The establishment of the CBE launched biofilms as a field of specialized research

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A B S T R A C T

The Center for Biofilm Engineering was the first center of excellence focused on biofilms and was originally funded through the Engineering Research Center Program from the U.S. National Science Foundation. After almost 30 years, biofilm continues to be a stand-alone scientific topic of inquiry that has broad implications for fundamental and applied science and engineering of bio-systems. However, much remains to be done, not only for research discovery but also education and outreach, to increase and grow the biofilm paradigm as well as our understanding of the microbial world.

A brief history of biofilms

In an evolutionary step falling between the primordial soup and the appearance of multicellular organisms, microbial associations emerged (i.e., colonies/aggregates), possibly as a safety-in-numbers means of survival, possibly to benefit from interactions, or merely as a consequence of microbial growth (or a combination thereof). Some 3.8 billion years later, scientists (some at the Center for Biofilm Engineering at Montana State University) would identify that the quorum sensing process was involved in the ability to sense the presence of other cells and collaboratively create an extracellular polymeric substance – the birth of a biofilm [1]. Today, it has been argued that in most studied environments, a greater number of the world’s microorganisms live in biofilm than in a planktonic state [2]. Microorganisms and the biofilms they form are known to play vital roles in ecosystem function, and most likely represent immense, as yet undiscovered, biochemical and physiological capacities. Additionally, microorganisms are known to play important roles in global biogeochemical cycling, industrial processes, and human health. It is becoming increasingly clear that attached microbial growth (i.e., biofilm) more closely resembles in situ conditions for many microorganisms in different environments and might likely be a universal feature that presents an important physiology to understand in addition to the more easily studied planktonic state [3].

Biofilms are ubiquitous. They have been observed in almost every known environment on the planet where liquid-solid, liquid-gas, gas-solid, or solid-solid interfaces exist. Yet, biofilm research is a relatively new branch on the tree of scientific inquiry. But long before the Center for Biofilm Engineering at Montana State University was established in 1990, biofilms had been observed by people who did not have the tools to study them in detail. In 1684, Anthony van Leeuwenhoek, often described as the father of microbiology, remarked on the vast accumulation of microorganisms in dental plaque, and most would agree this dental plaque was a biofilm and could also be considered one of the first human microbiome observations. “The number of these animicules in the scurf of a man’s [or woman’s] teeth are so many that I believe they exceed the number of men in a kingdom,” he wrote in a report to the Royal Society of London.

The study of microorganisms took an important turn in the mid-1800s when German microbiologist, Robert Koch, developed methods to create a solid nutrient medium in order to grow and isolate pure cultures of microorganisms. This development led to huge advances in medicine, agriculture, and industry. However, these advances were based on such a simplistic concept of microbial life that many “solutions” generated by these techniques are now being re-considered because of the notion of “isolated” microbial cultures. Microorganisms have proven to be much more diverse, much more complex, and much less tractable than Koch ever could have possibly imagined.

In a 1940 issue of the Journal of Bacteriology, authors H. Heukelekian and A. Heller wrote “Surfaces enable bacteria to develop in substrates otherwise too dilute for growth. Development takes place either as bacterial slime or colonial growth attached to surfaces” [3] and Claude ZoBell described many of the fundamental characteristics of attached...
microbial communities in the 1940s [e.g., [4]]. In the late decades of the 20th century, numerous articles were written about microbial films or slime layers, and German researchers sometimes used the term “Schmutzdecke.” The term can be translated as “dirt cover” or “dirty skin” and has come to mean the biological layer formed on the surface of a slow sand filter; however, the concept of “schmutzdecke” still holds for the general concept of microbial growth at interfaces.

As the unique properties of microbial communities versus planktonic microbes grew more apparent, it became helpful to use a special term to describe them. “Biofilm” was used colloquially among researchers for some years before it was considered a term acceptable for use in scientific publications. The term “biofilm” is believed to have first appeared in a 1975 Microbial Ecology article by W.N. Mack, J.P. Mack, and A.O. Ackerson titled, “Microbial film development in a trickling filter.” [5]. In the opening sentence of the abstract, the authors state, “The transmission and scanning electron microscopes were used to visualize the sequence of the biofilm development in the trickling wastewater filter.”

