

**Montana State University  
Center for Biofilm Engineering  
2005 Annual Report: “Postcards from the leading edge”**

**TABLE OF CONTENTS**

Director’s Message .....1  
Research.....2  
Education .....5  
Technology Transfer.....11  
Outreach.....13  
People.....14

**Image: Scanning Electron Micrograph of a chronic wound with an activated white blood cell (yellow) in the presence of bacterial cells below and behind it (green). Taken by Chemical & Biological Engineering undergraduate Ellen Swogger, colored by Peg Dirckx.**

**Image: Confocal Scanning Laser Microscope image of structurally heterogeneous 48-hour biofilm grown on glass under high fluid shear conditions. Imaged by CBE research staff member Kelli Buckingham-Meyer.**

**DIRECTOR’S MESSAGE**

*by Philip S. Stewart*

Even a quick look through this report will reveal that it has been a lively year, once again, at the Center for Biofilm Engineering. There have been grants awarded, students graduated, papers published, international visitors engaged, and new companies joined. Of the many balls in the air, the directorship is one that is still in flight. Here is an update.

Bill Costerton, director of the Center for Biofilm Engineering for the past 11 years, moved to the University of Southern California last September to start a new center focused on dental and medical biofilms. Early signs suggest that Bill is throwing himself into this opportunity with his characteristic optimism and relish. Though we will miss Bill’s exuberance and creativity here in Montana, we continue to interact with him and have access to his prodigious expertise. Bill still manages several projects running here at the CBE and will be attending our biannual TAC meetings, an arrangement that is anticipated to continue for the next few years. As this report attests, Bill has left the CBE in a very fine state of health.

As I compose this message, the University is working to make me an offer to become the permanent director of the CBE. Having served the CBE as Interim Director for the last nine months, I have had a good chance to see what I am getting into. Here is what I see: an exceptionally competent and dedicated staff, students pursuing research projects in an atmosphere that encourages interdisciplinary interaction and camaraderie, energetic visitors expanding the intellectual life of the Center, and supportive companies eager to learn about biofilms and develop commercial opportunities. It is a privilege to be considered to lead this Center.

Enjoy the postcards that illustrate this year’s annual report. Postcards are about staying in touch. One of my priorities will be to facilitate connections. I would like to see the CBE stay in touch with its alumni, with our constituents and collaborators around campus, with our many loyal friends in industry, and, I hope, with more of the hard-working researchers around the globe who are advancing the biofilm field.

## CBE 2005 ANNUAL REPORT : TEXT ONLY

### RESEARCH

CBE researchers brought in 3.8 million dollars for new research activity during the past fiscal year from a wide variety of funding sources. Notable awards this year include a federal earmark through the Army Research Office to examine the public health implications of biofilms in drinking water, a United States Department of Agriculture project to investigate microbial activities in wetlands, Department of Defense grants related to bioremediation, an NIH subcontract from University of Washington collaborators studying infection of transcutaneous devices, and an NSF grant probing biotic particles in polar ice. Thirty private companies, along with three private institutes, 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 MSU's Center for Biofilm Engineering.

One of the indicators of the CBE's leadership role in the biofilm research community is involvement of CBE staff in the organization of major biofilm meetings. In 2004, the main biofilm-related scientific meeting of the year was Biofilms 2004, held in Las Vegas under the auspices of the International Water Quality Association. This meeting, attended by delegates from 27 countries, was organized by Zbigniew Lewandowski, with extensive assistance and participation on the part of CBE faculty, staff, and students.

**TABLE: Ten 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.**

| Research Area                           | Activities   |
|---|--|
| Biofilm Control/Antimicrobials          | Biofilm resistance mechanisms, effective use of antimicrobials, alternative control strategies           |
| Biomineralization                       | 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                            |
| Cell-Cell Communication                 | Role of signal molecules in biofilm behavior, and disruption of biofilm formation by signal analogues    |
| 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   |

**Image: Microscopy by Allison Enos, undergraduate in Biomedical Sciences.**

*CBE researchers published 48 papers in peer-reviewed journals in the past year. See the 2005 Appendix for details.*

More information about the CBE's research program can be found on our web site at:  
[www.erc.montana.edu/CBEssentials-SW/research/](http://www.erc.montana.edu/CBEssentials-SW/research/)

## **Research highlights**

### ***Medical Biofilm Laboratory: Responsive and productive***

The Medical Biofilm Laboratory (MBL) has earned a reputation for being a university lab that responds quickly to real world needs in the area of health care as it relates to biofilms. Dr. Garth James (Ph.D., Microbiology), Randy Hiebert (MS, Chemical Engineering) and Dr. Elinor Pulcini (PhD, Microbiology) have been the innovative leaders and managers of this respected, flexible, and adaptable lab group. The MBL team includes three full-time technicians, a part-time statistician, and four undergraduate research assistants.

Activity in the MBL has expanded substantially during the last year. Fifteen companies, including CBE Industrial Associates, funded MBL projects in this reporting period, along with four subcontracts on Small Business Innovation Research Program (SBIR) grants. Projects have included developing a nine-species model of dental plaque, examining the role of biofilms in chronic wound infections, and inhibiting biofilm formation by natural plant extracts. The MBL is another example of integration at the CBE, bringing together applied biomedical science, industrial interaction, and student educational opportunities.

