From the time of its inception in 1990 as a National Science Foundation Engineering Research Center, the Center for Biofilm Engineering has practiced interdisciplinary scholarship; engagement with the natural, engineered, and human world beyond our campus; and integration of research with innovative educational experiences for students. How gratifying it is to find these same themes resonating in Montana State University’s new strategic plan (www.montana.edu/strategicplan/). As we report on our accomplishments from the past year in this annual report, look for the evidence of our success in contributing to the ideals of the University.

The CBE is also pleased to offer you a special feature in this report: “lessons” on key biofilm topics. We hope that, with this addition, our annual report will not only archive our activities and achievements of the past year, but endure as a living educational resource. Look inside to learn more about biofilm-mediated corrosion of materials, biofilm infections, and the enigmatic yet fundamentally important biofilm matrix. And then please share this report with colleagues who might appreciate and profit from instruction in biofilm concepts.

Finally, I would call your attention to the numerous faculty, staff, students, and visiting researchers pictured here, whose contributions in teamwork, excellence, inclusiveness, and creativity have once again made CBE a rewarding place to work and learn.

Phl Stewart

Access complete academic year 2012–13 information in the 2013 Appendix at: www.biofilm.montana.edu/resources/annual_reports/
CBE research impact, measured by citations per paper over the last decade, leads all MSU departments, centers, and institutes.

Publications in:

- Analyst
- Annals of Glaciology
- Antimicrobial Agents and Chemotherapy
- Applied Microbiology and Biotechnology
- Biofouling
- BioScience, Biotechnology and Bioengineering
- Biotechnology for Biofuels
- BMC Genomics
- Bulletin of Mathematical Biology
- Clays and Clay Minerals
- Computational and Structural Biotechnology Journal
- Discrete and Continuous Dynamical Systems
- Environmental Science & Technology
- European Physical Journal Applied Physics
- Geobiology
- Greenhouse Gases: Science and Technology
- International Journal of Uncertainty Quantification
- Journal of AOAC International
- Journal of Applied Phycology
- Journal of Bacteriology
- Journal of Chemical Technology and Biotechnology
- Journal of Inorganic Biochemistry
- Microbial Ecology
- MicrobiologyOpen
- Numerical Linear Algebra with Applications
- Physical Review E
- PLOS ONE
- PNAS: Proceedings of the National Academy of Sciences
- Subcellular Biochemistry
- Water Resources Research

Of the 39 articles published by CBE researchers this year, 35 (90%) name co-authors from at least two different disciplines. We are interdisciplinary.
In a very tough climate for federal funding, CBE investigators had an excellent year for new grants. We are **nationally competitive**.

**grants & projects**

**APPLIED**

*Energy Solutions*
- Bicarbonate induced microalgae lipid production, PI: Peyton, Funding: Church & Dwight
- Cultivation/characterization of oil producing algae, PI: Peyton, Funding: Little Big Horn College
- Alkaliphilic microalgae-based biofuels and products, PIs: Gerlach-Peyton-Fields, Funding: NSF
- Lignocellulosic feedstock conversion to lipids, PI: Macur, Funding: Sustainable Bioproducts
- Sustainable algal biorefineries, PIs: Gerlach-Peyton-Fields, Funding: DOE
  - Montana biodiesel initiative, PI: Peyton, Funding: DOE
  - Biofuels/bioproducts from extremophilic microalgae, PIs: Gerlach-Peyton-Fields, Funding: DOE
  - Fungal biofuels, PI: Peyton, Funding: NSF-ARRA

**Environmental Technologies**
- Renewable organic fertilizer, PI: Macur, Funding: DOE-AIREI
- Selenium biogeochemistry investigation, PI: Peyton, Funding: Teck Coal
- Low-cost NMR technologies to monitor subsurface processes, PI: Codd, Funding: DOE
- Environmental responses to CO₂ sequestration, PI: Cunningham, Funding: DOE
- Risk assessment, monitoring for geologic CO₂ sequestration, PI: Cunningham, Funding: DOE
- Zero Emissions Research & Technology II, PI: Cunningham, Funding: DOE-ZERT
- Complete denitrification in treatment wetlands, PI: Stein, Funding: NSF
- Porous media microbial activity in mixing zones, PI: Gerlach, Funding: DOE-ERSP

