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Microbial ecology to manage processes in environmental biotechnology

Bruce E. Rittmann

Professor and Director, Center for Environmental Biotechnology, Biodesign Institute at Arizona State University, PO Box 875701, Tempe, AZ 85287-5701, USA

Microbial ecology and environmental biotechnology are inherently tied to each other. The concepts and tools of microbial ecology are the basis for managing processes in environmental biotechnology; and these processes provide interesting ecosystems to advance the concepts and tools of microbial ecology. Revolutionary advancements in molecular tools to understand the structure and function of microbial communities are bolstering the power of microbial ecology. A push from advances in modern materials along with a pull from a societal need to become more sustainable is enabling environmental biotechnology to create novel processes. How do these two fields work together? Five principles illuminate the way: (i) aim for big benefits; (ii) develop and apply more powerful tools to understand microbial communities; (iii) follow the electrons; (iv) retain slow-growing biomass; and (v) integrate, integrate, integrate.

Introduction

Microbial ecology and environmental biotechnology are inherently tied to each other: microbial ecology provides the scientific foundation for the processes used to achieve the practical goals of environmental biotechnology, and processes in environmental biotechnology provide interesting ecosystems for microbial ecologists to study and advance their concepts and methods. Ultimately, environmental biotechnologists apply the concepts and tools of microbial ecology to manage their processes. But what are these two disciplines? How do they work together to enhance the well-being of society and the quality of the environment?

Providing services with environmental biotechnology

Microbial communities are self-organizing and self-sustaining assemblages of different microorganisms. When properly managed in an environmental biotechnological milieu, microbial communities provide a wide range of services, including those listed below, reliably, continuously, and economically:

- the detoxification of contaminated water, wastewater, sludge, sediment, or soil;
- the capture of renewable resources, particularly energy and water;
- sensing contaminants or pathogens in the environment; and
- protecting the public from dangerous exposure to pathogens.

Thus, environmental biotechnology can be defined as 'managing microbial communities to provide services to society.' It is particularly suited to such tasks because it is practical at large scales, it operates reliably for continuous use, it is economical to build and operate, and it is relatively simple to manage, being largely self-controlling and requiring only modest human intervention.

Processes relying on microbial communities have been around for nearly a century [1–3]. Figure 1 illustrates two long-standing processes – activated-sludge treatment of wastewater and anaerobic digestion of sludge – that came into widespread and successful use long before their microbiological bases were understood. This fact underscores the requirement for processes in environmental biotechnology to be simple to operate and largely self-controlling; however, vast improvements have become possible because the microbiological and ecological principles were recognized. A wonderful example is biological nutrient removal (BNR), in which cycling the microbial community through a series of aerobic, anoxic, and anaerobic stages enables complete removal of nitrogen (N) and phosphorus (P) from wastewater [1,2]. The different stages are used to select for three distinct groups of bacteria that are able to oxidize NH_3–N to NO_3–N, reduce NO_3–N to N_2 gas, and store extra PO_4–P, respectively.

Modern materials facilitate the development of new and better processes. For instance, during the 1970s the availability of lightweight, high-strength plastics made biological towers possible [1–3]: biological towers were the first biofilm processes with high surface areas and small footprints. The 1980s and 1990s brought lightweight biofilm carriers in the form of gravel-sized pellets, making even more compact 'high-rate processes' possible [1,2]. Today, the use of micro-filtration membranes is converting activated sludge systems (Figure 1) to membrane bioreactors (MBRs; Figure 2a), which improve effluent quality and are more reliable and compact [4,5]. Recently, the use of the membrane biofilm reactor (MBfR; Figure 2b,c) enables us to exploit the multiple advantages of using hydrogen gas as an electron donor to reduce nitrate, perchlorate, and a large range of oxidized contaminants in drinking water, ground water, and wastewater [6–9].

An important corollary to the advances in materials is that the microorganisms often live in close contact with

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Corresponding author: Rittmann, B.E. (Rittmann@asu.edu).