MSU wins NSF ERC grant

In 1990, when the National Science Foundation awarded Montana State University a prestigious, 11-year Engineering Research Center grant, the term “biofilm” was largely colloquial and not well understood. So much so that the Center for Biofilm Engineering was called the Center for Interfacial Microbial Process Engineering for the first three years of its existence. At the time, researchers predominantly studied bacteria in suspension and on agar plates, which formed the basis of standard methods used in research, industry, and regulatory laboratories. Although researchers suspected biofilms were part of the microbial ecosystem, there were limited research data that revealed biofilm’s fundamental role in most microbial ecosystems, including the link between biofilm and public health. This led directly to the advent of the CBE, and the research of biofilm as a specialized field of study in earnest.

The omission of the word “biofilm” was surely only a small compromise in the eyes of Center for Biofilm Engineering founding director, William Characklis. Widely regarded as a brilliant chemical engineer and a visionary leader with a Ph.D. in environmental engineering from Johns Hopkins University, Characklis passionately championed the study of biofilms [6]. Case in point, Characklis authored or co-authored 17 of the 19 chapters in the book Biofilms in 1990 [7]. Some contemporary views his application for ERC funding as a longshot at best. However, Characklis’ vision for a university-based research center dedicated to biofilms that would couple corporate interests and faculty from multiple disciplines brought risks that he thought worthy of staking his reputation upon. At the time, the benefits of cross-campus collaborations were still viewed with skepticism by many around the country, as was partnering with corporate sponsors — aka “industrial associates” — a practice some saw (and still do) as antithetical to independent scientific research that is conducted by academicians. But Characklis’ longshot paid off, and the involvement of industry has been crucial to the applied mission of the CBE. Montana State University was one of 48 applicants for ERC funding in 1989, and a recipient of one of just three grants awarded, resulting in what at the time was the largest grant in the nearly 100-year history of Montana’s land-grant institution.

Leadership at the CBE has been notably stable, having only four directors in 30 years. However, that was not the case early on. When CBE founding director William G. Characklis died from malignant lymphoma at the age of 50 in 1992, the NSF considered withdrawing the grant entirely. Fortunately, the CBE had already brought international recognition to MSU not only in the field of biofilm but also in higher education in general. So, the university’s leadership urgently undertook a robust search to bring an established researcher and dynamic leader to succeed Characklis. They found just that in John W. (Bill) Costerton, an accomplished microbiologist from the University of Calgary, and it was Costerton who renamed the Center for Interfacial Microbial Process Engineering as the Center for Biofilm Engineering. Part of Costerton’s considerable legacy included the incorporation of biofilm applications to infections and medical devices [8,9], which naturally brought related corporate interests into the Industrial Associates program. Costerton would lead the CBE until his retirement from MSU in 2004. The next year, Phil Stewart was named director. Stewart, a professor of chemical and biological engineering at MSU who earned his doctorate from Stanford University, had built an international reputation in biofilm research [10-12]. Stewart, whose papers greatly advanced the field, significantly increased the CBE’s exposure internationally and solidified its reputation as the world’s leading biofilm research center. Stewart is the most-cited researcher in the history of MSU and is arguably the most prominent biofilm control researcher worldwide, continues to work at the CBE. In 2019, the Montana University System Board of Regents named Stewart a Regents Professor, the highest honor bestowed upon professors by the state of Montana. Another component of Stewart’s legacy was bringing environmental microbiologist Matthew W. Fields to MSU as an affiliated faculty member in 2007. Fields, who earned his doctorate in microbiology from Cornell University, had a background in anaerobic physiology, biochemistry, genomics, and ecology, and his addition re-fortified CBE expertise in environmental and industrial processes that integrated well into the engineering expertise of the CBE [13-15]. When Stewart stepped down as CBE director in 2015 to focus exclusively on research and teaching, Fields, a professor in the department of microbiology and immunology, succeeded him. Four years into his tenure as CBE director, Fields’ most tangible impact thus far is arguably forging partnerships with biofilm-related researchers and centers around the world as well as growing focus on standardization of biofilm methods and impacts on technology innovation and regulatory policy. Fields along with CBE collaborators have also overseen significant funding increases, including from the Departments of Energy and Defense. His interests in environmental technologies and industrial ecology paralleled the environmental research interests of Characklis.