### ***Mass spectrometry facility***

Robin Gerlach was awarded an equipment grant totaling \$ 313,000 through the Department of Defense Fiscal Year 2005 Defense University Research Instrumentation Program (DURIP). The grant, for an Environmental and Biofilm Mass Spectrometry Facility, will fund the acquisition of a state-of-the-art high performance liquid chromatograph equipped with an ion trap mass spectrometer, and a gas chromatograph with an electron capture detector and flame ionization detector. Agilent Technologies has agreed to provide the CBE with a quadrupole mass spectrometer for the gas chromatograph for a one-year period, during which time the CBE will attempt to obtain additional funds (approximately \$ 40,000) to purchase the quadrupole mass spectrometer. Both instruments will be equipped with autosamplers capable of sampling 2 mL chromatography sample vials as well as 96-well plates (HPLC) or headspace vials (GC). Mass spectrometers are very well suited for unknown compound identification in environmental and biofilm research and will enhance the CBE's research capabilities significantly.

***CBE researchers contributed two papers to the prestigious journal Physical Review Letters in this reporting period:***

### ***The Tao of biofilm***

*“Yield and you need not break:  
Bent you can straighten,  
Emptied you can hold,  
Torn you can mend. . .”*

“Commonality of Elastic Relaxation Times in Biofilms,” by microbiologist Paul Stoodley, mathematician Isaac Klapper, and students Todd Shaw, Matt Winston and Cory Rupp, examines the mechanical properties of biofilm. Biofilms stretch and ooze in response to the fluid flow around them. Such viscoelastic behavior can be assigned a time scale, termed the elastic relaxation time. Measurements of this relaxation time suggest that it is approximately the same for very different types of biofilms—about 18 minutes. The authors note that this amount of time is similar to that required for microorganisms to mount a phenotypic response and suggest that the capacity of biofilms to deform and absorb transient mechanical stresses confers an evolutionary advantage. This result evokes the Taoist life strategy of “yield and overcome.”

**Image: Representative examples of creep test data. All show characteristic viscoelastic fluid response, with similar elastic relaxation times. From “Commonality of Elastic Relaxation Times in Biofilms,” Shaw, T., M. Winston, C.J. Rupp, I. Klapper and P. Stoodley, Phys. Rev. Lett., 93(9):096102 (2004).**

### ***The Tau of biofilm***

The second paper, a collaboration between chemical engineer Joe Seymour, physicist Sarah Codd, graduate student Justin Gage, and environmental engineer Robin Gerlach, investigates the effect of biofilms on flow in porous media. Water running through a groundwater aquifer or sand filter may carry enough nutrients to support the formation of a biofilm. When this happens, the biofilm can plug pores and alter the flow patterns and mixing behavior in the system. How can flow inside an opaque medium be imaged? This team has provided a beautiful answer to this question by using magnetic resonance imaging (MRI) to visualize microscale flow patterns. Their work shows that not only does fluid flow affect the shape of the biofilm, the biofilm can also profoundly affect the fluid flow around it. In mathematical treatments of fluid flow, the shear stress is often denoted by the Greek letter tau ( $\tau$ ).

**Image: Magnetic resonance microscopy map of fluid velocity in a cross section of a column packed with polystyrene beads. Flow paths and patterns change as the biofilm grows. NMR imaging by Justin Gage, Ph.D. candidate in Chemical & Biological Engineering. From "Anomalous Fluid Transport in Porous Media Induced by Biofilm Growth," Seymour, J.D., J.P. Gage, S.L. Codd and R. Gerlach, Phys. Rev. Lett., 93:198103 (2004).**

### ***Biofilms, bioterrorism, and drinking water***

Among many terrorist threats, one of the most troublesome involves the addition of chemical or biological agents to water distribution systems. While attention and effort has been devoted to vulnerability assessment of military (and municipal) drinking water systems, much work remains to be done on the transport, fate, and detection of these agents in water systems. With Army Research Office funding, the CBE has assembled a multidisciplinary program including students and staff from several departments on the MSU campus and partners at Texas A&M University, the University of Duisberg-Essen in Germany, and the Pacific Northwest National Laboratory. The team is becoming nationally recognized for research in this area, and is actively pursuing other collaborations with the US EPA, Homeland Security, Centers for Disease Control (CDC), and military branches outside of the Army Research Office.

Within the CBE, three groups have been working toward our goal of understanding the fate and transport of bacterial pathogens in drinking water systems. The experimental team, under the guidance of Anne Camper, is investigating how microbial pathogens interact with biofilms in drinking water environments. The goal of the modeling component of the project, led by Al Cunningham, is to develop the capacity to simulate bacterial (pathogen) transport through water systems, as well as the short- and long-term interactions between suspended bacteria and biofilms. The third group, lead by Warren Jones, consists of a senior design team directed to design a working prototype of a biofilm-based pathogen "trap" to be installed in drinking water systems for the detection of contamination events.

**Image: Confocal Scanning Laser Microscope image of *Pseudomonas aeruginosa* biofilm (green) and *Escherichia coli* (red), imaged by Ben Klayman, doctoral candidate in Civil & Environmental Engineering.**

### ***CBE cover-age***

Two CBE student-produced images were used as cover art in periodical publications. Undergraduate Amber Harrer, with assistance from CBE Microscopist Betsey Pitts and Principal Investigator Phil Stewart, obtained a confocal scanning laser microscope image of a *Pseudomonas aeruginosa* biofilm that was used on the August 2004 cover of *Biophotonics International*. The work of doctoral candidate Jason Chambless was chosen for the January 2005 cover of the SGM journal *Microbiology*. The image is a frame from an animated sequence of his computer model simulation of biofilm growth; it accompanied a paper published in the journal co-authored by CBE undergraduate Mark Roberts (Roberts, M.E. and P.S. Stewart, "Modeling Protection from Antimicrobial Agents in Biofilms Through the Formation of Persister Cells," *Microbiol.-UK*, 151:75-80, (2005).

