

2006 CBE ANNUAL REPORT

Montana State University
College of Engineering

Center for Biofilm Engineering 2006 Annual Report CBE Faces; Biofilm Surfaces

Director's message	1
Research	1
Education	6
Technology transfer	9
Visitors	11
Outreach	12
Awards	12
Contact information	13

The 2006 CBE Appendix—available in PDF format online, at the CBE's web site: www.erc.montana.edu—is loaded with information about CBE activities during the past year: publications, research projects, conference agendas, presentations, visitors, CBE collaborations, and more.

From Director Phil Stewart: CBE opens a window on the biofilm world

Here is a way of understanding what the Center for Biofilm Engineering is really all about: we bring people together in the biofilm world. As you read through this year's annual report, I ask you to look for the "connectivity" that the CBE facilitates. Examples include Center alumni who have gone on to faculty positions at other institutions yet maintain their ties to the CBE, the co-mingling of students from different backgrounds and disciplines, visitors from around the globe being welcomed into collaborative relationships, and companies networking with each other and tapping into the pool of biofilm expertise resident in our students, staff, and faculty.

Last year I articulated a vision for the Center for Biofilm Engineering with these four elements:

- mining the interdisciplinary boundaries of biofilm research;
- embracing a "synthesis" function that digests, analyzes, integrates, and interprets the diverse and copious information in the field of biofilms so that key trends, concepts, and insights can be accessed;

- building the CBE as an international hub for biofilm research, education, and technology transfer; and
- working with companies to translate the biofilm concept, and the exciting new science emerging from it, into new products and processes in industry and medicine.

Networking is central to realizing all four of these vision elements. Our researchers here in Bozeman must continue to work with each other, and also with collaborators at other institutions, to deliver the interdisciplinary dividend. A true synthesis of biofilm ideas requires us to look beyond the research and projects of our own campus and to take in the excellent work that is happening elsewhere. It makes sense for the CBE to build on its extensive connections around the world to cultivate partnerships and serve as a clearinghouse and hub. We can stimulate the development of new technologies by bringing industrial partners, researchers, regulators, and funding agencies together.

The CBE continues to work to enlarge our view of the biofilm universe. Come have a look.

RESEARCH

CBE researchers brought in \$3.2 million in new research activity during the past fiscal year from a wide variety of funding sources. Notable awards this year include a continuation of a federal earmark through the Army Research Office to examine the public health implications of biofilms in drinking water, an Office of Naval Research project to develop microbial fuel cells to power remote devices, a Department of Energy grant related to fate and transport of uranium and chromium, a National Institutes of Health grant to study dispersal of yeast from biofilms, a National Science Foundation grant to investigate biogeochemical cycling of heavy metals, and three awards or subcontracts from the American Water Works Association Research Foundation to study aspects of microbial activity in drinking water systems. Thirty-five private companies along with four private institutes or foundations sponsored research or testing projects at the CBE in the past year. This slate of activity, and its healthy diversity, shows that research is thriving at the CBE.

CBE researchers published 47 papers in the past year's reporting period. Since 1990, CBE researchers have published over 650 peer-reviewed papers.

2006 CBE ANNUAL REPORT

Research Area	Activities
Biofilm Control/Antimicrobials	Biofilm resistance mechanisms, effective use of antimicrobials, alternative control strategies
Bioelectrochemistry	Microbially influenced corrosion, deposition and electrochemistry of minerals at interfaces
Bioremediation	Degradation, removal or containment of contaminants in soil and groundwater
Bioterrorism	Persistence and detection of pathogens in drinking water distribution systems
Industrial and Drinking Water Treatment	Role of biofilms in water quality, corrosion and use of biological pretreatment to improve water quality
Medical Biofilms	Role of biofilms in disease
Souring	Control of bacterial hydrogen sulfide generation in petroleum production
Standardized Biofilm Methods	Development and dissemination of standardized methods for biofilm testing
Structure-Function	Relationship between biofilm structure, transport processes, biofilm rheology, and biological activity

Nine research areas supported by the CBE are summarized in the table above. This list encompasses those areas in which the CBE has significant, sustained activity.

Microbes generate energy and interest

Imagine if you could drop a sensor in a stream, collect data from it and transfer the data via satellite connection to your laboratory for 10 years without returning to the site. CBE researchers Zbigniew Lewandowski and Haluk Beyenal oversee a multidisciplinary team working to make this possible. Their research is funded by the Office of Naval Research.

Microbial fuel cells—devices that use catalytic reactions of microorganisms to convert chemical energy to electrical energy—play a key role in designing a “drop and forget” sensor. Because microbial fuel cells have an unlimited supply of energy from reduced substances dissolved in water and use bacteria that exist in the water, they do not need to be replaced or recharged as traditional batteries do. They also can be used where data collectors that rely on solar energy are not practical.

Graduate students Allison Rhoads, Avinash Shantaram, Raaja Raajan Angathevar Veluchamy, Enrico Marsili and Joseph Menicucci have worked on the project; ongoing support through the Undergraduate Scholars Program has also allowed

undergraduates Conrad Donovan, Alan Weeden and Jake Wiggs to contribute. Students have become familiar with the tribulations of field research during the project. In one instance, the device was stolen from Roskie Creek when it was left overnight. In another instance, students gathering satellite data in Hyalite Lake at midnight were questioned by police because of their “suspicious” behavior.

