaboration with MSU's Department of Visual Media, which is responsible for promoting MSU through photography and film. "The methods videos are a perfect example of the collaboration we enjoy at Montana State University. Our unit appreciates the amount of effort from the CBE team to streamline the production process. Now that we have established a style and workflow, we look forward to future collaborations," stated Kelly Gorham, director of MSU's Department of Visual Media.

The next project for the SBML will be to develop a similar training video series for the drip flow reactor, which is more frequently used for medically-relevant research.



Lindsey Lorenz, Research Assistant III, shown with a CDC biofilm reactor as part of a new online instructional video. Videos can be seen at www.**cbe**videos.com.

The CBE attracts visiting students, scientists, and faculty from around the nation and around the globe. CBE is an international hub for biofilm research, education, and technology transfer.

Visting Researchers

Dominik Ausbacher, Visiting postdoctoral researcher, The Arctic University of Norway, Tromsø, Norway

Greg Characklis, Visiting faculty, University of North Carolina, Chapel Hill, NC

Adam Hise, Visiting PhD candidate, University of North Carolina, Chapel Hill, NC

Charles Fosseprez, Visiting master's candidate, Agro Paris Tech, Paris, France

Luigi Frunzo, Visiting faculty, University of Naples Federico II, Naples, Italy

Johannes Hommel, Visiting postdoctoral researcher, University of Stuttgart, Stuttgart, Germany

Michael Lienemann, Visiting postdoctoral researcher, VTT Technical Research Centre of Finland, Ltd., Espoo, Finland

Suvi Månner, Visiting PhD candidate, Abo Akademi University, Turku, Finland

Mario Perez, Visiting master's candidate, Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico

Lea Chua Tan, Visiting PhD candidate, UNESCO-IHE, Delft, The Netherlands

Federica Villa, Visiting postdoctoral researcher, University of Milan, Italy

Yeni Yung, Visiting PhD candidate, University of Illinois at Chicago, IL

University of Montana earns cover image with a little help from their friends.

- Researchers at the University of Montana earned the cover image of the August 2015 issue of *Proceedings of The National Academy of Sciences (PNAS)*.
- Images were collected at the CBE using the confocal microscope.
- Researchers:
  - John McCutcheon, University of Montana
  - James Van Leuven, University of Montana
  - Betsey Pitts, CBE
  - Phil Stewart, CBE

Keeling PJ, McCutcheon JP, Doolittle WF, "Symbioses becoming permanent: Survival of the luckiest," *PNAS,* August 2015; 112(33): 10101–10103.

Most visiting students work at the CBE for a period of several months, while others - like Federica Villa, left, who is now a postdoctoral researcher - have visited the CBE several times over a period of years as they collaborate with CBE reseachers.

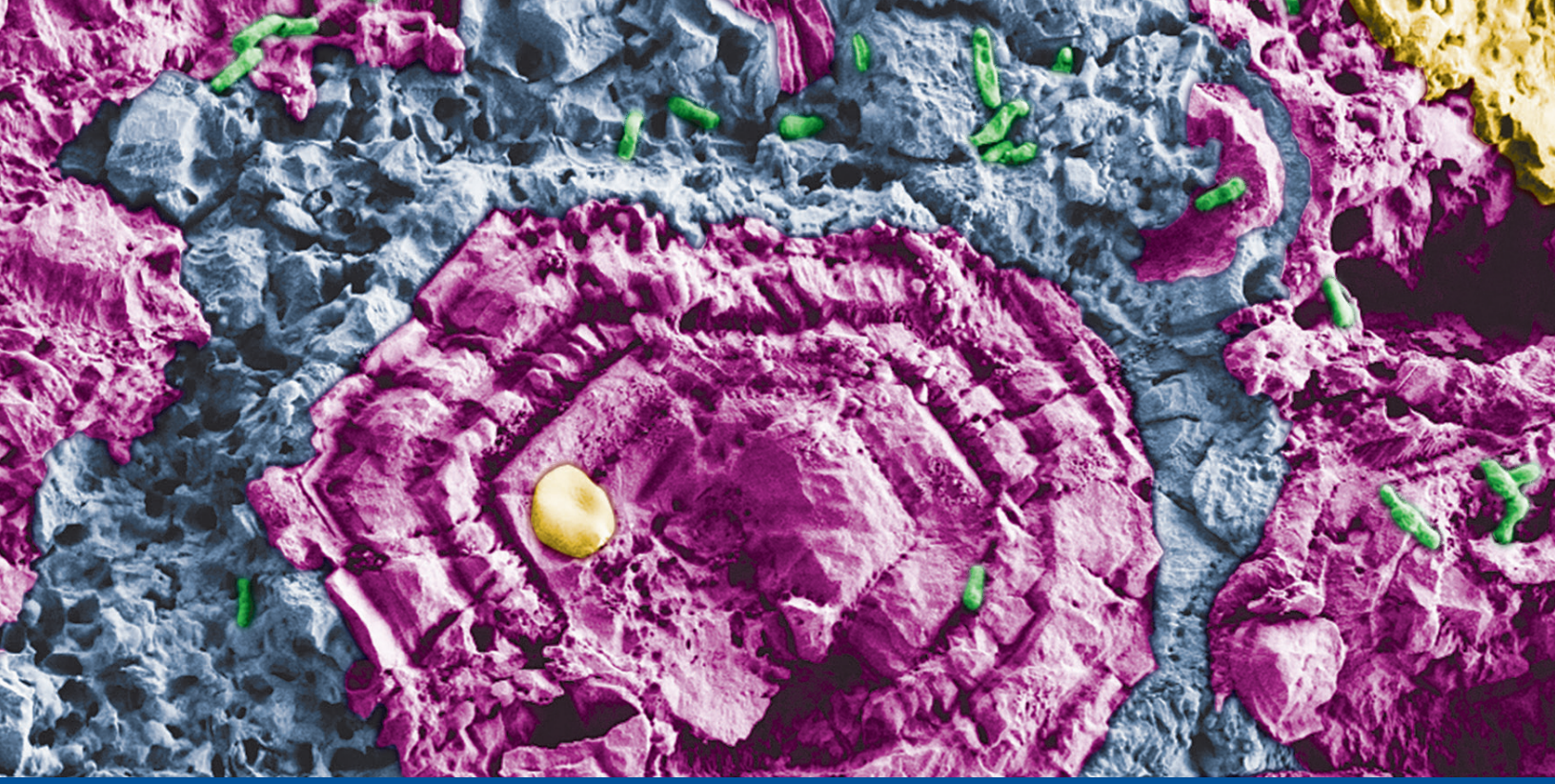
Villa F, Stewart PS, Klapper I, Jacob JM, Cappitelli F, "Subaerial biofilms on outdoor stone monuments: changing the perspective towards an ecological framework," *Bioscience,* 2016 April 1; 66(4): 285–294.