### EDUCATION

Education is at the heart of the CBE's mission. MSU students, both graduate and undergraduate, are fully integrated into the research and technology transfer programs of the CBE. In the past academic year, 32 graduate students from eight different home departments were actively pursuing advanced degrees (14 M.S. and 18 Ph.D. candidates) based on CBE research projects (table, page 10); 36 undergraduate students from 12 departments participated in our research projects (table, page 12). Student participation is a year-round activity at the CBE. Many undergraduates find summer employment at the CBE, and continue their research projects under the guidance of graduate students as well as CBE staff and faculty. The summer of 2004 marked the Center's 15th year as a provider of the NSF-funded Research Experience for Undergraduates program, bringing eight students to the Center from colleges and universities around the U.S. for an intensive 10-week immersion into a biofilm research project. The connection of genuine research with real-world industrial context is not only a great motivator for CBE students; it also gives them a head-start on their career choices and development. Students are also motivated by the Center's outstanding faculty. This year's 29 participating faculty members represent 10 departments and a wide variety of specialties in biofilm studies (table, page 22). We're proud of the opportunities our program offers and of our students' accomplishments, as the following pages will show.

More information about the CBE's education program can be found on our web site at:  
[www.erc.montana.edu/CBEssentials-SW/education/](http://www.erc.montana.edu/CBEssentials-SW/education/)

*"After ten months in the 'real world,' I've noticed many practical benefits to my undergraduate experience. Simply spending time in the lab and learning basic lab etiquette and practices has been a real advantage for me. The hands-on experience really helped me to grasp difficult concepts quickly."*

Amber Harrer Broadbent,  
B.S. graduate, 2004,  
Chemical and Biological Engineering

### Education highlights

#### ***Biofilms with class: "Fundamentals of Biofilm Engineering"***

Since 1991 CBE faculty have taught courses in biofilm engineering at MSU. The current course is called "Fundamentals of Biofilm Engineering" and is listed in the university catalog as Environmental Engineering 566 (ENVE 566). All Center graduate students, whatever their discipline, are encouraged to take this class.

The approach taken in ENVE 566 is to develop quantitative descriptions of the important processes occurring in biofilms and to use these to analyze biofilm phenomena. These processes include, for example, microbial growth, substrate utilization, solute diffusion, biofilm accumulation and detachment. This class exercises the less-than-intuitive skill of translating between the realms of biology and mathematics. CBE faculty guest-lecture during the semester, providing enriching perspectives on application areas such as bioremediation, drinking water, and medicine. One of the features of ENVE 566 appreciated by students in recent years is reviewing landmark papers in the biofilm field. Copies of 16 papers from the literature that have been highly cited by others are distributed to each student. Throughout the semester, students take turns reviewing these papers for the class by making a brief PowerPoint presentation. The formal presentation is followed by an informal discussion, in which the group works to identify the major contribution of the paper, to understand its impact, and to discern any weaknesses in the work.

Students learn to run a computer model of biofilm dynamics and use the model to explore an aspect of biofilm in individual projects. Often students are able to identify an aspect of their own thesis research that is amenable to a modeling study.

## CBE 2005 ANNUAL REPORT : TEXT ONLY

*It's always nice to hear from former staff and students that their experiences at the CBE have been beneficial and valued:*

### **Postcard from a friend. . .**

*“My experience at the CBE was of great help in the development of my career. The multidisciplinary program allowed me to broaden my vision of biofilm research and education, as well as to experience interaction with industries. I would like particularly to acknowledge the guidance of my postdoctoral mentors, Drs. Philip S. Stewart and Gordon A. McFeters. They demonstrated what a scientist and a group leader should be. Of course, I will never forget the outstanding administration team. I did not realize how fortunate I was at the CBE until I returned to Taiwan. The wonderful memory of the CBE and Montana will accompany my family for many years to come.”*

Ching-Tsan Huang, Ph.D.  
Associate Professor, Dept. of Biochemical Science and Technology  
National Taiwan University, Taipei, Taiwan

## **Graduate education highlights**

### **Theses from Academic Year 2004–05**

Ruifang (Grace) Xu, M.S. Chemical Engineering, July 2004  
“Spatial growth patterns of *Pseudomonas aeruginosa* biofilms”

Laura Purevdorj-Gage, Ph.D. Microbiology, November 2004  
“Influence of quorum sensing and hydrodynamics on *Pseudomonas aeruginosa* biofilm structure and behavior”

Patrick M. Norris, M.S. Mechanical Engineering, November 2004  
“Ultrasonically controlled antibiotic release from hydrogel coatings for biofilm prevention”

Paul Sturman, Ph.D. Civil Engineering, November 2004  
“Control of acid rock drainage from mine tailings through the addition of dissolved organic carbon”

Allison N. Rhoads, M.S. Environmental Engineering, December 2004  
“A microbial fuel cell using biomineralization of manganese as a cathodic reactant”

Mark McBroom, M.S. Environmental Engineering, December 2004  
“Kinetics and community profiling of sulfate-reducing bacteria in treated mine tailings”

Avinash Shantaram, M.S. Environmental Engineering, April 2005  
“Power management for microbial fuel cells”

**Image: *Staphylococcus* biofilm—labeled, cryosectioned and imaged with Confocal Scanning Electron Microscopy by M.S. candidate Suriani Abdul Rani.**

