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Practicing environmental biotechnology

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Abstract: Environmental biotechnology involves "managing microbial communities to provide services to society". Its success comes from partnering with prokaryotic microorganisms, whose wide-ranging metabolic capabilities can be harnessed to destroy pollutants and to generate renewable materials. Partnering with microorganisms requires that we understand them well, and important advances in molecular microbial ecology, analytical chemistry, and mathematical modeling are making it possible to look inside the black box of microbial communities. Also crucial is translating the understanding to biotechnological processes that "work for the microorganisms so that they work for us". Successful translation demands novel reactor designs, application of advanced materials, and partnering with practitioners and users. The Swette Center for Environmental Biotechnology, founded in at Arizona State University in 2005, brings together the science and engineering tools in an inter-disciplinary environment. The Center emphasizes teamwork and collaborations with research and practice partners around the world. Three new technologies illustrate how the Center applies these principles to "work for the microorganisms": the H_2-based membrane biofilm reactor (MBfR) for reducing many oxidized contaminants in water, the microbial electrochemical cells (MXCs) for converting organic wastes into renewable products, and Intimately Coupled PhotoBioCatalysis (ICPBC) to detoxify very difficult to biodegrade organic pollutants.

Key words: environmental biotechnology; biodegradation; renewable energy; microbial ecology
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1 Introduction

I define Environmental Biotechnology as "managing microbial communities to provide services to society"[1]. The services usually target improving environmental sustainability: cleaning up contaminated water or soils or producing renewable resources. Long-standing applications for environmental biotechnology include treating wastewater to remove BOD using the activated sludge process, removing nitrogen from wastewater by nitrification and denitrification, and stabilizing wastewater-treatment sludge by anaerobic digestion to produce methane gas [2]. In the past 20 years or so, many exciting new applications have arisen in environmental biotechnology. Examples include detoxifying a range of hazardous chemicals, making wastewater treatment much

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more energy efficient, and producing a wide range of high-value, renewable products from organic matter in wastewaters.

1.1 Small and Simple

Environmental biotechnology is able to take on such a wide and expanding arrayof services because it takes advantage of the metabolic activity of prokaryotic microorganisms, i. e., Bacteria and Archaea. Prokaryotes are small and simple compared to eukaryotic organisms, which range from algae to plants to animals. Being small and simple, prokaryoteshave low "metabolic overhead". Thus, prokaryotes are able to exploit almost any ecological niche that can provide them some energy to grow and sustain themselves. In contrast, mammals can gain energy only by oxidizing highly energetic organic molecules (e. g., carbohydrates) and respiring the electrons to the most favorable electron acceptor (O_2). While plants oxidize H_2O and fix CO_2, they require the input of copious energy from the sun.

Due to low overhead, prokaryotes are able to catabolize combinations of electron acceptors and electron donors that yield very small amounts of energy. Important examples are fermentation of acetate to form CH_4 and CO_2 (acetoclastic methanogens), oxidation of H_2 with reduction of CO_2 go CH_4 (H_2 - oxidizing methanogens), oxidation of acetate with reduction of SO_4^{2-} (heterotrophic sulfate reducers), and oxidation of acetate with respiration to an anode poised at a low potential (anode-respiring bacteria) [2-3].

1.2 Needs pull, science push

Environmental biotechnologyhas blossomed as a field in the past 20 years or so. Why has this happened? The answer is the convergence of a strong "needs pull" with a robust "science push".

Over the past few decades, human society has discovered many new environmental challenges that demand technological advancements to reduce risks and improve sustainability. This is the needs pull. Fortunately, many of the emerging challenges can be addressed effectively by technologies founded on environmental biotechnology. Examples that have become commercial successes include reductive dehalogenation of solvents such as trichloroethene (TCE) [4-5], anammox bacteria to remove nitrogen at lower energy cost [6], and direct delivery of H_2 to bacteria reduce a range of oxidized contaminants [7-8]. Many other exciting applications are at various stages of research and development. The benefits of these environmental biotechnologies include relatively low cost and simple operation, using no hazardous inputs, and producing no hazardous outputs.

