2014 Annual Report Student Success Success Stories from the

Center for Biofilm Engineering





ENGINEERING

From the Director



Phil Stewart

Montana State University's Center for Biofilm Engineering is widely known as a center of research excellence, but, as readers of previous annual reports and CBE visitors know, we also view ourselves as an education center.

In this year's report, we spotlight

CBE contributions to student learning by taking a student-centered look at activity from the past year. Our 41 graduate students and 48 undergraduate research assistants represent 11 departments and programs from 5 colleges across campus.

The CBE offers its students an interdisciplinary learning environment, diverse mentoring and leadership possibilities, opportunities to interact with industry, and a chance to co-teach our *Microbes in the Environment* course. These transformative experiences prepare our students to tackle real-world problems and to work effectively as team members.

The depth of opportunity in independent research afforded to our students is illustrated this year by the publication of four peer-reviewed journal articles crediting an undergraduate student as first author.

The CBE's education outreach is global in that we regularly host visiting students from around the world. In the past five years, 34 students from all over the United States and also from Brazil, Chile, France, Germany, Greece, Ireland, Italy, Korea, Mali, Mexico, Russia, South Africa, and Spain have spent time in CBE laboratories.

You can see in the following pages how students make research, education, and technology transfer come alive.

See the back cover for information about the CBE graduate and undergraduate programs.

See the 2011
Appendix online for additional details about CBE activity and people during the past year.

Credits:

Our thanks to MSU News Service and to the many CBE people who contributed to this report. Cover microscopy by Alessandra Agostinho. Editing/design: Peg Dirckx Photography by CBE staff and students unless otherwise noted. APPLIED RESEARCH AREAS AND PRENERGY SOLUTIONS | biofuels mid FILMS | chronic wound healing cat metal interactions | STANDARDIZI quality premise plumbing water train natural processes thermophiles sensing structure and function heation dynamics | CELLULAR/INTRACTECHNIQUES | INSTRUMENTATION DEVELOPMENT | experimental des modeling mathematics hydrodynamical counts | MOLECULAR BIOLOGY

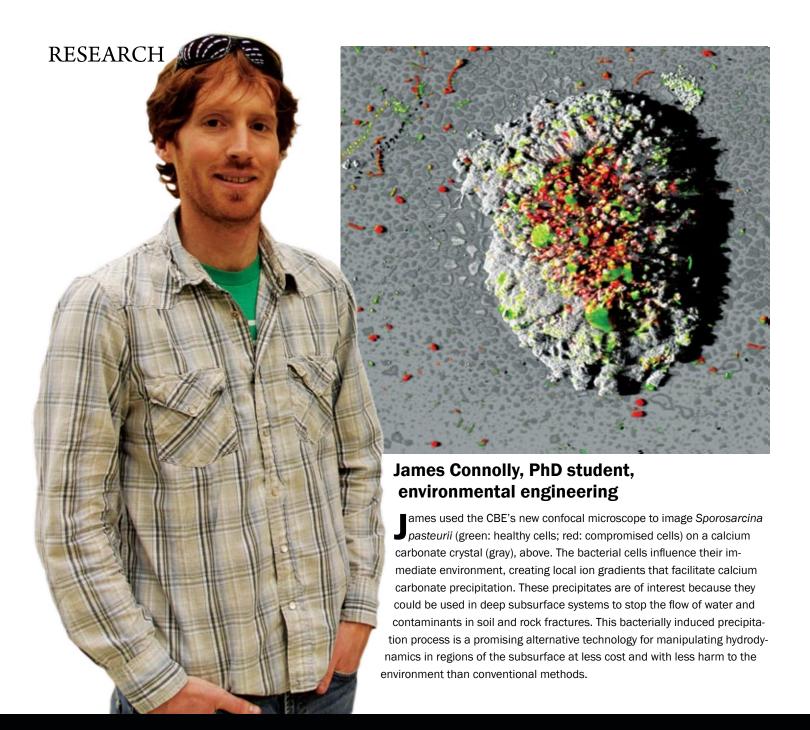
glance at this year's 52 publications (listed in the annual report Appendix, page 10) quickly conveys the breadth of ongoing research activity at CBE. Among the topics intersecting biofilms on this list are: chronic wounds, mathematical modeling, membrane fouling, microbial life in ice, transcriptomics, metal toxicity, viscoelasticity, antibiotic tolerance, hydrodynamic dispersion, and mineral precipitation. New grants from NSF, NASA, and DOE reinforce projects on microbial processes in constructed wetlands, bacterial ecology in icy niches, biofuels, and subsurface carbon sequestration. A pair of awards from NSF and the M.J. Murdock Charitable Trust, providing over \$900,000, funded a major overhaul of the CBE's confocal microscopy equipment.