Villa F, Pitts B, Lauchnor E, Cappitelli F, **Stewart PS,** "Development of a laboratory model of a phototroph-heterotroph mixed-species biofilm at the stone/air interface," *Front Microbiol.* 2015; 6:1251.

Cattò C, Dell'Orto S, **Villa F,** Villa S, Gelain A, Vitali A, Marzano V, Baroni S, Forlani F, "Unravelling the structural and molecular basis responsible for the anti-biofilm activity of zosteric acid," *PLoS ONE* 2015; 10(7): e0131519.

Villa F, Pitts B, Stewart PS, Giussani B, Roncoroni S, Albanese D, Giordano C, Tunesi M, Cappitelli F, "Efficacy of zosteric acid sodium salt on the yeast biofilm model *Candida albicans*," *Microb Ecol* 2011; 62(3):584–598.

Villa F, Albanese D, Giussani B, **Stewart PS,** Daffonchio D, Cappitelli F, "Hindering biofilm formation with zosteric acid," *Biofouling,* 2010 Aug; 26(6):739-52.



Pictured above: A formation of microbially induced calcite precipitation (MICP) grown in a wellbore analog reactor and stained pink. MICP technology has been successfully proven in the field to seal the microscopic cracks in oil and gas wells. See related story on page 8.

Image by Drew Norton and Abby Thane, colorized by Joey Parchen

GRADUATE PROGRAM

More than 200 master's and doctoral students have earned their degrees in the CBE's graduate research program since the CBE was founded in 1990. CBE graduate students acquire valuable experience by designing and performing research that crosses traditional academic discipline boundaries and has direct impact on current environmental, industrial and medical issues. In addition, the CBE's Industrial Associates program brings students into working relationships with potential employers. CBE graduate students are encouraged to develop their communication and leadership skills by presenting at research conferences, mentoring undergraduate students, organizing the CBE's seminar series, and assisting with outreach efforts. The CBE's standing in the international research community attracts visiting students and faculty from all parts of the world, providing a culturally diverse and stimulating academic environment. Graduate students pursue their degree in a discipline offered through one of the science, agriculture or engineering departments at Montana State University while conducting research in CBE laboratories.

For more information, go to:
www.biofilm.montana.edu/cbe-graduate-education.html

UNDERGRADUATE PROGRAM

Nearly 600 undergraduate students have participated in CBE research since 1990. Undergraduate students are highly valued team members in the MSU Center for Biofilm Engineering and are fully integrated into the research process. Our undergraduates learn to design and implement experiments that will provide results relevant to industry and the science community—and they develop the skills that will broaden their career opportunities and make them more valuable to prospective employers. For undergraduates who decide to pursue graduate degrees, their CBE research experience is often cited as a key component in being selected by their program of choice.

For more information, go to:
www.biofilm.montana.edu/cbe-undergraduate-education.html

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