## CBE 2005 ANNUAL REPORT : TEXT ONLY

**TABLE: 2004–05 CBE graduate students, by discipline.**

| Discipline                        | MS/Ph.D. | Male      | Female   | Total     |
|-----------------------------------|----------|-----------|----------|-----------|
| Chemical & Biological Engineering |          |           |          | <b>11</b> |
|                                   | MS       | 3         | 2        | 5         |
|                                   | Ph.D.    | 5         | 1        | 6         |
| Chemistry                         |          |           |          | <b>1</b>  |
|                                   | Ph.D.    | 1         |          | 1         |
| Civil & Environmental Engineering |          |           |          | <b>8</b>  |
|                                   | MS       | 5         | 1        | 6         |
|                                   | Ph.D.    | 2         |          | 2         |
| Computer Science                  |          |           |          | <b>1</b>  |
|                                   | Ph.D.    | 1         |          | 1         |
| Electrical Engineering            |          |           |          | <b>2</b>  |
|                                   | MS       | 1         |          | 1         |
|                                   | Ph.D.    | 1         |          | 1         |
| Mathematics                       |          |           |          | <b>3</b>  |
|                                   | Ph.D.    | 1         | 2        | 3         |
| Mechanical Engineering            |          |           |          | <b>2</b>  |
|                                   | MS       | 2         |          | 2         |
| Microbiology                      |          |           |          | <b>4</b>  |
|                                   | Ph.D.    | 2         | 2        | 4         |
| <b>Total</b>                      |          | <b>24</b> | <b>8</b> | <b>32</b> |

Over two dozen CBE graduates and undergraduates presented their research in prepared talks and research posters at a variety of conferences this year, including the Technical Advisory Conferences held for the CBE's Industrial Associates.

### ***CBE seminar series***

*“The speakers represent a wide range of interests and give very serious and carefully prepared talks, even though the atmosphere is informal and hence conducive to discussion and questions.”*

Cindy Morris  
Visiting Scientist, INRA, Avignon, France

The weekly CBE Seminar Series provides a great opportunity for CBE and MSU students, staff and faculty to keep up-to-date on biofilm research progress on campus and around the world. Seminar speakers include CBE researchers, long- or short-term visiting scientists, CBE alumni and specially invited experts. The Seminar Series is organized each academic year by CBE volunteers; this year graduate students Stewart Clark, Willy Davison, and Benjamin Klayman did an exemplary job of planning, coordinating and promoting the program. As organizers, these students took advantage of the opportunity to interact with scientists from around the world, to learn the challenge of coordinating schedules, and to give formal introductions for the speakers. Stewart, Willy and Ben made some great connections this year, and without a doubt, the whole CBE reaped the benefits from this year's student-organized seminar series.

*“The interdisciplinary diversity of the CBE is nowhere more evident than in the seminar series. These seminars provide the vital interaction point where clarifying questions that generate cross fertilization of ideas are asked. The diversity of the educational backgrounds of the speakers and audience enhances this interaction.”*

Dr. Joseph Seymour, Associate Professor,  
Chemical & Biological Engineering, CBE Faculty

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### **Visiting Seminar Speakers**

Volker Brozel, Department of Biology and Microbiology, South Dakota State University  
“Multicellular behavior of *Bacillus cereus* growing as a biofilm in soil”

Mark Dolan, Professor of Civil, Construction & Environmental Engineering, Oregon State University  
“Field bioaugmentation of a butane-utilizing culture to co-metabolize a mixture of problematic chlorinated solvents”

Garth Ehrlich, Center for Genomic Sciences, Pittsburgh, Pennsylvania  
“Developing experimental systems to test predictions of bacterial plurality with respect to chronic infections”

Ray Hozalski, Associate Professor, Civil Engineering, University of Minnesota  
“Development and application of a micro-cantilever method for measuring the cohesive strength of biofilms”

Brenda Little, Senior Scientist, Naval Research Laboratory, Stennis Space Center, Mississippi  
“Biofilm research: Past, present and future”

Cindy Morris, Visiting Scientist, INRA - Avignon, France  
“Biofilm on plant surfaces”

Garth Olds, M.D., Surgeon, Bozeman, Montana  
“Vascular graft infections”

Pradeep Singh, M.D., University of Iowa  
“Self-generated diversity produces ‘insurance effects’ in biofilm communities”

Joao Xavier, Postdoctoral Scholar, University of Delft, The Netherlands  
“Investigating the activity and structure of biofilms using mathematical modeling”

### **Undergraduate education highlights**

Undergraduates participating in CBE research frequently find that they get a head-start on career development. Since the year 2000, 33 peer-reviewed publications have listed CBE undergraduates as authors—one third of those have been published in the past year (see list at left). CBE undergraduates also have opportunities to present their work to various audiences at society conferences and to the CBE’s industrial representatives. This spring Ellen Swogger, an undergraduate working in the Biofilm Behavior Laboratory, was awarded 2nd place for her presentation “Evaluation of Wound Biofilms” at the Regional Conference for the American Institute of Chemical Engineers held at Oregon State University. Nick Ballor (Michigan Technological University), a 2004 REU student mentored by Robin Gerlach, won 1st place in the undergraduate student poster competition at the AIChE national conference in Austin, Texas, for his REU research project “Bioremediation and the Role of Mixed Pollution: TNT and Cr(VI).”

*“It is rewarding to look back and see that the papers I produced as an undergraduate are still being referenced and utilized by other researchers.”*

Jeff Anderl,  
B.S. Graduate, 1999, Chemical Engineering

## CBE 2005 ANNUAL REPORT : TEXT ONLY

**TABLE: 2004–05 CBE undergraduate students, by discipline**

| Discipline                        | Male      | Female    | Total     |
|-----------------------------------|-----------|-----------|-----------|
| Biological Science                |           | 1         | 1         |
| Biomedical Science                |           | 4         | 4         |
| Biotechnology                     |           | 1         | 1         |
| Cell Biology & Neuroscience       | 1         | 2         | 3         |
| Chemical & Biological Engineering | 6         | 5         | 11        |
| Chemistry                         | 3         |           | 3         |
| Civil Engineering                 | 1         | 1         | 2         |
| Computer Science                  | 2         | 1         | 3         |
| Environmental Engineering         | 3         |           | 3         |
| Mechanical Engineering            | 1         |           | 1         |
| Microbiology                      |           | 3         | 3         |
| Statistics                        | 1         |           | 1         |
| <b>Total</b>                      | <b>18</b> | <b>18</b> | <b>36</b> |

### **Ten peer-reviewed publications with undergraduate authors in 2004–05:**

Beyenal, H., *C. Donovan*, Z. Lewandowski and G. Harkin, “Three-dimensional biofilm structure quantification,” *J. Microbiol. Methods*, 59:395-413 (2004).