RESEARCH

OJECTS | BIOFILM CONTROL STRATEGIES | antimicrobial efficacy biocides bioelectric effect disinfectants inhibitory coatings nitrous oxide | crobial fuel cells | ENVIRONMENTAL TECHNOLOGIES | bioremediation wetlands CO2 sequestration biobarriers | HEALTH AND MEDICAL BIO-theter infections or all health food safety | INDUSTRIAL SYSTEMS AND PROCESSES | biofouling biocorrosion product contamination microbe-ED BIOFILM METHODS | biomineralization product claims regulatory issues ASTM methods acceptance | WATER SYSTEMS | drinking water



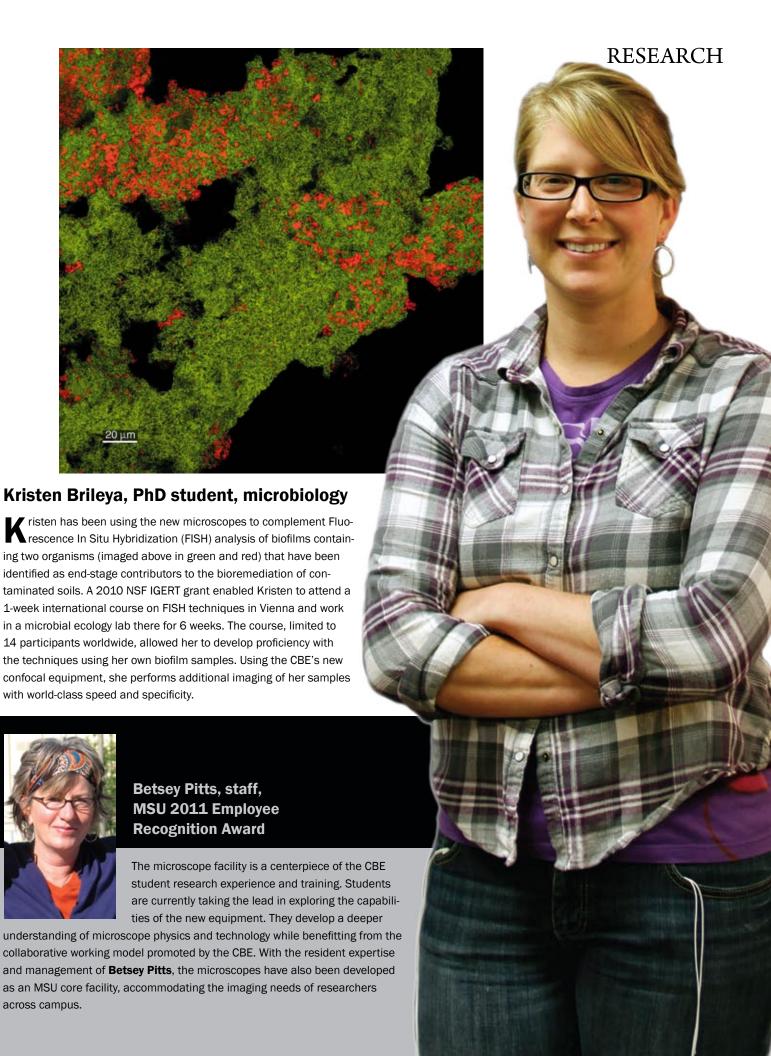
McLeod BR, Sandvik EL, "A biofilm growth protocol and the design of a magnetic field exposure setup to be used in the study of magnetic fields as a means of controlling bacterial biofilms," *Bioelectromagnetics*, 2010; 31(1):56-63.



Two brand-new customized confocal microscope systems will revolutionize biofilm study

n the fall of 2010, the CBE received a **National Science**Foundation award of \$498,400 to fund the purchase of new confocal microscope equipment. This award complemented a cost-sharing grant of \$406,500 from the **M.J.**Murdock Charitable Trust. Together, the awards have enabled the Center for Biofilm Engineering to purchase two customized Leica SP5 Spectral Confocal systems that will revolutionize the microscopic study of microbial biofilms.