Health, industry, and technology

Biofilms are everywhere and their impact on humanity is far reaching. For instance, biofilm buildup in urinary catheters – a common occurrence – is responsible for 30 percent of all hospital-acquired infections, leading to around 13,000 deaths and more than $340 million in treatment costs annually in the United States [16,17]. Biofilm is also a prime suspect when chronic wounds such as diabetes-related foot ulcers fail to heal [18], which leads to almost 300 amputations every day in the U.S. alone [19]. Biofilm is also known to harbor opportunistic pathogens and is attributed to increasing bacterial tolerance to antimicrobial agents and pathogen transmission [20-22]. Biofilm on hospital surfaces can put patients at risk for nosocomial infections, and consistent cleaning and disinfection are needed to maintain low biofilm levels [23,24]. Biofilms on food contact surfaces in restaurants as well as institutional and home kitchens may allow foodborne pathogens such as E. coli and Salmonella to survive and thus represent additional surfaces that require attention. Biofilm persists even in situations where disinfectants are applied. Multiple theories have been proposed to explain biofilm’s increased resistance to disinfection [25]. Although standard methods exist for growing biofilm and the determination of liquid anti-biofilm product efficacy [26-29], to date no standard methods exist for determining the removal of cells and biomass from a surface. Partially removed biofilms lead to rapid regrowth and the presence of biological “soil” on the surface may decrease the efficacy of the chemical disinfectants (e.g., re-used medical devices). Biofilms also wreak costly havoc in industrial, infrastructure, transportation, and military settings. In 2013, for instance, the U.S. government and private industry combined to spend ~$500 billion fighting biofilm-related metal corrosion – around 3 percent of the U.S. GDP [30].

But not all biofilms are harmful. Some are neutral, and others can be beneficial. For instance, in 2017, the CBE successfully field tested a biofilm technology that can be injected into leaking oil wells hundreds of
feet below ground and harden, sealing the cracks that are causing the leaks. This technology has significant commercial potential [31,32]. Beneficial biofilms have also been exploited in water pretreatment systems and in remediating contaminated soils [33–35]. In addition, fundamental research into biofilms provides insight into system processes important to almost every habitat/environment on the planet in terms of impacts of microbial interactions on resource allocation, biochemical processing, evolution, ecology, etc. [36].

Because so many aspects of biofilm are ripe for inquiry, research is burgeoning at the CBE and around the world. In a stark contrast to the era when the CBE launched, today there are dedicated research centers in Denmark, Singapore, the United Kingdom, Binghamton (NY), and, of course, Bozeman, Montana. Moreover, leading researchers at numerous universities and laboratories around the world are also studying biofilm (too many to list). These scientists are part of the biofilm community dedicated to understanding the role of biofilm in industrial, medical, and environmental processes, and the geographic distribution has contributed to the exponential growth of biofilm research since 1990. A PubMed search shows that 5558 articles pertaining to biofilm were published in 2018, compared to 45 articles published in 1990 (an almost 125x increase), the year the NSF funded the CBE as an Engineering Research Center. The total biofilm-related citations since 1990 of papers authored at the most-cited institutions total 177,677. In short, the more researchers dedicated to answering the challenges/questions presented by biofilm, the faster solutions to health, environmental, and industrial problems—and seeking opportunities to exploit them for our benefit—the better.