Science News interviewed Dr. Lewandowski about his microbial fuel cell research for the article, “Microbial Moxie; Bacteria-based fuel cells provide power,” Feb 4, 2006; Vol 169(5):72–73. Author: Aimee Cunningham.

2006 CBE ANNUAL REPORT

"Montana State University is now in the top tier of research universities in the United States.

A new classification system by the Carnegie Foundation for the Advancement of Teaching recognizes MSU as one of 94 research universities with "very high research activity." Other such institutions are Yale University, Harvard University, Johns Hopkins University, the University of Washington and Oregon State University."

*Evelyn Boswell,
MSU News Services*

New faculty reinforce biochemical engineering

Two new tenure-track faculty hired in the Department of Chemical and Biological Engineering have strong CBE ties and are already contributing to Center research projects. Brent Peyton did his graduate work at MSU with CBE founder Bill Characklis. He has returned to Bozeman after establishing a track record of success in environmental biotechnology, having worked five years at Pacific Northwest Laboratories and eight years at Washington State University. Brent conducts funded collaborations with microbiologist Gill Geesey and environmental engineer Robin Gerlach and is currently managing a project on biofilm-mediated uranium deposition for Saudi Aramco.

With a BS degree in biochemistry, an MS in microbial engineering, and a PhD in chemical engineering, Ross Carlson has just the right credentials to step into the CBE and go places. Ross is an expert in mathematical models of microbial metabolism and their application to predict microbial behavior. He has also been a key player in the development of chitosan coatings that have potent anti-biofilm activity.

Associated faculty & their specialties

NAME	DEPARTMENT	SPECIALTY
Haluk Beyenal	Chemical & Biological Engineering	Biochemical engineering
Anne Camper	Civil Engineering	Biofilms in environmental systems
Ross Carlson	Chemical & Biological Engineering	Metabolic engineering, metabolic networks
Sarah Codd	Mechanical Engineering	Magnetic resonance imaging
Bill Costerton	Microbiology	Biofilms in microbial pathogenicity
Al Cunningham	Civil Engineering	Subsurface biotechnology and bioremediation
David Dickensheets	Electrical & Computer Engineering	MEMs, confocal microscopy

Cover models. . . model covers!

CBE researchers use computer models to complement laboratory experimentation as tools to investigate biofilms. Two modeling studies from the past year resulted in technical articles with accompanying model-generated images on the covers of the journals. The first study was led by visiting researcher Joao Xavier, who simulated how a biofilm might be removed by enzymes or chemicals that attack the extracellular polymeric substances holding the biofilm together. The powerful model Joao used was developed in Delft in collaboration with Mark van Loosdrecht and Christian Picioreanu. The article was highlighted in a commentary that appeared in Nature Reviews Microbiology (Molloy, S., "Biofilms: you do the maths!" Nat. Rev. Microbiol. 4(1):4, 2006).

The second study was conducted by CBE graduate students Jason Chambless and Steve Hunt, working with Phil Stewart. They used the CBE's 3-D cellular automata model to explore four different protective mechanisms by which biofilms might evade killing by antimicrobial agents. Among the features noted in real biofilms that this model captures are: hollow cell clusters, antimicrobial tolerance of biofilm compared to the susceptibility of free-floating cells, and non-uniform, stochastic patterns of survival.

Xavier, J. B., Picioreanu, C., Abdul Rani, S., van Loosdrecht, M. C. M., and P. S. Stewart. (2005) "Biofilm-control strategies based on enzymic disruption of the extracellular polymeric substance matrix—A modelling study. Microbiol. 151(12):3817-3832.

Chambless, J.D., S.M. Hunt and P.S. Stewart. (2006) "A three-dimensional computer model of four hypothetical mechanisms protecting biofilms from antimicrobials." Appl. Environ. Microbiol., 72(3):2005-2013.

2006 CBE ANNUAL REPORT

Jack Dockery	Mathematical Science	Mathematical models of biofilms
Tim Ford	Microbiology	Drinking water, public health microbiology
Christine Foreman	Land Resources & Environmental Sciences	Microbial ecology in cold temperature environments
Michael Franklin	Microbiology	Molecular genetics, gene expression, alginate
Gill Geesey	Microbiology	Molecular and cellular interactions at interfaces
Robin Gerlach	CBE/Civil Engineering	Environmental biotechnology and bioremediation
Marty Hamilton	Statistics	Applied biostatistical thinking
Warren Jones	Civil Engineering	Water distribution systems
Taimur Khan	Center for Biofilm Engineering	Environmental engineering
Issac Klapper	Mathematical Science	Mathematical modeling
Zbigniew Lewandowski	Civil Engineering	Microsensors, chemical gradients, biofilm structure, hydrodynamics
Tom Livinghouse	Chemistry & Biochemistry	Organic synthesis, signaling analogues
Timothy McDermott	Land Resources & Environmental Sciences	Biofilms in extreme environments
Bruce McLeod	Electrical & Computer Engineering	Bioelectric effect
Andreas Nocker-Einsiedler	Center for Biofilm Engineering	Molecular microbiology
Brent Peyton	Chemical & Biological Engineering	Environmental biotechnology and bioremediation
Barry Pyle	Microbiology	Environmental, water and food microbiology
Rocky Ross	Computer Science	Web-based, active learning education
Joseph Seymour	Chemical & Biological Engineering	Magnetic resonance imaging
Otto Stein	Civil Engineering	Engineered waste remediation
Phil Stewart	Chemical & Biological Engineering	Biofilm control strategies
Paul Sturman	Civil Engineering	Biofilms in waste remediation and industrial systems
Peter Suci	Microbiology	Fungal biofilms
Brett Towler	Civil Engineering	Biofilm mechanics
Rick Veeh	Center for Biofilm Engineering	Bacterial identification using oligonucleotide probes