The new systems are so advanced that there are only five other similarly equipped confocal microscopes in the U.S. None of them are in the Northwest, and none of them have been configured with biofilm study as the primary intent. The microscopes will facilitate interdisciplinary science and engineering on diverse topics ranging from algal biofuels to biofilms on medical devices.



RESEARCH



RESEARCH





 $\textbf{Garret Vo}, \, \text{UG physics; MS mechanical engineering}$

One measure of the CBE's commitment to providing undergraduates with meaningful research experience is the number of peer-reviewed papers with undergraduate first authors.

This is a list of recent authors.



Chelsea Lipp, cell biology and neuroscience

Undergraduate first authors

Lipp C, Kirker K, Agostinho A, James G, Stewart P, "Testing wound dressings using an in vitro wound model," *J Wound Care*, 2010, 19(6):220–226.

Vo G, Brindle E, Heys JJ, "An experimentally validated immersed boundary model of fluid-biofilm interaction," *Water Sci Technol*, 2010, 61(12):3033–3040.

Vo GD, Heys J, "Biofilm deformation in response to fluid flow in capillaries," *Biotechnol Bioeng*, 2011, 108(8):1893–1899.



Trevor Zuroff, chemical and biological engineering; photo, MSU News Service

Zuroff TR, Bernstein H, Lloyd-Randolfi J, Jimenez-Taracido L, Stewart PS, Carlson RP, "Robustness analysis of culturing perturbations on *Escherichia coli* colony biofilm beta-lactam and aminoglycoside antibiotic tolerance," *BMC Microbiol*, 2010, 10:185.

EDUCATION

For over 20 years, the CBE has provided MSU students with opportunities for innovative, interdisciplinary, handson research experiences. This year more than 30 MSU faculty from 8 departments contributed expertise to biofilm projects. Their guidance allows CBE students to do research that is relevant outside the university, including work in chronic infections, remediating contaminated soil, mitigating fouling and corrosion in industry, and developing constructed wetlands to treat wastewater.

Matthew Sherick, undergraduate, chemical and biological engineering

"My project involves the extraction of bacterial alginate from *Pseudomonas* aeruginosa biofilms and the analysis of alginate gel formation. This project has applications in the biomedical field, particularly concerning cystic fibrosis (CF)





Hilary Fabich, undergraduate, chemical and biological engineering

In addition to excelling in research and mentoring, Hilary received MSU's Ethelyn C. Harrison Award, presented to a senior-class woman who exemplifies high standards and concern for others. Fabich is a member of Engineers Without Borders and the MSU Symphony Orchestra. She has been an undergraduate researcher in the Magnetic Resonance Microscopy Lab, with professors Sarah Codd, mechanical and industrial engineering, and Joe Seymour, chemical and biological engineering, for over three years.

EDUCATION

"Undergraduate **John Blaskovich** never puts himself in the limelight, yet has been very reliable, hardworking, and productive. He worked with doctoral student Erin Field and was instrumental in her progress in the past one-and-a-half years."

Robin Gerlach, associate professor, chemical and biological engineering





Anitha Sundararajan and Mari Eggers, both PhD candidates in microbiology, received the CBE's 2011 W.G. Characklis Outstanding Student Award in January. Mari (faculty mentor Anne Camper, civil engineering) was recognized for her dedication to community-based participatory research on water quality and the health of the Crow Reservation community. Anitha (faculty mentor Matthew Fields, microbiology) received the award in recognition of her original science at the interface between biofilms and microbial physiology.

Summary of undergraduate students 2010–11

 undergraduate students, Native American students; female/**26** male, representing **11** departments:

Cell Biology & Neuroscience
Chemical & Biological Engineering
Chemistry & Biochemistry
Civil Engineering
Immunology & Infectious Disease
Land Resources & Environmental Sciences
Mathematical Sciences
Mechanical & Industrial Engineering
Microbiology
Nursing (Bridges)
Physics

Summary of graduate students 2010–11

41 graduate students **24** female / **17** male; **31** PhD / **10** MS representing **10** departments and programs:

Cell Biology & Neuroscience
Chemical & Biological Engineering
Chemistry & Biochemistry
Civil/Environmental Engineering
Health Sciences
Land Resources & Environmental Sciences
Mathematical Sciences
Mechanical & Industrial Engineering
Microbiology
Molecular Biosciences



EDUCATION

Otto Stein, right, professor in the Department of Civil Engineering, was selected as one of two 2011 recipients of MSU's Provost's Excellence in Outreach Award. Stein is lead faculty adviser for the MSU student chapter of Engineers Without Borders. He has empowered students to be global thinkers and actors, while simultaneously

volution in the Khwisero

overseeing an infrastructural revolution in the Khwisero region of rural western Kenya. *Photo, MSU News Service*

WATER TREATMENT WETLANDS



Constructed wetlands team members Johnson, Burr, Hisey, Allen, and Collazo Ortiz.