Lynn Preston, one of the chief architects behind the NSF’s Engineering Research Centers program, said the CBE has proved its worth, pointing to the CBE’s groundbreaking research into how biofilms colonize to share nutrients and are linked to illnesses such as cystic fibrosis and medical-device infections. “It was clear from the start, these guys had this great passion for their mission, and there was no doubt about their commitment to working with industry partners,” Preston said. “And, despite Montana being ‘difficult’ to get to, industry loved being a part of the CBE because it basically nurtured a whole new field. Without question, we (the NSF) certainly got our money’s worth.”

**Foundational CBE biofilm work**

Since Bill Characklis landed that longshot NSF-ERC grant for Montana State University nearly 30 years ago, CBE researchers have published 1200 peer-reviewed papers through FY2018 and provided 21 journal cover images related to biofilms. Seminal among them were publications that demonstrated even monoculture biofilms can be complex and heterogenous with architectural structure that impacts flow around and into the components of the film to nurture the associated cells and this ability can be regulated through chemical communication that helps modulate micro-niches [37–47]. Appreciation of community diversity in biofilms and the complex interplay between species grew [15,48–51], as did a growing curiosity of the biofilm matrix [52–55]. Other major findings over the years from CBE researchers included substrate gradients [56], diffusion limitations [57], and viscoelastic properties that contribute to detachment/dispersal [58]. The listed citations are not meant to be exhaustive (either for the CBE or abroad), but merely representative of the breadth of interdisciplinary biofilm work at the CBE that was and remains to be foundational to the biofilm discipline.

As the breadth and depth of biofilm research grew at the CBE and abroad, the importance of standardized methods emerged as an essential component that linked innovation and regulation. Much of the work and research in biofilms is, and has been, about the prevention, killing, and/or removal of biofilms. One of the laboratories that evolved as the CBE grew is focused on the standardization of methods for biofilm cultivation, anti-biofilm efficacy testing, and the prevention/killing of biofilms [26–29,59,60]. These efforts include biofilm testing and research with broad relevance from environments that range from medical devices to industrial production plants (and everything in between) [61]. As the methods are developed and data produced, the relevance has expanded to the crossroads linking regulation policy and technology innovation. At these crossroads, the industry perspectives are paramount to motivate the standardization of methods that enable the testing of needed technology that can be integrated with current and future needs of both industry and the regulatory bodies that oversee the materials and chemicals.

For example, data provided by the CBE provided the scientific backbone for new antimicrobial testing standards recently adopted by the U.S. Environmental Protection Agency [62,63]. The standards are the first to apply specifically to bacterial biofilms. The standards—an outgrowth of research by CBE faculty member Darla Goeres and the standards biofilm laboratory, provide a certification framework for companies to verify that their products are effective against biofilm bacteria and to label them accordingly, with a statement similar to the “Kills 99.9% of bacteria” found on bottles of bleach and other cleaners. Antimicrobial manufacturers are eager to attain the certification because of growing awareness about bacterial biofilms and the increasing importance to public health entities such as hospitals.

This, and other advancements by CBE scientists, could not have been realized without technologies such as confocal microscopy, which has been the centerpiece of the CBE Microscopy Facility at Montana State University. Optical microscopy is extremely well suited for the study of biological systems because of the non-invasive nature and compatibility with live samples, and confocal scanning laser microscopy (CSLM) has been an essential instrument for biofilm researchers around the world (including the CBE) because of the ability to provide 3D information of hydrated samples non-invasively and in real-time. In the last two decades, advances in (meta)genome and transcriptome sequencing, mass spectrometry-based metabolomic and proteomic methods, and cell isolation/separation have had huge impacts on biofilm research. Nonetheless, due to the inherent heterogenous complexity of most biofilms, systems-level, mechanistic understanding remains a challenge, and correlative techniques that enable exploration of spatial and temporal scales are needed. For example, the CBE recently acquired a Confocal-Raman spectroscopy that will enable chemical and cellular composition discrimination at the micro-scale. Moreover, additional technologies are being applied to biological systems, including multi-photon light-sheet confocal microscopy. With these significant advancements in the biofilm field coming in a relatively short period of time, the Center for Biofilm Engineering and other centers (and researchers) around the world will continue to be interdisciplinary hubs assembling the expertise of materials scientists, microbiologists, chemists, engineers, bioinformaticists, and physicists.