Scanning this year's publications reveals the wide diversity of CBE research interests and activities. Below is a sampling from recent titles:

- wireless sensors
- inhibition by plant extract
- constructed wetlands
- bacterially derived wood adhesive
- reductive transformation
- orthopaedic biofilm infections
- calcium-induced virulence factors
- marine *Pseudoalteromonas* sp.
- epimerase active domain
- quorum sensing inhibitor RIP
- neurosurgical device-related infections
- chronic suppurative otitis media
- bioelectric effect
- ultrasonically controlled release of ciprofloxacin
- dynamic patterns of flow, biofilm growth and activity in porous media

2006 CBE ANNUAL REPORT

- uranium removal
- three-dimensional computer modeling
- antimicrobial action
- rapid display of bacterial diversity
- effects of starvation
- cerebrospinal fluid shunt infection
- transport
- biofilm inhibitors from *Diospyros dendo*
- effect of substrate concentration on population densities
- sustainable power generation by microbial fuel cells
- removal of DNA from dead cells
- biological material in ice cores
- magnetic resonance microscopy
- activity in stratified biofilms
- membrane filtration
- endotoxin level measurement
- adaptive responses
- souring
- hemodialysis
- enzymic disruption of EPS
- seeding dispersal
- in situ biofilm barriers
- nylon corneal suture infection
- oral biofilm

CBE alumni in academic positions

One of the indicators of the prominent role that the CBE plays in the biofilm research community is the number of CBE alumni who go on to faculty positions at other institutions where they continue to pursue research related to biofilms. Here are the Center graduate students and post-docs who are helping to expand the world of biofilms. Many continue to collaborate with colleagues at MSU.

Rune Bakke, Telemark University College, Porsgrunn, Norway

Current research interests: Methane and hydrogen production by anaerobic digestion, including biofilm membrane effects

Ongoing collaboration: Course development with Al Cunningham

Thomas Borch, Colorado State University, Fort Collins, Colorado

Current research interests: Biodegradation of mixed-waste contaminants

Ongoing collaboration: With Robin Gerlach, investigating the fate of explosives in the presence of heavy metals and soil organic matter by a fermenting soil bacterium

Nick Cogan, Florida State University, Tallahassee, Florida

Current research interests: Mathematical biology, biofluids, biofilms, fluid/structure interactions

David G. Davies, State University of New York at Binghamton

Current research interests: Bacterial biofilm development, including an examination of the genes

and gene-products involved in transitional stages in biofilm maturation; development of a novel dispersion inducer for use against developing and existing biofilms in clinical applications

Ongoing collaboration: Two papers published with CBE faculty while at Binghamton; presented research results at the 2005 Summer TAC

Dirk de Beer, Max-Planck-Institute for Marine Microbiology, Bremen, Germany

Current research interests: Eco-physiology of environmental microbial communities, especially marine gradient systems

Ongoing collaboration: Current CBE staff: Peter Suci, hyperspectral imaging of benthic microbial communities. Former CBE staff: Paul Stoodley, 1) microsensor and molecular studies on medical biofilms, 2) hyperspectral imaging of benthic microbial communities, 3) single cell sequencing of very big filamentous nitrate storing sulfur bacteria, 4) transport of biofilms/biomass through porous matrices

Ching-Tsan Huang, National Taiwan University, Taipei, Taiwan

Current research interests: Use of liposomal and novel photosensitizers for photodynamic inactivation against bacterial biofilms with multiple drug resistance

Jeff G. Leid, Northern Arizona University, Flagstaff, Arizona

Current research interests: Mechanisms of bacterial biofilm resistance to the human host and the role of biofilms in biothreat pathogens

Ongoing collaboration: With a number of biofilm researchers who either used to work at the CBE or have been through the Center over the years

Tim Magnuson, Idaho State University, Pocatello, Idaho

Current research interests: Enzymology of microbial mineral transformation; microbial ecophysiology

Ongoing collaboration: With Gill Geesey, includes molecular detection of mineral transforming bacteria

Andy Neal, Savannah River Ecology Laboratory, Aiken, South Carolina

Current research interests: Adhesion of, and electron transfer by, iron reducing bacteria to iron oxide surfaces

Ongoing collaboration: Continuing to work with Tim Magnuson

Satoshi Okabe, Hokkaido University, Sapporo, Japan

Current research interests: Polyphasic analysis of microbial eco-physiology in complex multispecies biofilms

Karin Sauer, Binghamton University, Binghamton, New York

Current research interests: Elucidating the mechanism of biofilm development and how biofilm