Borriello, G., *E. Werner*, F. Roe, *A.M. Kim*, G.D. Ehrlich and P.S. Stewart, “Oxygen limitation contributes to antibiotic tolerance of *Pseudomonas aeruginosa* in biofilms,” *Antimicrob. Agents Chemother.*, 48(7):2659-2664 (2004).

Hunt, S.M., *E.M. Werner*, B. Huang, M.A. Hamilton and P.S. Stewart, “Hypothesis for the role of nutrient starvation in biofilm detachment,” *Appl. Environ. Microbiol.*, 70(12):7418-7425 (2004).

Lewandowski, Z., H. Beyenal and *D. Stookey*, “Reproducibility of biofilm processes and the meaning of steady state in biofilm reactors,” *Water Sci. Technol.*, 49(11-12):359-364 (2004).

Shaw, T., *M. Winston*, *C.J. Rupp*, I. Klapper and P. Stoodley, “Commonality of elastic relaxation times in biofilms,” *Phys. Rev. Lett.*, 93(9):098102 1-4 (2004).

Vinogradov, A.M., *M. Winston*, *C.J. Rupp* and P. Stoodley, “Rheology of biofilms formed from the dental plaque pathogen *Streptococcus mutans*,” *Biofilms* 1(1):49-56 (2004).

*Werner, E.*, F. Roe, *A. Bugnicourt*, M.J. Franklin, A. Heydorn, S. Molin, B. Pitts and P.S. Stewart, “Stratified growth in *Pseudomonas aeruginosa* biofilms,” *Appl. Environ. Microbiol.*, 70(10):6188-6196 (2004).

*Abdul Rani, S.*, B. Pitts and P.S. Stewart, “Rapid diffusion of fluorescent tracers into *Staphylococcus epidermidis* biofilms visualized by time lapse microscopy,” *Antimicrob. Agents Chemother.*, 49(2):728-732 (2005).

*Roberts, M.E.* and P.S. Stewart, “Modelling protection from antimicrobial agents in biofilms through the formation of persister cells,” *Microbiol.-UK*, 151:75-80 (2005).

*Rupp C.J.*, C.A. Fux and P. Stoodley, “Viscoelasticity of *Staphylococcus aureus* biofilms in response to fluid shear allows resistance to detachment and facilitates rolling migration,” *Appl. Environ. Microbiol.*, 71(4):2175-2178 (2005).

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### ***Undergraduate Scholars Program brings out the best***

Engineering and science undergraduates at MSU get a chance to plan Center research projects in collaboration with CBE faculty members through the Undergraduate Scholars Program (USP). The USP supports undergraduate research by awarding grants and/or providing academic credit to students who undertake an investigative or creative project in collaboration with a faculty mentor.

USP applicants submit a mini-proposal detailing methods and techniques that will be involved in the proposed research project. At the completion of the project, students submit a final report. In some cases, awardees are asked to present their work. At the Spring 2005 USP Conference on March 30th, CBE students presented their research results with posters and presentations. Dr. Phil Stewart, last year's winner of the Provost's Award for Undergraduate Research/Creativity Mentoring, presented the keynote address at the USP conference luncheon.

#### **Undergraduates participating in the USP program during this academic year were:**

Chelsea Grace

Major: Chemical & Biological Engineering

USP Project: "Structure and interactions of *Pseudomonas aeruginosa* biofilms"

Amy Martin

Major: Chemical & Biological Engineering

USP Project: "Screening plant extracts for biofilm inhibition"

Pat Secor

Major: Biochemistry

USP Project: "Fluorescent *in situ* hybridization"

David Stepler

Major: Biochemistry

USP Project: "Influence of co-contaminants and natural organic matter on the reduction of 2,4,6-trinitrotoluene (TNT), chromium (VI), and carbontetrachloride by zero-valent iron"

Ellen Swogger

Major: Chemical & Biological Engineering

USP Project: "Evaluation of wound biofilms"

Alison Ziegler-Enos

Major: Biomedical Science and Secondary Education

USP Project: "Response of biofilm to toxic gas exposure"

## **TECHNOLOGY TRANSFER**

### ***The CBE Industrial Associates program drives relevant research***

More information about the CBE's industry program can be found on our web site at:

[www.erc.montana.edu/Ind-Col99-SW/](http://www.erc.montana.edu/Ind-Col99-SW/)

Industrial partnerships have been critical to the CBE's research focus and technology transfer for over two decades. In 2004–05, the CBE had 26 subscribing Industrial Associate (IA) companies, representing the health care, household products, specialty chemical, and drinking water industries (listed on page 16). New members over the past year include Cumbre, Ecolab, GlaxoSmithKline, Masco, NASA, and Novozymes North America. Semi-annual Technical Advisory Conferences (TAC) in Bozeman provide the latest biofilm information and lots of interaction. In keeping with our goal of making the TAC an international forum for the latest in biofilm research worldwide, invited speakers for 2004–05 included Dr. Peter Gilbert (University of Manchester), Dr. Bonnie Bassler (Princeton University), and Dr. Per Nielsen (University of Aalborg). The Industrial Associates program accounts for a large portion of the CBE total budget, not only through direct contributions (in the form of annual dues) but also through specific projects sponsored by IA companies. These contributions enhance our ability to maintain a high profile internationally, and to lead in the science and engineering of biofilm-related research. Noteworthy examples of CBE technology transfer are highlighted on the following pages.