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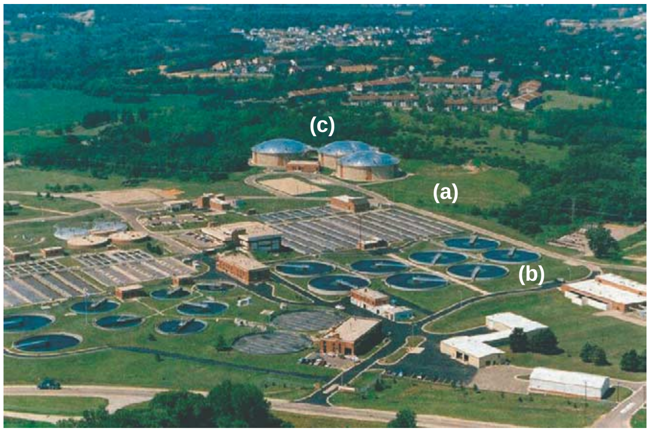

Figure 1. Traditional biological processes used in full-scale wastewater treatment. The wastewater is treated, biologically, in a battery of aeration basins **(a)** linked to another battery of settling tanks **(b)**. Aerobic biomass is retained in activated sludge by taking advantage of the natural aggregation of bacteria into flocs, which settle out in the quiescent settling tanks. These settled flocs are recycled to the aeration tanks, where the aeration system provides the electron acceptor (O_2), ensuring that the electron donors, the oxygen-demanding pollutants, are removed at a rapid rate. Excess biomass produced in activated sludge is sent to the aerobic digestors **(c)**, where strictly anaerobic conditions are imposed to force the fermentation of the organic matter in the sludge to CH_4, a valuable energy product. Photograph courtesy of George Sprouse. Reproduced with permission.

the materials as biofilms. Biofilms self-assemble to provide the community with optimal access to substrates in addition to protecting them from a variety of environmental insults, such as toxicity, predation, desiccation, and wash out. Biofilms also establish gradients of substrates, creating specialized niches where microorganisms with different and seemingly incompatible metabolic functions can co-exist in the same biofilm. Obvious examples are the high-rate biofilm processes for removal of organic and nitrogenous pollutants, anaerobic biofilm processes for ammonia oxidation, and the hydrogen-based MBfR for reducing several oxidized contaminants at the same time. An exciting and contemporary example is the microbial fuel cell (MFC), which can be used to generate electricity from organics: the bacteria live attached to an anode and transfer electrons to the anode instead of directly to a soluble electron acceptor [10–12]. Figure 3 illustrates how the biofilm works in an MFC.

The science core of microbial ecology

The foundation of environmental biotechnology is microbial ecology, a long-standing scientific discipline that aims to understand microbial communities and how communities interact with their environment [1,13,14]. The science of microbial ecology addresses four fundamental issues:

- the microorganisms present in the community – the structure of the community;
- the capabilities of the microorganisms for carrying out reactions – the phenotypic potential of the community;
- the reactions that the community members actually perform – the function of the community; and
- the inter-relationships among the members of the community and between it and their environment.

The beginnings of microbial ecology date back to around 1950 [15,16], with great conceptual advances made in the 1960s and 1970s [13,14]. Nonetheless, microbial ecology labored in relative obscurity for decades because the tools to address the four fundamental issues either did not exist or were unreliable. Since 1985, the application of tools from molecular biology began to change the prospects for microbial ecologists. Selective and rapid amplification of DNA using the polymerase chain reaction (PCR) and hybridization with DNA oligonucleotides made it possible to interrogate, directly, the genetic information of individual microorganisms and entire communities [17,18]. This can be performed in several ways: the small sub-unit rRNA (SSU rRNA) gives information on the phylogenetic identity of the microorganisms; amplifying and detecting specific genes in the chromosome catalogues the phenotypic potential [19–21]; the mRNA reveals which genes are being expressed and, therefore, which functions are occurring [22]; and microscopic visualization of RNA targets with fluorescence *in situ* hybridization (FISH) explores spatial organization [23,24].

Table 1 summarizes some of the emerging genomics tools that are becoming common in molecular microbial ecology.

A pull from societal needs

Environmental biotechnology depends on more than exciting advances in science and materials. Today, environmental biotechnology is getting a powerful pull from one of the most pressing needs of human society: sustainability. At the top of the list of sustainability needs are two resources that environmental biotechnology addresses directly: water and energy. Water providers often must tap sources whose quality is compromised and requires substantial treatment to eliminate public-health risks, taste, odor, and color. Likewise, energy will become an even more precious resource than it is today, and future consumers must shift away from fossil fuels to renewable sources. Environmental biotechnology has great potential for upgrading poor water sources and for converting renewable energy sources – particularly biomass and sunlight – to useful forms, including natural gas (or biomethane), bioethanol, biohydrogen, and bioelectricity. Table 2 illustrates how the energy and electrons in organic matter can be captured in each of the useful forms.