2006 CBE ANNUAL REPORT

formation contributes to antimicrobial resistance and virulence

Robert R. Sharp, Manhattan College, Bronx, New York

Current research interests: Fundamental and applied aspects of biofilm processes in water and wastewater treatment and bioremediation

Ongoing collaboration: With Al Cunningham and Robin Gerlach, impacts of biofilm processes on reactive transport in porous media (DOE-ZERT). This ongoing collaboration has resulted in multiple publication and conference presentations. With Anne Camper, studying the impacts of chloramination on corrosion and regrowth in drinking water distribution systems (NYCDEP)—our third large collaborative drinking water project

Mark E. Shirliff, University of Maryland-Baltimore, Maryland

Current research interests: Using animal models and transcriptomic and proteomic techniques to study biofilms in order to identify vaccine candidates and antimicrobial targets as well as to understand host-pathogen interactions in chronic infections

Ongoing collaboration: With Anne Camper's research group, in the understanding of the component bacterial species in polymicrobial biofilms. With Phil Stewart, understanding of phage mediated lytic responses in staphylococcal biofilms

Paul Stoodley, Center for Genomic Sciences Allegheny-Singer Research Institute, Allegheny General Hospital, Pittsburgh, Pennsylvania

Current research interests: Biofilm involvement in infections of host tissue and foreign materials

Ongoing collaboration: With Marty and Cord Hamilton, detachment patterns in staphylococcal species; with Al Cunningham and Rocky Ross, development of a biofilm hypertext book; with Peter Suci, hyperspectral imaging of biofilms (in collaboration with Dirk deBeer at the MPI Bremen)

EDUCATION

Education at the CBE goes far beyond student experiences in a typical university setting. In addition to excellent classroom instruction, our graduate and undergraduate students have the opportunity to interact with each other, staff, and faculty on multidisciplinary projects. This past year, our 32 graduate students (12 MS and 20 PhD candidates) from 8 departments interacted with 38 undergraduate research students from 12

departments (another 6 undergraduates from 3 more departments contributed to the CBE as technical or administrative assistants).

Our 31 participating faculty members represent 10 departments from across campus. As in the past, our students also have the opportunity to interact with companies by working on industrially funded projects and also by participating in our semi-annual Technical Advisory Conferences.

Our graduate students and staff learn valuable mentoring skills by supervising undergraduates and other students who participate in minority programs aimed at increasing diversity. They also gain experience in teaching through their involvement in undergraduate classroom education. And all of our students, staff and faculty are educated by, and enriched through, interactions with visiting students and faculty who become part of our extended CBE family.

2005-2006 Graduates

Doctoral:

Darla Goeres,

Civil Engineering/Environmental Engineering
"Design of model reactor systems for evaluating disinfectants against biofilm bacteria"

Masters:

Suriani Abdul Rani, Chemical Engineering

"Spatial patterns of DNA replication, protein synthesis, and oxygen concentration within bacterial biofilms reveal active and inactive regions"

Rajaa Rajaan Angethevar Veluchamy

Civil Engineering/Environmental Engineering
"Structure and activity of *Pseudomonas aeruginosa* PAO1 biofilms"

Bachelor of Science:

Heidi Cicon, Cell Biology & Neuroscience, Minor in Biochemistry

Chelsea Grace, Chemical Engineering

Amy Martin, Chemical Engineering

Holly Reinemer Berg, Microbiology/Medical Lab Science

Pat Secor, Chemistry/Biochemistry

David Steppler, Chemistry/Biochemistry

Luke Strutz, Chemical Engineering

Mike Sutton, Mechanical Engineering

Ellen Swogger, Chemical Engineering

Ryan Wallace, Civil Engineering/Bioresources Engineering

Jackie Whitaker, Microbiology/Medical Lab Science

2006 CBE ANNUAL REPORT

CBE graduate students, 2005–2006

Discipline	MS/PhD	Male	Female	Subtotal	TOTAL
Chemical & Biological Engineering					11
	MS	1	2	3	
	PhD	6	2	8	
Chemistry					1
	PhD	1		1	
Civil & Environmental Engineering					9
	MS	5	1	6	
	PhD	3		3	
Computer Science					1
	PhD	1		1	
Electrical Engineering					1
	PhD	1		1	
Land Resources & Environmental Sciences					2
	MS		1	1	
	PhD	1		1	
Mathematics					2
	PhD	1	1	2	
Microbiology					5
	MS		1	1	
	PhD	1	3	4	
TOTALS		21	11		32

MSU senior David Stepler discussed his innovative CBE research on treatments for groundwater contaminants with Catherine Hunt (President Elect for the American Chemical Society) and Pat Dennis (NSF program director in microbial genome sequencing) at a poster session co-hosted by the American Chemical Society held on Capitol Hill in Washington, D.C., April 2006. David's project was one of only 75 chosen nationwide for the honor by the Council on Undergraduate Research. The council represents more than 900 colleges and universities. David and CBE faculty member and mentor Robin Gerlach also visited Montana Senators' offices during their trip.