**Medical Biofilms**
- Novel anti-biofilm compounds to treat chronic wounds, PI: James, Funding: NIH
- Biofilm mediation of keratinocyte apoptosis, PI: Kirker, Funding: NIH

**Methods Development**
- Antimicrobial test methodology, PI: Goeres, Funding: EPA

**FUNDAMENTAL**

*Biofilms in Nature*
- Molecular level characterization of microbial metabolism and dissolved organic matter from Antarctica, PI: Foreman, Funding: NSF
- Integrated chemical and biological measurements in Antarctica, PI: Foreman, Funding: NSF

**Education**
- Improving Montana community health, PI: Camper, Funding: NIH
- Graduate Fellowship (H Smith), PI: Foreman, Funding: NASA

**Physiology & Ecology**
- Virtual institute for microbial stress and survival, PI: Fields, Funding: LBNL
- Phototroph-heterotroph interactions, PI: Carlson, Funding: DOE PNL
- Role of non-coding RNAs in biofilm development, PI: Franklin, Funding: NIH
- Role of IbpA in viability of biofilm persistor cells, PI: Franklin, Funding: NIH

**TOOLS & TECHNIQUES**

*Modeling*
- CMG research, PI: Klapper, Funding: NSF

**New grant awards for FY 2013 totalled $3,535,635**

Learn more about our research areas at:  
[www.biofilm.montana.edu/research-program.html](http://www.biofilm.montana.edu/research-program.html)
EXPERTISE

associated faculty

Jennifer Brown ChBE Rheology and biofilm mechanics
Mark Burr LRES Microbial community analysis
Anne Camper CE Biofilms in environmental systems
Ross Carlson ChBE Metabolic engineering, metabolic networks
Sarah Codd M&IE Magnetic resonance imaging
Kevin Cook MET Tool and machine design
Al Cunningham CE Subsurface biotechnology and bioremediation
Jack Dockery MathSci Mathematical models of biofilms
Matthew Fields Micro Physiology and ecology
Christine Foreman ChBE Microbial ecology in cold temperature environments
Michael Franklin Micro Molecular genetics, gene expression, alginate
Gill Geesey Micro Molecular and cellular interactions at interfaces
Robin Gerlach ChBE Environmental biotechnology and bioremediation
Darla Goeres ChBE Standardized biofilm methods
Marty Hamilton Stat Applied biostatistical thinking
Jeff Heys ChBE Fluid-structure interactions
Garth James ChBE Medical biofilms
Warren Jones CE Water distribution systems
Isaac Klapper MathSci Mathematical modeling
Zbigniew Lewandowski CE Microsensors, chemical gradients, biofilm structure
Richard Macur ChBE Biofuels, geochemistry, geomicrobiology
Aurélien Mazurie Micro Bioinformatics
Bruce McLeod E&CE Bioelectric effect
David Miller M&IE Experimental mechanics
Andy Mitchell CE Geomicrobiology
Al Parker Stat Statistical models in biofilm systems
Brent Peyton ChBE Environmental biotechnology and bioremediation
Elinor Pulcini ChBE Medical biofilms
Barry Pyle Micro Environmental, water, and food microbiology
Abbie Richards ChBE Environmental biotechnology
Rocky Ross CS Web-based, active learning education
Joseph Seymour ChBE Magnetic resonance imaging
Otto Stein CE Engineered waste remediation
Phil Stewart ChBE Biofilm control strategies
Paul Sturman CE Biofilms in waste remediation and industrial systems
Peter Suci Micro Fungal biofilms
Tianyu Zhang MathSci Mathematical modeling

www.biofilm.montana.edu/people/faculty

The CBE is led by award-winning faculty from 10 academic departments.

faculty awards & news

Sarah Codd: College of Engineering Lloyd Berg Faculty Mentorship Award
Christine Foreman: appointed College of Engineering Associate Dean of Student Success
Robin Gerlach: 2013 Cox Family Award for Creative Scholarship and Teaching
Darla Goeres: Awarded Fulbright Scholarship to study in Finland
Warren Jones: Lifetime Achievement Award from the Montana Water Environment Association
MSU 2013 Awards for Excellence
Al Parker: CBE Outstanding Faculty Award
Brent Peyton: appointed Director of the MSU Thermal Biology Institute
Abbie Richards: 2013 College of Engineering Faculty Award for Excellence in Advising
2012 National Outstanding Advisor, Tau Beta Pi engineering honor society
Otto Stein: Student Organization Advisor of the Year (Engineers Without Borders)
This graph illustrates the typical time course of pH in dental plaque after a rinse with a sugar solution. This drop and recovery in pH is known as the Stephan curve.