Principles of good management

Managing a microbial community means creating a technology that ‘works for the microorganisms, so that they work for us.’ It is the epitome of a ‘win–win’ situation. Creating a ‘win–win-technology’ requires that the knowledge gained from microbial-ecology research be translated to a practical setting. How is this accomplished? Five principles illuminate the way: (i) aim for big benefits; (ii) develop and apply more powerful tools to understand microbial communities; (iii) follow the electrons; (iv) retain slow-growing biomass; and (v) integrate, integrate, integrate.

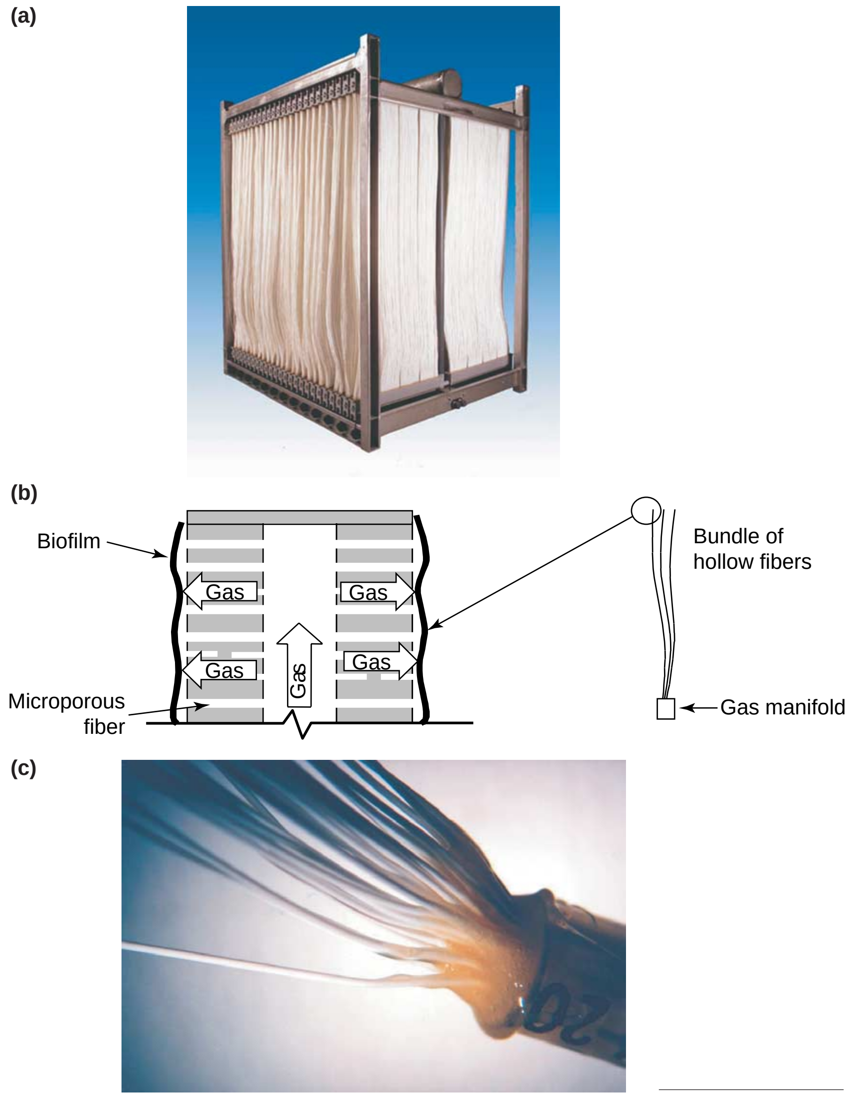

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Figure 2. Two examples of new treatment processes using membranes in totally different ways. **(a)** View of a Zenon (www.zenonenv.com) membrane module being placed in a membrane bioreactor (MBR), in which the membrane separator replaces the settling tank in activated sludge (Figure 1c). **(b)** Schematic of how H_2 gas is supplied to biofilms that reduce oxidized contaminants in a MBfR. **(c)** Picture of hollow-fiber membranes used in an MBfR.