In the fall of 2010, Drs. **Otto Stein, Anne Camper**, and **Mark Burr** were awarded a three-year, \$303K grant from NSF: "Plant, season, and microbial controls on com-

plete denitrification in treatment wetlands."

Additional team research explores the mechanics of *E. coli* removal in treatment wetlands. Five undergraduates and two graduate students—representing microbiology, civil/environmental engineering, and land resources and environmental sciences—contributed to the research this year.

Chris Allen, PhD student, environmental engineering



10



Brent Peyton (left), Department of Chemical and Biological Engineering, and Matthew Fields (below), Department of Microbiology, were two of four MSU faculty members who won this year's Charles and Nora L. Wiley Faculty Awards for Meritorious Research and Creativity. Photos, MSU News Service

ALGAL BIOFUELS



Rob Gardner, with MSU faculty Keith

Cooksey and Brent Peyton, discovered that baking soda, added at a specific time in the growth cycle of algae, dramatically increases the production of the key oil precursors for biodiesel. It turns out that this common substance is the elusive chemical trigger that scientists have sought since the early 1990s to increase algal oil production. When added at just the right time in the growth cycle, algae produce two to three times the oil in half the time of conventional growth models.

ENERGY PRODUCTION

Methane is a natural gas alternative that can be extracted from wells drilled into subsurface coal beds. While high-grade coal beds produce methane thermally, all of the methane in sub-bituminous coal beds is microbially produced. A native of Broadus, Montana, Elliott is well acquainted with how the coal-bed methane industry has impacted land-owners, ranchers, and the landscape in eastern Montana and Wyoming. With Matthew Fields, he has been investigating microbial communities acquired from existing coal beds and hopes to find ways to make the methane production

process more efficient and less destructive to the environment. With his masters degree work completed in August 2011, he plans to pursue a PhD degree at MSU in the fall.

Elliott received MSU's Torlief Aasheim
Community Involvement Award in 2009.
He earned Academic All-American honors in football, received the Montana Athletes in Service Award, and was the 2006 Offensive Player of the Year and 2007 Bobcats team captain. He was also a member of the University Choir in the spring of 2008.
Off-campus, as a youth group leader and as a student athlete, Barnhart has been an active volunteer in the President's Day Plunge for Special Olympics and the Relay for Life.

Elliott Barnhart, MS, microbiology

Rob Gardner, PhD student, chemical & biological engineering

INDUSTRY

Industry highlights

In 2011 the CBE's Industrial Associates program participation remained at a robust 32 subscribing members (26 full members and 6 small business members). Participation remains strong among healthcare and biomedical companies, consumer products manufacturers, specialty chemical companies, testing laboratories, and US Government-funded agencies and laboratories. After more than a decade hiatus, ExxonMobil rejoined the CBE industrial program, and

other energy-related companies have shown recent interest as well. The Montana Biofilm Science & Technology Meetings continue to be the major venue for industrial interaction, with over 140 current and prospective member scientists attending one or both of the meetings over the past year. Industry-sponsored testing and research project work continued at a very high level in 2011, with 33 total projects for 25 different companies, 60% of whom were CBE industrial partners. Through our semi-annual meetings (February and July of each year), our biofilms methods workshops, visits to companies, and regulatory outreach, the CBE seeks to expand the value of membership to our partner companies.

Sabrina first came to the CBE in 2005 as a visiting undergraduate student from Germany, at the urging of her microbiology professor there. She enjoyed the friendliness and team spirit at the CBE during her visit and decided to pursue her graduate studies at Montana State University. During her tenure at the CBE, she won the Montana Water Center Research and Fellowship Award (January 2008) and the CBE's W.G. Characklis Award (February 2010). Her work on chlorine disinfection, under the mentorship of Anne Camper, civil engineering, was sponsored in part by Unilever UK Central Resources Ltd.