2006 CBE ANNUAL REPORT

CBE undergraduate student participation, 2005–2006

Discipline	Male	Female	Total
Biomedical Science		3	3
Business	1	1	2
Biotechnology	1		1
Cell Biology & Neuroscience		2	2
Chemical & Biological Engineering	9	6	15
Chemistry	2	1	3
Civil Engineering	3	1	4
Computer Engineering	1		1
Ecology		1	1
Mechanical Engineering	1		1
Microbiology		3	3
Nursing		2	2
TOTAL	18	20	38

Since 1990 over 400 undergraduate students have participated in research projects at the Center for Biofilm Engineering.

Effective mentoring forms lasting connections in the CBE's education program

Montana State University and the College of Engineering are committed to increasing the participation of American Indian students and easing the transition for them from their high school communities to campus life. The CBE has been a long-time supporter of the Montana Apprenticeship Program (MAP) administered out of the American Indian Research Opportunities office. One of these MAP students was Cinnamon Spear from Lame Deer. As a result of her work at the CBE, she is a co-author on a peer-reviewed publication (see reference below). Though Cinnamon has chosen to pursue her undergraduate degree at Dartmouth College, she will be returning to work with us in the summer of 2006 as a visiting undergraduate research student. We are proud of her accomplishments and consider her to be part of our CBE family.

From Mark Burr, Cinnamon's MAP mentor during the summer of 2004:
 "Cinnamon adapted quickly to our lab. A camaraderie with all the lab members was quickly established. After the program was over, we invited Cinnamon to work an extra few weeks. Then she returned to her senior year at Lame Deer High School. In May 2005, I received an invitation to her high school graduation and immediately dragged lab members Ben Klayman and Stewart Clark into the plan: we would not tell Cinnamon we were coming, we would just show up. Before the ceremonies began, Cinnamon passed Stewart and Ben in the hallway and did a double-take. She was completely surprised that we were there. The graduation ceremony was touching. Cinnamon won several scholarships and, as valedictorian, gave a speech. I felt so proud of her that night and was honored to have been part of that special event."

Burr M.D., Clark S.J., Spear C.R., Camper A.K.
 "Denaturing Gradient Gel Electrophoresis Can Rapidly Display the Bacterial Diversity Contained in 16S rDNA Clone Libraries," *Microb. Ecol.* 2006 Apr 28; [Epub ahead of print].

UNIV 125 class: Where art can meet science, & undergrad can meet graduate

The CBE's interdisciplinary interactions sometimes bring unexpected benefits. Graduate students Stewart Clark, Willy Davison, and Ben Klayman honed their professional skills by team teaching "UNIV 125: Microbes in the Environment" in the 2005 fall semester. The course, developed for non-science major undergraduate students, focuses on microbiology and biofilm engineering as they relate to everyday life. Each of the graduate students organized material in one of three modules—environmental, medical and industrial science—and addressed topics such as swimming pools, hot tubs, Yellowstone National Park, bioterrorism, forensics, and food-borne diseases. Willy Davison, PhD candidate in chemical engineering, said, "The skills I gained by teaching difficult material to a group of mixed-major students are invaluable, but the greatest reward for me was seeing the students acquire knowledge about the world of science that they can take with them for the rest of their lives."

The CBE's emphasis on recruiting high caliber undergraduate students prompted Willy to facilitate a connection between one of his students and Peg Dirckx, who directs the CBE's visual communications. "One of the highest grades on the final exam was earned by Skye Saylor, a studio arts major, who demonstrated her talents in the classroom on a daily basis and showed a strong interest in microbiology. Skye's interests in both art and science, and her attention to detail, made her a perfect candidate for Peg's student assistant." From the undergraduate student perspective, Skye said, "The class was taught entirely by graduate students who were approachable, with outgoing and fun personalities. Instead of feeling like I had to attend the class, I felt

2006 CBE ANNUAL REPORT

excited to be there." She also says that she is enjoying her new position, the variety of personalities and the CBE's atmosphere of cooperation.

2006 Characklis Award goes to William Davison

Willy Davison's uncommon leadership and dedication to the CBE was recognized in February 2006, as he received the annual W.G. Characklis Award, named in honor of the CBE's founding director. He has been a co-organizer of the CBE seminar series and was one of three graduate student instructors for the CBE-based course "Microbes in the Environment." Willy's heart and soul investment in this course was recognized and appreciated by students. He has also regularly volunteered to assist with CBE workshops. Willy has shown a generous willingness to train others in laboratory techniques and equipment use, and his constructive relationships with other students, staff, and faculty exemplify the level of teamwork to which the CBE aspires.

TECHNOLOGY TRANSFER

In keeping with the mission of the CBE to provide synthesis of the myriad biofilm-related research advances published across the globe, and to act as an international hub for biofilm research, education, and technology transfer, our Technical Advisory Conferences (TAC) are intended to inform, enlighten, and inspire attendees from sponsor companies and collaborating research institutions. Over the past year, we've hosted internationally recognized invited speakers, including Dr. Pete Greenberg (University of Washington), Dr. Pradeep Singh (University of Iowa), and Dr. Rod Donlan (Centers for Disease Control). These semi-annual conferences provide the foundation of communication with the CBE's group of 23 Industrial Associate member companies. They feature sessions on medical biofilms, industrial biofilms, and environmental biofilms, as well as a mix of fundamental and applied research. Our conferences are regularly attended by approximately 50 representatives from sponsor companies, invited guests and members of the regulatory community (EPA and FDA).