Aim for big benefits

Environmental biotechnology should aim for the biggest benefits it can deliver to society; and the biggest benefits will come when environmental biotechnology is directed towards the most daunting challenges facing society: water and energy sustainability. Wastewaters, sludges, residues, and other 'wastes of today' must be viewed as resources that can yield renewable water, energy, and, in some cases, other materials of value. Of special note are the water and energy needs of developing countries. On the one hand, developing countries produce organic matter that can provide important forms of renewable energy if environmental biotechnology reliably transforms them into convenient energy carriers such as methane, hydrogen, and electricity (Table 2). On the other hand, uncontrolled release of the organic matter constitutes serious threats to water quality and human health. Thus, developing countries have the potential to gain a double benefit by investing in environmental biotechnologies that simultaneously capture energy value and eliminate pollution hazards.

Develop and apply more powerful tools to understand microbial communities

Although a strong science push comes from the explosion of new molecular tools (Table 1), existing tools remain inadequate for elaborating the complexity of microbial ecosytems and supporting the design and operation of innovative processes in environmental biotechnology. New molecular tools must have higher throughput, be quantitative, and focus better on revealing structure and function in parallel. To do this, the new tools need to expand beyond genomics and must exploit transcriptomics (the study of mRNA as an indicator of gene expression), proteomics (protein and/or enzyme identification, characterization, and quantification as it relates to cellular function), and metabolomics (the study of metabolic intermediates of cellular functions).

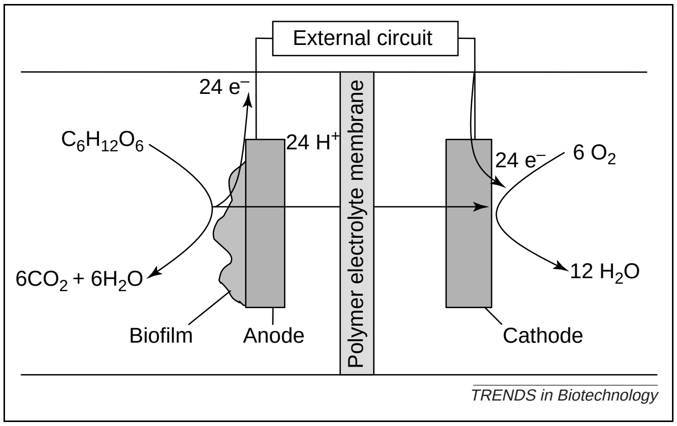

Figure 3. Principles of the MFC. The organic fuel, represented as glucose, is oxidized by bacteria living as a biofilm on the anode. The bacteria transfer the electrons to the anode, which is an electron collector. The electrons move through the electrical circuit and reduce O_2 to H_2O at the cathode. The corresponding protons (H^+) move to the cathode by another path, using a polymer electrolyte membrane in this example. The net reaction is $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ but the oxidation and reduction reactions occur at different electrodes, creating electrical energy. A major advantage of an MFC over a conventional fuel cell is that organic fuels, such as $C_6H_{12}O_6$, can be used directly. This opens up the possibility of using renewable organic materials, including wastes, directly as fuels to generate electrical energy. In a conventional fuel cell the only viable fuel is high-grade H_2, which is generated, today, almost exclusively from non-renewable fossil fuels.

Follow the electrons

Whatever the goal is, whether biodegrading pollution or capturing energy, the microbially catalyzed reactions that define the process are usually oxidations and reductions. Thus, following the electrons as they move through the microbial ecosystem is the surest way to translate knowledge about the structure and function of the microbial community into practice.

For example, the complete oxidation of glucose yields 24 electron (e^-) equivalents (eq.) (Equation 1):

$$C_6H_{12}O_6 + 12H_2O \rightarrow 6H_2CO_3 + 24H^+ + 24e^- \qquad \text{[Eqn 1]}$$

The microorganisms can send the electrons to one of several electron acceptors to gain energy. A common and familiar acceptor is oxygen (Equation 2):

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \qquad \text{[Eqn 2]}$$

The overall reaction – the sum of the electron-donating and electron-accepting half reactions – yields energy (-120 kJ/e^- eq.), which the microorganisms can use for synthesis or other energy-consuming reactions (Equation 3):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2CO_3 \qquad \text{[Eqn 3]}$$