In parallel, environmental biotechnology has benefited from a strong science push that began in the 1980s and really "took off" in the past two decades. The core of the science push is from the amazing advancements in molecular microbial ecology [1,9]. Environmental biotechnologists were able to adapt the rapidly advancing tools of molecular biology to address pressing questions that had been unanswerable. These are the core questions of microbial ecology: What microorganisms are present? What metabolisms can they do? What metabolisms are they doing? How do they interact with each other and their environment? Today, researchers and practitioners of environmental biotechnology can look inside the "black box" of microbial communities, understand who are the crucial microorganisms, and begin to figure out what each type is doing and why it is important. I call this being able to "think like the microorganisms", the first step towards being able to manage them to provide desired services.

The environmental biotechnologist needs more than clever tools of molecular biology to think like the microorganisms. Advancing alongside molecular microbial ecology has been analytical chemistry able to provide deeper and more precise tracking of what the microorganisms are consuming and producing. Traditional measures remain as valuable as ever and should never be ignored: e. g., chemical oxygen demand, dissolve organic carbon, nitrogen species, alkalinity, and pH. At the same time, advanced methods of gas and liquid chromatog-

raphy, mass spectrometry, X-ray analysis, and more make it possible to dissect the details of metabolic pathways and materials produced by the microorganisms.

The vast amount of information from old and new methods has to be integrated if the environmental biotechnologist isto have an accurate picture of how the microbial community works. The ultimate tool for integrating the microbiological, chemical, and transport processes in a microbiological system is mathematical modeling. More powerful computers and more convenient modeling software have promoted more sophisticated models of environmental biotechnologies [10-12]. They can provide 1-, 2-, or 3-dimensional representations of complex microbiological systems, such as biofilms and granules with multiple species. Linking model outputs to visualization tools makes it possible to be "inside" microbial communities as they evolve in time and space. These powerful models, developed in the past ten years or so, open up a window on how microorganisms "think". However, good modeling does not require a high-powered 3-dimensional simulation with Disney-like animation. Often the most valuable modeling is a simple mass-balance model that makes an accurate accounting of what is produced and consumed by a microorganism or a community. Basic stoichiometry is the most valuable tool in the toolkit of the environmental biotechnologist, and it can be carried out with a simple spreadsheet.

1.3 Working for the microorganisms

Finally, a successful environmental biotechnology - one that actually provides the service to society - must go well beyond "thinking". The understanding achieved with the powerful research tools must be brought to practice. This means creating an engineered process that "works for the microorganisms so that they work for us". The process must be technically reliable, safe to operate, and cost effective. Achieving this for the challenging new applications means designing novel process configurations and using advanced materials. It is fortunate that materials science is generating new materials that can work well with microorganisms. It is up to the environmental biotechnologist to develop novel processes that take advantage of new materials, allowing the microorganisms to provide the service we want and that they want to do, too.

2 The Swette Center for Environmental Biotechnology

Research and development in environmental biotechnology demands a special working environment. I was fortunate to have the opportunity to create such an environment when I joined the Biodesign Institute at Arizona State University in January 2005. The Biodesign Institute (www.biodesign.asu.edu) had just been established as a location to foster biology-based, use-inspired research, and I was asked to build a research center that was inter-disciplinary and able to link fundamental research with its practical application. Over its ~9-year lifetime, the Swette Center for Environmental Biotechnology has become a living organism dedicated to "thinking like the microorganisms" (the fundamental research) so that it can create processes that "work for the microorganisms so that they work for us" (the practical applications).