TAC member companies provide critical feedback to the CBE on project relevance and future research directions. New members of the previous year include Unilever, NovaCal Pharmaceuticals and American Air Liquide, Inc.

Medical Biofilm Laboratory bridges the gap between in vitro and in vivo study

The Medical Biofilm Laboratory (MBL) works with Industrial Associates and other companies to develop customized in vitro laboratory model systems for evaluating a variety of products for killing and removing biofilm. In vitro models are a more cost effective method for evaluating products than animal

models or clinical trials. Furthermore, the results of testing in in vitro models can be useful in designing subsequent animal model tests and clinical trials. Models developed in the MBL include those for oral biofilms, urinary catheter biofilms, and central venous catheter biofilms. The MBL also performs analysis of samples obtained from animal models and clinical trials. These analyses include evaluating samples for the presence of biofilms using microscopic and molecular techniques. Projects in the MBL are sponsored by a wide variety of clients ranging from large manufacturers of personal care products and medical devices to small businesses developing novel products for dentistry and medicine. In the past year the MBL has conducted over 15 projects for industrial clients.

CBE team develops new standardized biofilm methods

A new standardized method—for the Drip Flow Biofilm Reactor—has been added to the collection developed by the CBE's Standardized Biofilm Methods (SBM) research team. The distinguishing features of the drip flow reactor (DFR) are mild fluid shear, a biofilm grown close to the liquid-air interface and a short media residence time. Rigorous testing of the standard operating procedure for growing a *Pseudomonas aeruginosa* biofilm in the DFR demonstrated that the reactor and method were both repeatable and rugged. The protocol has been submitted to ASTM Subcommittee E35.15 for acceptance as a standard method.

The DFR method is the third CBE method proposed for standardization. The first method (ASTM #E-2196-02) was approved in 2002 and uses the rotating disk reactor. The second method, which is based on the CDC (Centers for Disease Control) biofilm reactor, is currently in the approval process. The SBM team is also in the process of developing a standard method for growing a *Staphylococcus aureus* biofilm in the DFR. A standard method for assessing the efficacy of dental unit water line antimicrobials is also nearing completion.

In September 2005, the Antimicrobials Division of the US-EPA accepted the final report titled "A LABORATORY HOT TUB MODEL: Engineering Design, Standard Operating Procedure and Performance Characteristics." The SBM team and the EPA are now deciding where to submit the method for standardization. In addition, the SBM team is working on developing two check methods for the EPA. These methods will determine biofilm removal and disaggregation efficiency.

2006 CBE ANNUAL REPORT

The CBE's Industrial Associates

American Air Liquide, Inc.
Aramco Services Company
Church & Dwight Co., Inc.
Colgate-Palmolive
C.R. Bard, Inc.
Cumbre, Inc.
Dow Chemical Company
DuPont
Eastman Kodak Company
Ecolab, Inc.
Edstrom Industries, Inc.
*Electric Power Research
Institute (EPRI)*
Genencor International, Inc.
GlaxoSmithKline
Idaho National Laboratory (INL)
Masco
NASA
NovaCal Pharmaceuticals
Novozymes North America, Inc.
Tyco Healthcare
Unilever
U.S. Bureau of Reclamation
W.L. Gore & Associates

MSU offers CBE's slime-based inventions

Researchers from the CBE have five new inventions ranging from trapping atmospheric carbon to stopping slime from growing on medical implants. Montana State University is now licensing these inventions:

- a special coating that may inhibit biofilms from growing on the surfaces of medical implants;*
- a compound that disaggregates disease-causing biofilms, allowing traditional antibiotics a shot at killing the infection;*
- chemicals that can be released at a controlled rate to limit the growth of slimy bacteria in industrial water systems, spas and swimming pools;*
- combining bacteria and nutrients to create a gas-tight seal in geologic formations. The method could be used to trap gases, such as carbon dioxide, and sequester them underground;*
- a compound that improves the ability of antibiotics to kill biofilm infections.*

CBE continues to build rapport with regulatory agencies

The CBE continued its outreach to the EPA and FDA over the past year, with CBE on-site visits at regulatory offices and regulator attendance at Technical Advisory Conferences. In September 2005, Marty Hamilton and Darla Goeres visited the Antimicrobials Division of the EPA and Paul Sturman presented to a group of 30 FDA regulators. Marty and Darla continue to provide support to EPA in assessing the statistical validity of antimicrobial claims.

25 years of CBE friends say 'so long and farewell'. . . Rick Rosser retires from Saudi Aramco

For over 18 years, Rick Rosser made the annual journey from Dhahran, Saudi Arabia, to Bozeman, Montana, to attend our Technical Advisory Conferences. Announcing his retirement in December 2005, Rick was the longest serving designated representative from our longest standing Industrial Associate company. Thanks, Rick and Saudi Aramco!

Workshops: popular and productive

During the past year the CBE hosted 14 workshops. For the convenience of TAC participants, three of the workshops were scheduled to coincide with our Technical Advisory Conferences. A special highlight was the Biofilm Methods Workshop, held in February 2006, to which Diane Gray from Invitrogen Molecular Probes™ brought 11 fluorescent stains for CBEers and TAC participants to use for biofilm imaging. The results of this imaging workshop were then shared during a session of the TAC—immediate research gratification!