Sabrina Behnke, PhD student, microbiology

"A unique part of the education at the CBE is the interaction with national and international companies. Here, students have the opportunity to present their research, discuss it with scientists from various fields of expertise, and potentially collaborate with them. During my time at the CBE, I was able to conduct a project with an international company that eventually helped me fund my graduate research. This collaboration was an extremely valuable experience in addition to the education I got through my classes, teaching assignments, and the interaction with other CBE scientists. I feel confident that the very comprehensive and interdisciplinary education I received at the CBE will help me be a valuable addition to the R&D team at Reckitt Benckiser."

STANDARDIZED METHODS

"In October 2008, Innovotech began working with the CBE's Standardized Biofilm Methods group to perform the testing necessary to have the company's MBEC Assay™ approved as an ASTM International standard for high throughput disinfectant efficacy testing against *Pseudomonas* biofilms. In April 2011 that standard was approved as ASTM E2799-11. The process was extremely smooth and efficient. The expert advice, professional service and guidance received from this group were critical to the success of this method's rapid development and review process."

Nick Allan, Contract Research Manager, Innovotech Inc.

Ken Boutilier, president and CEO of Innovotech, Inc.:

"This approval paves the way for a new generation of disinfectants in the food industry and in hospitals, designed specifically to address the issue of biofilms, thereby reducing the number of deaths and sicknesses resulting from foodborne and infection related illnesses."

FROM GRADS IN INDUSTRY



Jennifer Hornemann PhD, 2009, chemical & biological engineering, employed by ExxonMobil

"Being part of the CBE team at MSU gave me a unique opportunity to learn the value of collaboration. Having diverse teams of people from various technical, cultural and geographical backgrounds working towards the same goal gave necessary and beneficial perspectives to approach research challenges. This experience has proved imperative in my current job, as we work to solve multifaceted, costly, immediate research needs for the oil and gas operating environment."



Willy Davison
PhD, 2008, chemical & biological engineering, employed by
W.L. Gore & Associates

"The CBE's Industrial Associates program allowed me to develop relationships with many industry representatives, including my current employer, W.L. Gore & Associates, Inc. (Gore). At Gore, we believe in the power of small teams and ultimately rely on our Associates' expertise and knowledge to help solve problems. Similarly, the **CBE** fosters collaboration with a multidisciplinary, cross-functional approach to microbial biofilm research. The knowledge I gained and skills I developed during my time at the CBE have translated directly into my current role at Gore."



Myles Perkins, undergraduate, chemical and biological engineering assisted with the development of the MBEC Assay™.

NEW MEMBERS IN ORANGE 2010-2011 INDUSTRIAL ASSOCIATES

3M | Agile Sciences | BASF | Bausch & Lomb
Baxter Healthcare | Bayer MaterialScience
BD Medical | Bridge Preclinical Testing Service
CareFusion | Church & Dwight Company
Colgate-Palmolive | Covidien
Dow Microbial Control | Embro Corporation
ExxonMobil | Glanbia Nutritionals | ICU Medical
Johnson & Johnson Consumer and Personal Products
Kane Biotech | Kimberly-Clark | Masco Corporation
NASA | Novozymes A/S | Procter & Gamble
Reckitt Benckiser | Sandia National Laboratories
Semprus BioSciences | STERIS
The Sherwin-Williams Company | Unilever
W.L. Gore & Associates | WuXi AppTec

INDUSTRY

SPONSORED PROJECTS

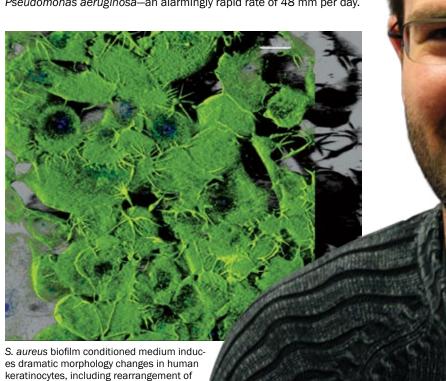
Industry-sponsored project work is focused in both the Standardized Biofilm Methods Laboratory and the Medical Biofilm Laboratory. Both labs use students and full-time staff to ensure project continuity and a broad understanding of methods that yield field-relevant results and cost-effective solutions.

Needle-free connectors

The CBE's Medical Biofilm Laboratory (MBL) team, under the direction of **Garth James**, has been working on needle-free connectors with member company **ICU Medical, Inc.** since 2005. This work has included comparing bacterial transfer through a wide range of different connector types and, more recently, downstream effects (e.g., catheter colonization) of bacterial passage through connectors. The MBL has also tested a variety of prototype connectors and provided data included in a successful 510K application to the FDA. The results have been presented at a variety of national and international meetings, including the Society for Healthcare Epidemiology of America (SHEA). This work was conducted in collaboration with Marcia Ryder, PhD, RN, of Ryder Science.