C O R R O S I O N

This is a cutaway view of equipment from an oilfield pipeline. The crusty deposit is a mixture of biofilm and abiotic corrosion products that have become incorporated in the sticky matrix of the biofilm. Microorganisms in the biofilm, in this case likely including sulfate-reducing bacteria, influence the corrosion process. Biocides are used in such systems to control fouling, souring, and corrosion problems.

A pit in the surface of a tooth—imaged here once the dental plaque was removed—is a cavity. Bacteria such as Streptococcus mutans in biofilms on teeth ferment sugars to a mixture of low molecular weight organic acids. This decreases the pH at the tooth surface and promotes demineralization of the enamel. Dental caries is a biofilm infection and also an example of mineral corrosion by attached microbes.

The Stephan Curve

Stoichiometry of sugar fermentation to acidic products by S. mutans

\[
\begin{align*}
100 \text{ glucose} & \rightarrow 7 \text{ D-lactate} \\
& \quad + 169 \text{ ethanol} \\
& \quad + 73 \text{ acetate} \\
& \quad + 289 \text{ moles acid}
\end{align*}
\]
biofilm lesson 1:

Microrganisms in biofilms, through their localized metabolic activity, can cause corrosion of mineral and metal materials.

Here, a diagram depicts one mechanism of metal corrosion by bacteria. **A)** When metallic iron corrodes, soluble Fe$^{2+}$ is released to the surrounding water and electrons are conducted through the metal. **B)** For the corrosion process to continue, the electrons have to be consumed in a reaction somewhere on the metal surface. Manganese dioxide is an excellent electron acceptor. **C)** Manganese-oxidizing bacteria, such as those of the genus *Leptothrix*, provide this outlet by depositing manganese dioxide on the metal surface.

Brownish manganese dioxide on a stainless steel coupon. The mineral was deposited on the surface by manganese-oxidizing bacteria.

**BIBLIOGRAPHY**


CBE students excel.

Alissa Bleem, ChBE
Goldwater Scholar
2012-13 Hughes Scholar

Eric Dietrich, CE (bio-resources)
Rhodes National finalist
MSU 2013 Award for Excellence
MT Society of Engineers Gold Medal Finalist

Mandi Durch, ChBE
internship at Procter & Gamble

Justin Nagy, Micro
participant, 2012 Complex Biological Systems
Summer Undergraduate Research Program

Breana Pabst, ChBE
MT Society of Engineers Gold Medal Finalist

Matthew Sherick, ChBE
Goldwater Scholar
inducted into Septemviri honorary society

Amber Schmit, ChBE
ASM research fellowship honorable mention

Erika Whitney, Micro
2012-13 Hughes Scholar
participant, 2012 Complex Biological Systems
Summer Undergraduate Research Program

Neerja Zambare, ChBE, presented her research at the Council on Undergraduate Research’s Posters on the Hill program in Washington D.C. April 23–24, 2013.

www.biofilm.montana.edu/msu-cbe-educational-experience.html

Note: Not all students are pictured.
Summary of graduate students 2012–13

52 graduate students
29 female / 23 male;
40 PhD / 12 MS
representing 8 departments:
Cell Biology & Neuroscience
Chemical & Biological Engineering
Chemistry & Biochemistry
Civil/Environmental Engineering
Health & Human Development
Land Resources & Environmental Sciences
Mathematical Sciences
Microbiology

Elliott Barnhart, PhD student, microbiology, was one of four recipients of an Outstanding Student Oral Presentation award at the Secondary Biogenic Coal Bed Natural Gas International Conference in Laramie, Wyoming.