### ***Biofilms in dentistry***

Biofilms are of concern to dentists and patients alike from the standpoint of both periodontal disease and dental chair cleanliness. Through a donation from Industrial Associate Church & Dwight, Inc., the CBE has developed and performed preliminary testing on an oral biofilm model that emulates tooth and gum surfaces in the mouth as well as incorporating both facultative and obligate anaerobic bacteria implicated in tooth decay. In addition, the CBE continues to work with the American Dental Association (ADA) and the Organization for Safety and Asepsis Procedures (OSAP) in developing a standard method for testing the efficacy of dental unit water line antimicrobials. The method is currently undergoing robustness testing at the CBE with the intention of going out for round-robin testing and validation in the coming months.

### ***The CBE regulatory connection***

In the dental chair, in products on the supermarket shelf, and in industry, biofilms are a hot topic. With the rapidly growing understanding of the importance of biofilm comes the need for sound science in the regulation and approval of new product claims. Through CBE expertise, the EPA and FDA have a partner in the task of developing the necessary methods to test these claims. Both agencies maintain close contact with CBE researchers, and in the past year, have spoken at TAC conferences. The result is better communication between regulatory decision makers and industry scientists.

### ***Microbes in space***

Controlling microbial growth in closed-loop cooling water systems in the International Space Station (ISS) is extremely important to the ISS mission and maintenance of a healthy environment for the crew. The National Aeronautics and Space Administration (NASA) has tasked the CBE with assisting staff microbiologists and Boeing Inc. engineers in developing protocols for biofilm control and eradication in this sensitive application. As one of the CBE's newest associates, NASA brings both interesting challenges and the potential for international exposure for our research team.

**Image: Confocal Scanning Laser Microscope images collected by CBE Microscopist Betsey Pitts. Upper image: Gfp-expressing *P. aeruginosa* biofilm grown on a contact lens surface as part of a microscopy workshop held for academic collaborators from Rome. Lower image: A thermophilic biofilm grown by Trish Ball, a former CBE REU student, now working on her M.S. degree at the University of Idaho.**

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### **A note from one of our sponsors**

*“Association and membership with the Center for Biofilm Engineering has been extremely beneficial for our company. Through the expertise of the faculty, staff, students and guest speakers, we have been exposed to not only state-of-the-art, but relevant applied research of bacterial biofilms. We have used this information in product development, including updated regulatory strategies, for outreach to our clinical customers, and in our environmental manufacturing and cleanroom areas. The Center’s willingness to communicate biofilm issues to the scientific, industrial, regulatory and clinical world, and to establish standardized biofilm methods, has been invaluable. The networking between the Center and industrial technical associates, visiting scientists and regulatory agencies has been critical for our success and our product development priorities. The CBE is well respected worldwide for its scientific excellence and its commitment to solving real problems with real biofilms.”*

Ruth Cutright,  
Corporate Microbiologist,  
Technology Champion,  
W.L. Gore and Associates Inc.  
Flagstaff, Arizona

### **CBE Industrial Associates**

Aramco Services Company  
Church & Dwight Co., Inc.  
Colgate-Palmolive  
C.R. Bard, Inc.  
Cumbre, Inc.  
DePuy, Inc.  
Diversa Corporation  
Dow Chemical Company  
DuPont  
Eastman Kodak Company  
Ecolab, Inc.  
Edstrom Industries, Inc.  
Electric Power Research Institute (EPRI)  
Gambro Corporate Research  
Genencor International, Inc.  
GlaxoSmithKline  
Idaho National Laboratory (INL)  
Masco  
NASA  
Novozymes North America, Inc.  
Philips Oral Healthcare  
S. C. Johnson & Son (formerly S.C. Johnson Wax)  
Smith & Nephew, Inc.  
Tyco Healthcare (formerly Kendall Healthcare Products Company)  
U.S. Bureau of Reclamation  
W.L. Gore & Associates

### **Postcard from a friend. . .**

*“The training I received at the CBE was not only world-class but of a true interdisciplinary nature. PNNL is a multiprogram laboratory that is often in need of researchers capable of interdisciplinary research. For me this is a natural way to do research and, because of my background from the CBE, I can easily work at the interface between disciplines.”*

Chris Wend, Ph.D., P.E.  
Pacific Northwest National Laboratory, Richland, Washington

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### ***Workshops that work***

The first impression many people have of the CBE environment comes from attending one of our semi-annual hands-on biofilm methods workshops. The two-day workshop is an immersion course in biofilms from the basic growth theory to advanced microscopy analysis techniques. In two cases, workshop participation has directly resulted in Industrial Associate membership for attendee companies. In addition, the workshops routinely generate funding opportunities such as Small Business Innovation Research (SBIR) projects and sponsored research.

Understanding biofilms is the first step in recognizing their importance in a wide variety of industrial systems. Over the past year, the CBE has also implemented a new facet of interaction: the strategic planning workshop. Typically taking place at a company's place of business and involving senior scientific and management staff from the company, the strategic planning workshop is a forum for CBE researchers to provide focused and confidential feedback and assistance on business plans involving biofilm-related issues. For CBE member companies, this is a benefit of membership.

**Image: Deb Berglund provides instruction on the use of the flow cytometer.**

**Image: The workshops organized by Zbigniew Lewandowski and Haluk Beyenal are well-attended by researchers from top universities such as the University of California–Berkeley, Yale University, The Pennsylvania State University, University of Ottawa and University of Helsinki, Finland.**

## **OUTREACH**

### ***BiofilmsOnline helps the CBE reach new audiences***

Through [www.BiofilmsOnline.com](http://www.BiofilmsOnline.com) the Center has truly been able to perform world-wide outreach. Since October 2000, this website has been a valuable web resource of current biofilm-related information for a broad audience. Supported by the CBE's Industrial Associates, it provides information to the public and to professionals in industry, academia, and the government. This web site is searchable and covers biofilm news, research, patents, and conferences around the world. It quickly provides what could otherwise only be found through time-consuming searching of multiple resources. The information is carefully gathered and reviewed by CBE personnel and collaborators with credentials and experience in biofilm research and education. Biweekly, an electronic newsletter of the latest information (educational materials, journal references, patents, press releases, employment opportunities, etc ) is disseminated to over 1,000 subscribers. Subscribership gives the CBE the opportunity to establish relationships with readers who have an expressed interest in biofilms and have given permission to be contacted.