Two workshops were hosted "on the road" by CBE personnel, and two workshops were conducted in response to industry requests. The Biofilm Structure-Function research group continued its program of three information-packed biofilm summer workshops and had full attendance for each. The Structure-Function workshop topics included: microsensors, biofilm structure quantification and image analysis, and bioelectrochemistry: microbially influenced corrosion and microbial fuel cells.

2006 CBE ANNUAL REPORT

VISITORS

The CBE continues to be a magnet for visiting scholars. This past year we had 19 visitors whose stays varied from a few weeks to a full year. They learned from us, we learned from them, and often long-term collaborations resulted. As an example, during 2005, 11 published manuscripts and 5 grant proposals with current and former visitors were produced. Two recent examples of productive visiting collaborators are highlighted below.

Alessandra Agostinho, University of Sao Paolo, Brazil

Sabrina Behnke, University of Duisburg-Essen, Germany

Vildan Caner, University of Pamukkale, Turkey

Audrey Corbin, National Institute of Applied Sciences (INSA), France

John Cotter, University College, Dublin, Ireland

Martin Eschenhagen, TU Dresden, Institute of Microbiology, Germany

Christopher Groth, Manhattan College, NY

Ray Hozalski, University of Minnesota

Laura Jennings, Cornell University, Ithaca, NY

Jae-eun Kim, Seoul National University, Korea

Elisa Korenblum, Federal University of Rio de Janeiro, Brazil

Jessica Levinson, Manhattan College, NY

Cindy Morris, Recherche Agronomique (INRA), France

Jessica Richard, University of Duisburg-Essen, Germany

Deepak Sharma, University of Ottawa, Canada

Katherine Sossa-Fernández, Universidad de Concepción, Chile

Shoji Takenaka, Niigata University, Japan

Kyung-Min Yeon, Seoul National University, Korea

Jeyong Yoon, Seoul National University, Korea

Visiting researchers play an essential role in the CBE experience

Dr. Christoph Fux, M.D.
Division of Infectious Diseases,
University Hospital Bern, Switzerland

"Clinicians caring for patients with bacterial infections generally are confronted with rather faceless enemies. I knew little about what really was going on in the microcosm, where bacteria interact with each other and the host. At the Center, I got the chance to dive down to the micrometer scale and actually observe how bacteria behave: What an experience to realize in my own experiments, how much antibiotic tolerance depends on growth phase, bacterial density ... and the antibiotic class used! I am deeply thankful to all the people at the CBE that shared the excitement of bringing clinical experiences and basic research together—you have taken me down to where infections happen and have given bacteria a face."

Papers co-published with Christoph Fux

Dr. Fux has published 8 papers co-authored with CBE personnel, including the papers below, published in this reporting period.

Balaban, N., P. Stoodley, C.A. Fux, S. Wilson, J.W. Costerton and G. Dell'Acqua, "Prevention of Staphylococcal Biofilm-associated Infections by the Quorum Sensing Inhibitor RIP," *Clin. Orthop. Relat. R.*, 437:48-54 (2005).

Braxton, E.E., G.D. Ehrlich, L. Hall-Stoodley, P. Stoodley, R. Veeh, C.A. Fux, F.Z. Hu, M. Quigley and J.C. Post, "Role of Biofilms in Neurosurgical Device-Related Infections," *Neurosurg. Rev.*, 28:249-255 (2005).

Fux, C.A., M. Quigley, A.M. Worel, C. Post, S. Zimmerli, G. Ehrlich, and R.H. Veeh, "Biofilm-Related Infections of Cerebrospinal Fluid Shunts," *Clin. Microbiol. Infect.*, 12(4):331-337 (2006).

Ray Hozalski, Associate Professor, Civil Engineering, University of Minnesota

My experience at the CBE was phenomenal from both the personal and professional perspectives. Professionally, I found the atmosphere at the lab to

2006 CBE ANNUAL REPORT

be open and intellectually stimulating. The weekly seminars and other informal discussions resulted in a lively exchange of ideas. I was also able to return to the laboratory after a 7-year hiatus, and I generated sufficient data concerning a new water distribution system corrosion control chemical to write an unsolicited proposal with Anne Camper that was funded by the American Water Works Association Research Foundation. Anne and I also teamed up on a solicited proposal, funded by AwwaRF, to investigate the biodegradation of haloacetic acids in water distribution systems. In addition, Phil Stewart and I teamed up to write a proposal to the National Science Foundation concerning a fundamental investigation of biofilm cohesive strength and approaches for manipulation of cohesive strength. The extent of collaborations was exciting and very rewarding, and I expect collaborative activities with CBE staff and faculty to continue for many years to come.

Grants co-authored with Ray Hozalski

Anne Camper/AwwaRF; The Investigation of the Mode of Action of Stannous Chloride as an Inhibitor of Lead Corrosion (\$192,351)

Anne Camper/AwwaRF; Biodegradation of HAA's in Distribution Systems (\$400,000)

Phil Stewart/NSF; Investigation of the Mechanisms of Biofilm Cohesive Strength (pending)

OUTREACH

Tours de force

We love it when people come to visit! And nearly everybody at the CBE—faculty, staff and students—plays a role in welcoming visitors to the Center and sharing with them what we do and how we work. Below are just a few examples of this year's tours and "tour directors."