Bacterial migration

Member company **Covidien, Inc.** sponsored a project in the MBL to evaluate bacterial migration along electrocardiogram (ECG) wires. Surprisingly, non-motile *Staphylococcus aureus* (in this case, MRSA) migrated along the wires at approximately the same rate as the motile *Pseudomonas aeruginosa*—an alarmingly rapid rate of 48 mm per day.



Pat Secor, PhD 2010, cell biology and neuroscience

at began working at the CBE in 2004 as an undergraduate researcher in the Medical Biofilm Laboratory, characterizing bacterial communities in chronic wounds. By December 2010, he had completed his doctoral work on the impact of Staphylococus aureus biofilm-conditioned medium on human keratinocytesspecialized cells that are active in the early stages of wound healing. He found that exposure to medium in which biofilms had formed decreases keratinocyte viability dramatically and increases their likelihood of undergoing apoptosis, or programmed cell death. Exposure to biofilm medium also upregulates genes for cytokines and chemokines associated with inflammation. Pat's association with medical collaborators on the CBE's NIH chronic wound project led to his current postdoctoral position at the University of Washington's Center for Lung Biology.



the actin cytoskeleton (green) and nuclear fragmentation (blue). Microscopy, P Secor

OUTREACH

VISITING RESEARCHERS

Debora Barbosa, Visiting postdoctoral researcher and dentist. Universidade Estadual Paulista. Brazil

Mery De La Fuente, Visiting PhD student, Universidad de Concepción, Chile

Anozie Ebigbo, Visiting postdoctoral researcher, Universität Stuttgart, Germany

Megan Elam, Visiting MS student, Oregon State University, Corvallis, OR

Marion Fontagneu, Visiting MS student, Université de Pau, France

Buntu Godongwana, Fulbright visiting researcher, Cape Peninsula University of Technology, South Africa

Mijeong Jang, Visiting postdoctoral researcher, University of Seoul, Korea

Irina Khilyas, Visiting PhD student, Kazan Federal University, Russia

Danielle Kinsey, Visiting undergraduate, Fort Belknap College, Harlem, MT

Fidel Martínez Gutiérrez, Visiting scholar, Universidad Autónoma de San Luis Potosí, Mexico

Emma Mean, Visiting undergraduate, Notre Dame University, South Bend, IN

Trond Møretrø, Visiting research scientist, Nofima MAT, Ås, Norway

Sai Nagarajan, Visiting postdoctoral researcher, University of Montana, Missoula, MT

Indra Sandal, Visiting research scientist, Virginia Tech, Blacksburg, VA

John Tobiason, Visiting faculty,
University of Massachusetts, Amherst, MA

Yi Wang, Visiting faculty,

Xi'an University of Architecture &

Technology, Shaan'xi Province, China



Above, Federica Villa, University of Milan, Italy, a student visitor in 2009; and, left, visiting researcher Anozie Ebigbo, Universität Stuttgart, Germany.

Visiting graduate student first authors

Most visiting students work at the CBE for a period of several months, while others—like Anozie Ebigbo, above, who is now a postdoctoral researcher—have visited the CBE several times over a period of years as they collaborate with CBE researchers.

Cotter JJ, O'Gara JP, Stewart PS, Pitts B, Casey E, "Characterization of a modified rotating disk reactor for the cultivation of *Staphylococcus epidermidis* biofilm," *J Appl Microbiol*, 2010, 109(6):2105–2117.

Ebigbo A, Helmig R, Cunningham AB, Class H, Gerlach R, "Modeling biofilm growth in the presence of carbon dioxide and water flow in the subsurface," *J Adv Water Resour*, 2010, 33:762–781.

Kim J, Hahn JS, Franklin MJ, Stewart PS, Yoon J, "Tolerance of dormant and active cells in *Pseudomonas aeruginosa* PAO1 biofilm to antimicrobial agents," *J Antimicrob Chemother*, 2009, 63:129–135.

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.

Graduate program

ore 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



Center for Biofilm Engineering 366 EPS Building Montana State University Bozeman, Montana 59717-3980 USA

Phone: 406-994-4770 Fax: 406-994-6098





Microscopy, B Pitts

Undergraduate program

early 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

www.biofilm.montana.edu

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