### ***Web watch. . .***

The CBE web site has such a wealth of biofilm information that readers usually visit it more than once (85% of web visitors) to glean information. The web site has truly become an educational resource for those learning about biofilms and for those trying to teach the biofilm concept to students, to peers and to the public.

The CBE's web Resource Library proves to be the most popular section of the web, providing 65,702 accesses to CBE publications, biofilm movies, and an image library. The CBE's Publications List is the most frequently downloaded pdf file, with over 1,000 requests.

The CBE's web site Image Library prompted image use requests originating in 29 states and 21 countries around the world. The number of image requests more than doubled in 2004–05, underscoring the demand for biofilm educational materials world wide and at every level. Image requestors include instructors, students, medical health professionals and textbook or magazine publishers.

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### ***CBE tours***

One way that the CBE reaches out to the Montana community is through tours. Our staff and students are adept at introducing biofilms to a variety of audiences. Tours have been given to homeschoolers, local business owners and industrial representatives, all with a warm enthusiasm to show and to explain the real-world problems that scientists at the CBE are trying to solve.

### ***PASI brings South America to Bozeman***

Two biofilm workshops—coordinated by Dr. Richard Veeh, CBE Senior Research Associate, and Dr. Homero Urrutia Briones, Associate Professor, Microbiology, at the University of Concepción in Chile—took place in 2004: the first in January in Concepción and the second in July in Bozeman. Both workshops were funded by a grant from the NSF/DOE-sponsored Pan-American Advanced Studies Institutes (PASI) Program. The goal of these workshops was to broaden student researchers' knowledge-base through lectures and hands-on application of techniques critical to the study of biofilms. The workshops focused on the interdisciplinary nature of biofilm studies—highlighting, in particular, the fields of biochemistry, microbiology, and engineering. Students were introduced to techniques that allow microscale investigation of microbial biofilms; after the course, students had gained understanding sufficient for independent application.

Two other important aspects of the workshops were to foster interactions among students from diverse educational and cultural backgrounds, and to expose students to industrial perspectives on real-world applications related to biofilm processes and on academic/industrial collaborations.

### ***CBE team organizes IWA Biofilms 2004 international conference***

CBE people made huge contributions to the Biofilms 2004 Conference held in Las Vegas in October, 2004, and sponsored by the International Water Association. The conference focused on structure and activity of biofilms, including physiology and genetics, modeling and applications. Over 180 people attended the conference from 27 countries. Dr. Zbigniew Lewandowski was named Chair of the IWA Specialist Group on Biofilm Processes. Ten CBE faculty and students presented research results at the conference.

## **PEOPLE**

### ***New CBE employees***

New staff and faculty have brought specialized skills and energy to the CBE's endeavors. There were some familiar faces among the new hires at the CBE this year. Elinor Pulcini, Brett Towler and Diane Walker all received advanced degrees in the Center's graduate program.

#### **Faculty**

Christine Foreman  
Brett Towler

#### **Postdoctoral Researchers**

Ross Carlson  
Andreas Nocker-Einsiedler

#### **Senior Research Staff**

Elinor Pulcini

#### **Research Staff**

Deb Berglund  
Paul Perry  
Diane Walker

Sara Martin  
Adrienne Phillips

#### **Administrative Staff**

Jeanne Olsen

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### ***Visiting students and researchers***

Visiting student and faculty researchers help to keep the exchange of ideas, information and techniques lively and fresh at the CBE. Visitors seeking collaborative opportunities in biofilm research came from nine countries as well as various locations in the U.S.

Alessandra Agostinho, University of São Paulo, Brazil  
Nahit Atkas, Van University, Turkey  
Sabrina Behnke, University of Duisberg-Essen, Germany  
Elena Deligianni, University of Ulster, Ireland  
Goksel Demir, Istanbul University, Turkey  
Christoph Fux, M.D., University Hospital, Bern, Switzerland  
Ray Hozalski, University of Minnesota, Minneapolis, Minnesota  
Albert Jacobs, Lyon University, France  
John Lennox, Pennsylvania State, Altoona, Pennsylvania  
Volker Maczek, University of Duisberg-Essen, Germany  
Nishma Manandhar, University of Duisberg-Essen, Germany  
Enrico Marsili, La Sapienza University, Rome, Italy  
Cindy Morris, Institut National de la Recherche Agronomique, Avignon, France  
Brent Peyton, Washington State University, Pullman, Washington  
Jessica Richard, University of Duisberg-Essen, Germany  
Patrizia Russo, La Sapienza University, Rome, Italy  
Deepak Sharma, University of Ottawa, Ontario, Canada  
Eoin Syron, University College, Dublin, Ireland  
Bastian Wittstock, University of Stuttgart, Stuttgart, Germany  
Joao Xavier, Delft University of Technology Delft, The Netherlands  
Kyung-Min Yeon, Seoul National University, Seoul, Korea

### ***2004-05 Awards***

The 2004 CBE Outstanding Researcher Award was presented to Linda Loetterle on June 30, 2004, at the Summer Technical Advisory Conference. This award recognizes individuals who have not only demonstrated exceptional creativity and productivity in their research, but who have also made special contributions to research life at the Center for Biofilm Engineering.