Leadership Bozeman, a program of the Bozeman area Chamber of Commerce, brought Gallatin County business people and entrepreneurs to visit the CBE October 26, 2005 (Tour leaders: Phil Stewart, Tricia Cook, John Neuman).

CBE faculty, staff and students hosted a workshop for a group of grade-school girls as part of the "Expanding Your Horizons" program on Saturday, April 15, 2006 (Leaders: Anne Camper, Diane Walker,

Linda Loetterle, Sutapa Barua, Jackie Whitaker and Raffaella Pulcini).

Over 30 members of the Northwest Academic Forum (NWAFF)—provosts, academic vice presidents, CEOs and CAOs from graduate-level university system offices in the northwestern U.S.—visited April 28th, 2006. (Tour leaders: Phil Stewart, Holly Reinemer Berg, Ellen Swogger, Suriani Abdul-Rani, Stewart Clark, Willy Davison, Laura Jennings and Ben Klayman).

BiofilmsOnline presents new "Perspectives"

BiofilmsOnline subscribership has continued to grow—up 20% this past year. This online resource for the latest biofilm information has added a new feature: "Biofilm Perspectives." These brief articles, published six times per year, are written by prominent scientists and industry practitioners from around the world. They identify current trends, concepts and hypotheses in the biofilm field. Topics will include biofilm research, applications to real-world problems and regulatory issues. See the web site and sign up! www.biofilmsonline.com

CBE web attractions

CBE web traffic has increased to over 126,000 visits this year. Our Resource Library continues to provide publications, movies and images that are requested by academics, industry representatives and clinicians worldwide. Image use requests came in from 22 countries and more than 20 U.S. states. Our "Bioglyphs" and "Biofilm Basics" modules experienced large increases in visits this year, indicating that more people are seeking to learn the 101's of biofilm and the intriguing phenomenon of bioluminescent microorganisms.

AWARDS

Tim Ford receives Gen-Probe Joseph Award

Timothy E. Ford, Professor and Head of the Department of Microbiology at Montana State University received the Gen-Probe Joseph Award from the American Society for Microbiology in May, 2006. This award honors Ford as a distinguished microbiologist for his exemplary leadership and service in the field of public health. Ford's specialty is environmental health and water. Recent biofilm-related work has focused on survival of opportunistic pathogens in drinking water and routes of exposure.

2006 CBE ANNUAL REPORT

His work on drinking water has resulted in elevated awareness of health risks from water in countries around the world.

2006 College of Engineering Awards Recipients

Three CBE-affiliated faculty and staff were recognized for research excellence at the May 2006 College of Engineering awards luncheon. Outstanding Research Award recipients were: Betsey Pitts, Center for Biofilm Engineering; Joe Seymour, Chemical and Biological Engineering; and Sarah Codd, Mechanical and Industrial Engineering. Dr. Codd was appointed to the College of Engineering's Department of Mechanical and Industrial Engineering as assistant professor in the fall of 2005; she is also co-director of the Magnetic Resonance Microscopy (MRM) laboratory at MSU.

2005 CBE Outstanding Researcher Award

Masters candidate in Mechanical Engineering Patrick Norris was presented with the CBE's Outstanding Researcher Award at the summer 2005 Technical Advisory Conference. Patrick was recognized for the contributions he made to the CBE in research and leadership, especially as they pertain to advances made in achieving Center goals. Throughout his work, he exemplified the CBE's interdisciplinary ideal in research, showing high standards in work and all in the spirit of cooperation, helpfulness and creativity.

VOLUNTEERS

Stereo-microscope project volunteers (see PDF version of the report for this imaging project.)

Thanks to the volunteers who led the stereo-microscope project: Alessandra Agostinho, Diane Walker, Suriani Abdul-Rani, Ben Klayman, Kelli Buckingham-Meyer, Willy Davison and, especially, to Betsey Pitts, for coordinating the team's efforts. Thanks also to contributors: Garth James, Laura Jennings, Adrienne Phillips, Kathy Sossa, Phil Stewart, and Diane Williams.

PARTING SHOTS (captions) (see PDF version of the report for these photos)

Elizabeth Sandvik and Alex Hilyard greeted incoming students last fall and introduced them to the Center for Biofilm Engineering at MSU's "Catapalooza" event.

Margie Hansen gave Susan Cooper some assistance during a special microscopy workshop for the CBE's administrative staff, taught by Betsey Pitts.

The weekly seminar series gives CBE students, staff and faculty the opportunity to hear about new research and to interact. Pictured are Paul Sturman and Taimur Khan.

Why was everyone clapping? It was the horse, of course (actually Betsey Pitts and Willy Davison). During a celebration of the appointment of Phil Stewart as CBE director, the party got a surprise visit from his new steed: "CBE Biscuit." Once he got them sorted out, Paul Sturman invited Phil to 'take the reins!'

**The Center for Biofilm Engineering
366 EPS Building
P.O. Box 173980
Montana State University
Bozeman, Montana 59717-3980
USA**

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

www.erc.montana.edu

**The Center for Biofilm Engineering is a
National Science Foundation Engineering Research Center
founded in 1990. It is part of the
Montana State University College of Engineering.**