Today, the Swette Center (www.biodesign.asu.edu/research/research-centers/swette-center-for-environmental-biotechnology/) has 5 professors and about 60 researchers. Besides me, the other professors are Drs. Rosa Krajmalnik-Brown, César Torres, Hinsby Cadillo-Quiroz, and Andrew Marcus. The researchers include PhD students in seven academic programs, post-doctoral associates, research scientists, visiting scientists, and under-graduate and high-school interns. The Center has over 800 m^2 of high-quality laboratory and office space, extensive instrumentation for chemical and microbiological analyses, and specialized research space and equipment. The Center is "named" after Mr. Brian Swette, an alumnus of Arizona State University who provided a substantial gift to support the university's mission to promote sustainability.

The key to the Center's success is its culture, which features being inter-disciplinary and making partner-

ships. Almost every project in the Center marries molecular microbial ecology, chemistry, and modeling. The Center has inter-disciplinary teams in which multiple researchers work together so that the productivity of each project and each person is multiplied. And, the partnering extends far beyond the research staff. The Center has collaborations with many universities and private companies around the world. The partnerships with private companies are especially important, because these partners allow us to get our technologies into practice much faster. Of course, we also have all of our microbial partners: the ideal "win-win" situation where "we work for them so that they work for us".

3 Examples of environmental biotechnologies

I provide three examples of environmental biotechnologies in the Center. Each of them illustrates the ways that needs pull, science push, and novel reactor designs come together to create the process that works for the microorganisms who happily work for us.

3.1 Membrane biofilm reactor

The membrane biofilm reactor (MBfR) targets the broad class of oxidized contaminants in water. Prime examples are nitrate(NO_3^-), perchlorate (ClO_4^-), selenite (SeO_4^{2-}), chromate ($Cr_2O_4^{2-}$), bromate (BrO_3^-), TCE (C_2HCl_3), and chloroform ($CHCl_3$). Except for nitrate, all are relatively new contaminants for which no satisfactory technology is established. The goal is to have bacteria reduce them to innocuous products, such as N_2 gas, Cl^- ion, Se^0 solid, $Cr(OH)_3$ solid, Br^- ion, ethane, or methane. The oxidized contaminant is the bacteria's electron acceptor, which allows them to grow as long as a suitable electron donor is supplied.

The technological breakthrough of the MBfR is that it is a simple and efficient means to deliver H_2 gas as the electron donor [7-8]. H_2 is the ideal electron donor because it is relatively inexpensive, is non-toxic, and works for all of the oxidized contaminants. Before the MBfR, using H_2 as the donor was technically intractable, because H_2 has very low water solubility (only about 1.6 mg/L in equilibrium with 101.325 kPa of H_2). The MBfR overcomes this limitation by delivering H_2 directly to a biofilm that adheres to the outer wall of hollow-fiber gas-transfer membranes. H_2 is supplied to the interior of the non-porous membranes, diffuses through the wall, and is then consumed by the biofilm. The oxidized contaminants diffuse into the biofilm from the water side of the biofilm. Thus, the biofilm is the meeting place for the bacteria's electron donor and electron acceptor. This creates an ideal habitat for H_2-oxidizing bacteria - a perfect example of "working for the microorganisms".

The MBfR has been extensively studied at the bench and pilot scales, and Center researchers have published more than 50 peer-review papers on its performance and the mechanisms underlying the performance. One of the Center's partners for the MBfR is APTwater (Long Beach, CA), which has commercialized the MBfR undertheir brand name ARoXXXX. ARo stands for Autotrophic Reduction of, since the H_2-oxidizing bacteria are autotrophs, or use CO_2 (not organic compounds) as their carbon source. XXXX stands for the particular application, such as NITE to reduce nitrate and PERC to reduce perchlorate. A strategic partner is Teijin, Ltd (Tokyo), which produces custom hollow fibers that are tailored to meet H_2-delivery, durability, and cost targets. The Center also has partnered with leading environmental consulting firms to pilot-test the MBfR: e. g., Montgomery-Watson-Harza, CH2M-HILL, and CDM-Smith.