#### **The College of Engineering presented the following awards to CBE staff and faculty in the spring of 2005:**

Outstanding Instructor Award  
**Otto Stein**, Civil Engineering  
**Joe Seymour**, Chemical & Biological Engineering  
Outstanding Researcher, CBE  
**Haluk Beyenal**  
Outstanding Professional Employee  
**Tricia Cook**

Former CBE director, **J. William (Bill) Costerton** is the recipient of the 2005 Procter & Gamble Award in Applied and Environmental Microbiology for his work demonstrating the importance of microbial biofilms in industrial, medical and natural environments.

**Ben Klayman**, doctoral candidate in Civil Engineering, received the 2005 W.G. Characklis Award. The award, created in honor of the Center's founder, is presented to a Center for Biofilm Engineering Ph.D. student based on his or her contributions in research, especially as it pertains to advances made in achieving Center goals. From leading tours, to organizing seminars, to creating 3D microscope movies, Ben has made contributions with enthusiasm. His excellent work, broad perspective, volunteer ethic, good heart and great sense of humor have affected just about everyone at the CBE.

## **CBE 2005 ANNUAL REPORT : TEXT ONLY**

### **Postcards from friends. . .**

*“The CBE has had an incredible influence on both my professional and personal life. After almost ten years, I still maintain active collaborations with a number of researchers at the CBE. Various collaborations over the last ten years have resulted in novel biofilm research, graduate and undergraduate student exchanges, and numerous publications. The original founder of the CBE, Bill Characklis, and other faculty have inspired and mentored me throughout my career. My close ties and continued involvement with the Center have resulted in life-long friendships with a number of the faculty and staff at the CBE. I feel extremely lucky to have been involved with the CBE.”*

Robert R. Sharp Ph.D., P.E.  
The O’Connor Endowed Faculty Fellow of Environmental Engineering  
Dept. of Civil and Environmental Engineering  
Manhattan College

*“Because of my research experience at MSU, I gained a better understanding of what research is. My research experience helped me develop many skills that are necessary in a career: technical writing, speaking, problem solving, working with a team, and thinking in new ways. Because of my experience at the CBE, I decided to pursue a graduate degree in pharmaceutical research.”*

Erin Werner,  
B.S. graduate, 2003, Chemical and Biological Engineering

### **Glowing reviews**

The Bioglyphs project continues to attract interest both at the CBE and via its web site ([www.erc.montana.edu/Bioglyphs/](http://www.erc.montana.edu/Bioglyphs/)). It has been featured on the home pages of *USA Today* (“Hot Sites,” June 25, 2004) and *Yahoo!* (“New and Notable Sites,” May 3, 2005). A short article and image were published in the January, 2005, issue of *BioTechniques: The International Journal of Life Science Methods* (“Web Watch”), as well as in the December, 2004, issue of the student-oriented magazine *ChemMatters*, published by the American Chemical Society. The Bioglyphs image at right is part of an installation prepared with support from Paul Sturman and Phil Stewart to coincide with the Summer 2004 Technical Advisory Conference by CBE staff and students: Suriani Abdul-Rani, Andrew Anacker, Willy Davison, Peg Dirckx, Kevin Grealish, Betsey Pitts, Christy Rabinovitch, and Allison Rhoads.

### **Want more information?**

The 2005 CBE Appendix is loaded with information about CBE activities during the past year: publications, research projects, conference agendas, presentations, CBE collaborations, and more.

## CBE 2005 ANNUAL REPORT : TEXT ONLY

### Faculty

| 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 network analysis  |
| Sarah Codd            | Chemical & Biological 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  |
| Jack Dockery          | Mathematical Science                    | Mathematical models of biofilms  |
| Tim Ford              | Microbiology                            | Drinking water, public health microbiology, pollutant fate and transport   |
| Michael Franklin      | Microbiology                            | Molecular genetics, gene expression, alginate biosynthesis   |
| Gill Geesey           | Microbiology                            | Molecular and cellular interactions at interfaces  |
| Marty Hamilton        | Statistics                              | Applied biostatistical thinking  |
| Warren Jones          | Civil Engineering                       | Water distribution systems   |
| Isaac 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   |
| Barry Pyle            | Microbiology                            | Environmental, water and food microbiology   |
| Rocky Ross            | Computer Science                        | Web-based, active learning education; design and analysis of algorithms for practical problems; information technology |
| Joseph Seymour        | Chemical & Biological Engineering       | Magnetic resonance imaging   |
| Otto Stein            | Civil Engineering                       | Engineered waste remediation   |
| Phil Stewart          | Chemical & Biological Engineering       | Biofilm control strategies   |
| Paul Stoodley         | Microbiology                            | Biofilm mechanics, cell-cell communication   |
| Paul Sturman          | Civil Engineering                       | Biofilms in waste remediation and industrial systems   |
| Peter Suci            | Microbiology                            | Biofilm mechanics  |
| Brett Towler          | Civil Engineering                       | Biofilm mechanics  |
| Rick Veeh             | Land Resources & Environmental Sciences | Bacterial identification using oligonucleotide probes  |
| Aleksandra Vinogradov | Mechanical & Industrial Engineering     | Biofilm mechanics  |

#### Center for Biofilm Engineering

**366 EPS Building**

**P.O. Box 173980**

**Montana State University**

**Bozeman, Montana**

**59717-3980**

**Phone: 406-994-4770**

**Fax: 406-994-6098**

**Web site: [www.erc.montana.edu](http://www.erc.montana.edu)**

*The Center for Biofilm Engineering at Montana State University is a National Science Foundation Engineering Research Center founded in 1990.*

**Image: Confocal Scanning Laser Microscopy of a *Staphylococcus epidermidis* biofilm, grown as part of an industrial project. Image provided by Betsey Pitts, Paul Perry and Phil Stewart.**