**Education is central to CBE’s mission**

The CBE has trained more than 1100 students since 1990, and in 2018 we had 60 undergraduates and 54 graduate students from 10 departments at Montana State University working in our labs. Our students continue to contribute to major advancements toward combating harmful biofilms, as well as finding ways to exploit beneficial ones and to have the opportunity to present their research during our annual Montana Biofilm Meeting that brings together scientists, industrial representatives, and regulatory officials. Importantly, these students get to put into practice the basic science and engineering fundamentals learned in class. Also, crucial to the applied aspect of their work, interactions with industry provide needed context to integrate education and application.

This work is done with the goal of having a global impact. Since its inception, the CBE has become an international hub for biofilm-related research, industrial development, and education, thereby providing the intellectual matrix for cross-disciplinary teams to advance fundamental and applied knowledge. A guiding principle has been the development of a systems approach to understand, control, and commercially exploit microbial, chemical, and biochemical processes. The CBE does this
through an organizational structure that offers decentralized control while promoting distributed problem-solving and multiplicity of interactions, which benefits CBE researchers and students alike. Ultimately, the students learn in an environment that is powered by self-organization and cumulative cooperation that results in diversity of knowledge all with the unique perspectives and insights from industry. We aim to further promote these attributes in the application of training to broader impacts for academic and non-academic STEM careers alike.

Industrial associate program ensures research has practical applications

Part of what makes the CBE unique is its success in an area that other research centers have struggled—establishing partnerships with industry. Not only does this relationship provide funding for biofilm research, but industry input ensures a portion of our independent research focuses directly on real-world problems facing industries as well as national infrastructure and human health. The CBE has had 122 companies participate in our membership-based “Industrial Associates” program, including Fortune 500 companies representing consumer products, pharmaceutical, energy, medical device and biocide manufacturing. Testing laboratories and government labs also actively engage CBE researchers and support research on the latest in biofilm research, technology and analytical methods. Companies support the CBE because knowledge on growing, exploiting and controlling biofilm increases the market potential of their products. These companies understand that their success depends on scientifically validated, unbiased standard methods that enable the development, testing and regulatory registration of antibiofilm technologies. These companies recognize that a neutral third party that has no interest in marketing antibiofilm products must develop the testing methods. This ensures the effort is focused on providing scientifically based methods to mitigate a public health risk. Unbiased, reproducible standard methods are the tools industry and regulatory bodies need for making informed decisions on moving technologies forward.

Looking forward, insight into the fundamentals of biofilm biology and chemistry will be far-reaching—especially given the ubiquity of biofilms and the important impact on health and environment. As we learn and appreciate more the complexity in biofilm systems and how interacting microbial communities are selected to maximize resource processing and allocation, we seek the ability to promote and utilize designed biofilms with specific functions and outcomes. Novel and new techniques are needed to provide insight into the internal and external biochemical environments that promote differentiated, localized, and shared metabolisms in biofilm systems, and the new fundamental understanding can motivate innovation. Case in point, despite great efforts and some advances, little is still known about cohesion mechanisms of biofilms and how the decision to grow at interfaces is mediated or modulated beyond a handful of chosen microorganisms and systems. Moving forward, biofilm researchers will no doubt expand our current knowledge of biofilm science with phenotypically and physiologically diverse organisms and develop multi-domain biofilm models that more closely emulate naturally occurring systems. The CBE is excited to continue on-going work and developing new areas in fundamental and applied biofilm science and engineering.

COI statement

The authors do not have conflicts of interest to report.

CRediT authorship contribution statement

Matthew W. Fields: Conceptualization, Writing - review & editing.
Paul Sturman: Conceptualization, Writing - review & editing.
Skip Anderson: Conceptualization, Writing - review & editing.

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