Kristen Brileya, PhD 2013, microbiology
2012 CBE Student Citizen Award

Kara De León, PhD 2013, microbiology
2013 W.G. Characklis Award
2012 CBE Student Citizen Award

Catherine Kirkland, PhD student, EnvE
National Science Foundation Graduate Research Fellowship

Adie Phillips, PhD 2013, ChBE
Outstanding Student Paper Award, 2012 American Geophysical Union (AGU)

Liz Sandvik, PhD 2013, ChBE
W.G. Characklis Award

Sarah Jane Vogt, PhD 2012, ChBE
Special award for Exceptional Publication Productivity

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, and environmental contexts.

CBE production of doctoral degrees relative to total research expenditures is more than 3.5 times the MSU average. CBE is a model for integration of research and PhD level education.
biofilm lesson 2:
Microorganisms in biofilms cause diverse, slow-moving, yet persistent infections.

This is a scanning electron microscope view of a biofilm-encrusted pacemaker lead. The device came out of a man who experienced repeated bouts of septicemia: staph bacteria were cultured from his blood. He was hospitalized and placed on a strong IV antibiotic. He got better and was discharged. After a week or so, he was back with fever, chills, and localized tenderness in his upper torso. In the end he went through three rounds of treatment and relapsing infection before the entire pacing unit was surgically removed. The biofilm of coccoid bacteria on the lead wire is unmistakable. These are the classic sequelae of a biofilm infection. It is recurrent and difficult to clear with antibiotics or antiseptics.

Periodontitis is caused by a biofilm infection below the gum line. You do not have to be a physician to see the chronic inflammation of the gums here. In the standoff between the biofilm and the host, the biofilm persists despite the body’s continuing reaction to it while this assault takes a toll on neighboring healthy tissue. In untreated periodontitis this stalemate results in slow but progressive bone loss; teeth eventually fall out.
Some dermal wounds such as diabetic foot ulcers and bedsores (pressure ulcers) fail to heal even over periods of months. The biofilm hypothesis, diagrammed here, offers an explanation for this failure.

### A few examples of biofilm infections
- Catheters
- Prosthetic joints
- Rhinosinusitis
- Dental caries
- Chronic wounds
- CF pneumonia
- Acne

### Common characteristics of biofilm infections
- Form preferentially on foreign bodies, dead or damaged tissue
- Slow to develop, but persistent
- Respond poorly or only temporarily to antibiotics, antiseptics
- Collateral damage to neighboring healthy tissue

### Market size for selected products (annual)

<table>
<thead>
<tr>
<th>Product</th>
<th>Market Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catheters</td>
<td>$21 B</td>
</tr>
<tr>
<td>Orthopedic implants</td>
<td>$9 B</td>
</tr>
<tr>
<td>Oral care</td>
<td>$5 B</td>
</tr>
<tr>
<td>Advanced wound care</td>
<td>$3 B</td>
</tr>
</tbody>
</table>

### BIBLIOGRAPHY


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.

visiting researchers, 2012–13

CBE visiting researchers include students, staff, and faculty from USA and international academic institutions, as well as health clinicians and industrial researchers from the USA and abroad. Visiting researchers may stay for a few weeks to a year or more. In each case, the CBE emphasizes learning, productivity, and collaboration.

In the 2012–13 period, CBE hosted 21 visiting researchers, including 12 students at the high school, undergraduate, and graduate levels. International visitors hailed from Brazil, China, Finland, Germany, Italy, Mexico, and Spain. USA visitors included researchers from the University of Connecticut, Rensselaer Polytechnic Institute, and Utah State University.

Five visitors in summer 2012 were participants in a collaborative research project between MSU’s Department of Chemical and Biological Engineering, the CBE, and Little Big Horn College in Crow Agency, Montana. Participants investigated nitrogen-fixing strains of cyanobacteria that might be used to extract waste carbon dioxide from the coal liquefaction process on the Crow Reservation in the hope of developing an organic fertilizer product for crop production. Project Principal Investigators were MSU faculty members Brent Peyton and Rich Macur.

Note: Not all visitors are pictured.