3.2 Microbial electrochemical cells

Microbial electrochemical cells (denoted MXC, which is explained below) address the pressing need to generate renewable energy and chemical feedstock. High-strength organic wastes embody a large amount of renewable energy, but it is in a non-usable form. Examples are animal manures, wastewater from the food and

beverage industries, and sewage-treatment sludge. Today, it is possible to convert some of the energy value of these waste streams to methane gas (CH_4) , but this approach has serious limitations. Perhaps the biggest limitation is that the output – CH_4 gas – does not have high economic value.

The MXC is platform technology that takes advantage of the recent discovery that certain bacteria are able to oxidize certain organic compounds and then respire the electrons to an anode using extracellular electron transport [13] . The bacteria are called anode-respiring bacteria (ARB) , and they naturally form electrically conductive biofilms on the outside of anodes made of conductive materials, such as graphite. Controlling the anode potential makes it possible to select for very efficient ARB that transmit the electrons to the anode with no loss of electrical potential [14] . The electrons that enter the anode are conducted to a cathode, where they can be used to reduce a range of different electron acceptors.

Selecting the reduction reaction at the cathode determines how the energy value of electrons in the organic substrate is captured as a renewable product. If O_2 is reduced to H_2O, then the energy in the electrons is captured as electrical power through the current going from low potential at the anode to high potential at the cathode. This is called a microbial fuel cell, or MFC. If H_2O is reduced to H_2 gas at the cathode, the energy value is captured in the H_2. This option is a microbial electrolysis cell, or MEC. Valuable chemicals also can be produced at the cathode. A well-documented example is partial reduction of H_2O to hydrogen peroxide (H_2O_2) , which is the main product, although a small amount of electrical power is possible [15-16] . It also is possible, at least in principle, to produce organic products that can be used for fuel or the chemical industry [17] . And, services, such as desalination of seawater and reduction of oxidized contaminants also are possible [18] ; this is a microbial desalination cell, or MDC. The meaning of the MXC acronym should now be clear: All are electrochemical cells (the C) catalyzed by microbes (the M) , but can perform different functions indicated by the middle letter (the variable X) .

In the Swette Center, our MXC team works on fundamentals: e. g. , the physiology of ARB [19-20] , the ecology of biofilm anodes [21-23] , kinetics and modeling of the biofilm anode [12,24-26] , and cathode kinetics [27] . We also are active in scaling up MXCs towards pilot studies and then commercialization. To accelerate this activity, the Center "spun out" a start up company, Arbsource (www. arbsource. us) . Arbsource and the Center also partner with CDM-Smith, Honeywell, Dow, and Aquatech on various aspects of MXC materials and design.

3.3 Intimately coupled PhoBioCatalysis

One of the large, unsolved challenges of environmental technology is treatment of the hazardous and poorly biodegradable organic contaminants from many industries. Industry examples include paints and dyes, pesticides, pharmaceuticals, petrochemicals, pulp and paper, and munitions. Most of the recalcitrant organics are comprised of complex chemical structures that contain rings andsubstitutions that make attack by bacterial enzymes difficult and slow, if not impossible. One means to make these complex, recalcitrant organics biodegradable is to treat them with advanced oxidation, which uses free radicals to attack the chemical structures by breaking rings and adding hydroxyl (– OH) groups. Both features make the molecules biodegradable. Advanced oxidation works well in this situation because the free radicals act powerfully and indiscriminately [28] .

The most obvious way to use advanced oxidation is as a pre-treatment before biodegradation. While this method has shown some success, it has serious drawbacks that have prevented it from being widely adopted. The drawbacks stem from its advantages: powerful and indiscriminate attack. When used as a pre-treatment, advanced oxidation produces a range of products that may be oxidized more than necessary (wasting expensive oxidant and energy) , toxic to the microorganisms, and even less bioavailable. A better approach would be to have advanced oxidation do as little attack as possible – just enough to make the compounds bioavailable and

no more.