In the past five years, 18 CBE visiting researchers (5 US and 9 foreign countries) have been co-authors on 23 peer-reviewed publications. CBE emphasizes substantive interactions.

workshop on biofilm-induced mineralization

CBE faculty members Al Cunningham (CE), Robin Gerlach (ChBE), Issac Klapper (MathSci), and Tianyu Zhang (MathSci) organized a two-day workshop on biofilm-induced mineralization in the summer of 2012, co-sponsored by NSF and the CBE. Microbiologically induced calcium carbonate precipitation has been proposed for a number of engineered applications including carbon dioxide binding, protection of construction materials, soil stabilization, and environmental remediation. Participants, including experts in both laboratory experiments and mathematical modeling, presented and discussed the current state of knowledge and concepts in this field.

Visiting presenters were:
- Benito Chen, University of Texas at Arlington
- Yohan Davit, Oxford University, UK
- Bruce Fouke, University of Illinois
- Markus Hilpert, Johns Hopkins University
- Andy Mitchell, Aberystwyth University, UK
- Cristian Picioreanu, Delft University of Technology, The Netherlands
- Marcel Schaap, University of Arizona
- Tim Scheibe, Pacific Northwest National Laboratory
- Qi Wang, University of South Carolina
- Dorthe Wildenschild, Oregon State University

www.biofilm.montana.edu/visiting-researchers-cbe.html
CBE engages with industry across a wide spectrum of markets and application areas.

**Industrial Associates, 2012–13**

Small business member * Bold, new

3M
Bard Access Systems
BASF
Bausch & Lomb
Baxter Healthcare
BCG Solutions *
Bend Research *

**BP**
CareFusion (formerly Cardinal Health)
Church & Dwight Company
Colgate-Palmolive
Covidien
Dow Corning Corporation
Dow Microbial Control / Rohm and Haas

**Ecolab**
ExxonMobil
ICU Medical, Inc.
Johnson & Johnson Consumer and Personal Products
Kane Biotech, Inc.*
KCI
Kimberly-Clark
Masco Corporation
Microbial Defense Systems *
NASA
NCH Corporation
Novozymes
Procter & Gamble
Reckitt Benckiser
Sample6 Technologies *

**Sani-Marc, Inc.**
Sealed Air Corporation
Semprus BioSciences *
STERIS

**The Clorox Company**
The Sherwin-Williams Company
Unilever
W.L. Gore & Associates
WuXi AppTec, Inc. *

Memberships and sponsored projects through CBE have brought over $7M in industrial funding to Montana during the past 5 years.

www.biofilm.montana.edu/cbe-industry-program.html
An uncharacterized constituent of the matrix of this *Pseudomonas aeruginosa* biofilm was stained with an amine-reactive dye (red). Bacterial cells (green) were tagged with a fluorescent protein. The matrix extends between the cells in a web-like fabric.

Biofilm matrix polymers, also known as extracellular polymeric substances (EPS), reported for one bacterial species, *Escherichia coli*. Matrix polymers are chemically diverse, and a single organism may synthesize several polymers with differing properties.

This mixed species river biofilm was stained with multiple lectin probes. The marvelous variety of colors seen in the image reflects diversity in the specific chemistry of sugar residues of the extracellular polysaccharides.

**BIBLIOGRAPHY**


biofilm lesson 3:
Biofilm cohesion depends on a crosslinked network of extracellular polymers that form a hydrogel.

Biofilm cohesion requires that extracellular matrix polymers interact to form a gel network. Crosslinks could be formed by: A) cation bridging between negatively charged polymer strands, B) direct electrostatic interaction between polymers of opposite charge—a polyelectrolyte complex, C) hydrogen bonding interactions between repeated units of the same polymer, or D) binding of an extracellular polysaccharide chain by a cell-surface associated lectin.

Gel theories predict a power law dependence of the gel elastic modulus ($G'$) on polymer concentration ($C$), i.e., $G' = C^n$. This example with a common biopolymer, agarose, conforms to one of the theories in which the exponent $n = 9/4$ (2.25). One thing this means is that modest changes in the local concentration of EPS can lead to large changes in the strength of the biofilm. Polymer concentrations on the order of magnitude of 1% (w/v) are sufficient to form gels with mechanical properties similar to those of biofilms.
Over 600 undergraduate students have participated in CBE research since 1990. Undergraduate students are highly valued team members in the CBE 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 students also 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

More than 220 master’s and doctoral students have earned their degrees in the CBE’s graduate research program since the Center 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, 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. 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