Intimately Coupled PhotoBioCatalysis (ICPBC) is an approach that achieves the desired goal. It does this by having advanced oxidation and biodegradation occur in the same reactor [29]. At first glance, this seems to be an impossible goal, because the components involved in advanced oxidation are used to kill microorganisms: e. g., free radicals and UV light. ICPBC works, however, by protecting the microorganisms from the radicals and UV light using specially designed reactors that allow the advanced oxidation and biodegradation to occur simultaneously in the same reactor: i. e., intimately coupled.

We demonstrated the concept of ICPBC in the photocatalytic circulating bed biofilm reactor, or PCBBR [29]. In the PCBBR, the bacteria that biodegrade the products of advanced oxidation grow as a biofilm on the interior of macroporous carriers that circulate in an air-lift reactor. The advanced oxidation reactions, photocatalyzed by TiO_2 and UV light, occur at the outer surface of the carriers or in the bulk liquid. The biodegradable products then diffuse into the carriers and are biodegraded by bacteria that are not exposed to UV light or free radicals, which cannot penetrate inside the carriers. The PCBBR is patented [30] and has been demonstrated for chlorinated phenolics, reactive dyes, and dinitrophenol [29,31-33]. We have partnered with Samsung, who supplied special macroporous carriers that work well in the PCBBR.

Another important partnership for ICPBC is with Professor Zhang Yongming of Shanghai Normal University. After spending a year working on ICPBC as a visiting scientist in the Center, Prof. Zhang initiated research on two other variations of ICPBC. They are theintegrated photocatalytic-biological reactor (IPCBR) and the internal loop photocatalytic biofilm reactor (ILPBR). In both cases, water is rapidly circulated between an advanced-oxidation zone that can be illuminated with UV light (with or without a TiO_2 photocatalyst) and a biofilm biodegradation zone. Professor Zhang's team has documented intimate coupling for sulfamethoxazole, phenol, chlorinated phenolics, quinoline, and N-containing natural organic matter [34-38].

4 Conclusions

Environmental biotechnology is a rapidly advancing field that has almost unlimited promise for addressing many of society's emerging environmental challenges. The most important key is partnering with microorganisms, whose wide-ranging metabolic capabilities can be harnessed to destroy pollutants and to generate renewable materials. Partnering with microorganisms requires that we understand them well, and important advances in molecular microbial ecology, analytical chemistry, and mathematical modeling are making it possible to look inside the black box of microbial communities. The next key is translating the understanding to biotechnological processes that "work for the microorganisms so that they work for us". Successful translation demands novel reactor designs, application of advanced materials, and partnering with practitioners and users. The Swette Center for Environmental Biotechnology was founded in 2005 to bring together the science and engineering tools, as well as the partnerships with microorganisms and many scientific and practice collaborators. The value MBfR, MXCs, and ICPBC exemplify the success of this approach.

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环境生物技术的实践

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摘 要: 环境生物技术所从事的是“使微生物群落能为人类社会提供服务”,它的成功来自与原核微生物的协同作用,其广泛的代谢能力可以被利用来破坏污染物的结构并产生可再生材料. 要想发挥微生物的作用,需要通过微生物分子生态学、分析化学和数学建模等学科的发展去很好地理解它,从而看清微生物黑箱模型内部的本质. 同样重要的是将对生物技术的理解转化为“利用好微生物以使他们为我们工作”. 这种转化需要新颖的反应器设计,先进材料的利用,以及与用户的良好合作. 成立于 2005 年的亚利桑那州立大学 Swette 环境生物技术中心,就是将相关的科学与工程领域中的多种方法和手段汇集在一起,形成了一种跨学科的研究中心. 该中心强调团队协作精神,并与世界各地的研究者和实践者进行合作. 介绍了 3 个新的技术: 用于消减水中许多氧化性污染物的 H_2 基膜生物膜反应器(MBfR);用于有机污染物转化为可再能源的微生物电化学电池(MXCs);以及用于加速难生物降解有机污染物脱毒的光催化与生物降解紧密结合的光-生物催化技术说明了中心是如何将基本原理应用于“利用好微生物”的.

关键词: 环境生物技术; 生物降解; 可再生能源; 微生物生态

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