If the electrons from glucose are transferred to methane to generate energy (by different microorganisms), the overall reaction yields much less energy for the cells (-18 kJ/e^- eq.), but produces a valuable energy carrier, biomethane (Equation 4):

$$C_6H_{12}O_6 + 3H_2O \rightarrow 3CH_4 + 3H_2CO_3 \qquad \text{[Eqn 4]}$$

Some of the electrons from the donor, in this case glucose, are invested to synthesize biomass, which is represented simply by $C_5H_7O_2N$ [2] (Equation 5):

$$5CO_2 + NH_3 + 20H^+ + 20e^- \rightarrow C_5H_7O_2N + 8H_2O \qquad \text{[Eqn 5]}$$

Thus, biomass is a storehouse for electrons. It is a particularly large storehouse for high-energy reactions, such as the aerobic oxidation of glucose, but it is a small storehouse for low-energy reactions, such as methanogenesis. In the end, the electrons and the energy value originally present in those electrons ends up somewhere; during aerobic respiration, some of it ends up in newly synthesized biomass, but most of it ends up in CH_4 during methanogenesis.

A traditional water pollutant is the biochemical oxygen demand (BOD), which is simply a misplaced electron donor. When BOD enters receiving water, bacteria oxidize it, consuming dissolved oxygen in stoichiometric proportion. Each electron equivalent removed from BOD and transferred to oxygen consumes 8 g (0.25 mole) of O_2 (Equation 6):

$$0.25O_2 + H^+ + e^- \rightarrow 0.5H_2O \qquad \text{[Eqn 6]}$$

If too much BOD is oxidized, dissolved oxygen is depleted, leading to serious water-quality problems, including odor, color, fish mortality, and mobilization of metals and nutrients.

Oxidized contaminants take an opposite role to BOD because they are electron acceptors. Removing oxidized contaminants requires transferring electrons to them in proportion to the change in oxidation state from the oxidized pollutant to a reduced form, which is harmless [8]. Two common examples are nitrate (NO_3^-; Equation 7)

Table 1. Examples of genomics tools commonly used in microbial ecology

Name	Target	Goal	Refs
Hybridization after RNA extraction	rRNA	Phylogenetic identity of individual strains or coherent groups	[17]
FISH (fluorescence *in situ* hybridization)	rRNA	Phylogenetic identity and spatial relationships of individual strains or coherent groups	[23,24]
DGGE (denaturing gradient gel electrophoresis)	Specific genes	Fingerprint of community structure (cDNA gene) or phenotypic potential (other genes)	[21]
T-RFLP (terminal restriction fragment length polymorphism)	Specific genes	Fingerprint of community structure (cDNA gene) or phenotypic potential (other genes)	[19]
Cloning and sequencing	Specific genes	Catalog of phenotypic potential for a certain type of gene	[20]
qRTm PCR (quantitative real-time polymerase chain reaction)	Specific genes	Quantification of community structure (rDNA gene) or phenotypic potential (other genes)	[26]
RT—PCR (reverse-transcription polymerase chain reaction)	mRNA	Community function according to expression of a target gene	[22]

Table 2. Analysis of the three energy-carrier outlets from organic matter[a]

Energy carrier	Standard free energy[a]	Electrical energy-conversion efficiency	Net energy yield[a]
Biomethane	−2450		−730
$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$			
$\underline{3CH_4 + 6O_2 \rightarrow 3CO_2 + 6H_2O}$		~33% (Combustion)	
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$			
Bioethanol	−2630		−870
$C_6H_{12}O_6 \rightarrow 2C_2H_5O + 2CO_2$			
$\underline{2C_2H_5O + 6O_2 \rightarrow 4CO_2 + 6H_2O}$		~33% (Combustion)	
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$			
Biohydrogen + Biomethane	−2590	~55% (Fuel cell for H_2 [25])	−1090
$C_6H_{12}O_6 \rightarrow 4H_2 + 2C_2H_4O_2 + 2CO_2 + 2H_2O$		~33% (Combustion for CH_4)	
$4H_2 + 2O_2 \rightarrow 4H_2O$			
$2C_2H_4O_2 \rightarrow 2CH_4 + 2CO_2$			
$\underline{2CH_4 + 4O_2 \rightarrow 2CO_2 + 4H_2O}$			
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$			
Biohydrogen alone	−2850		−1570
$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$			
$\underline{12H_2 + 6O_2 \rightarrow 12H_2O}$		~55% (Fuel cell [25])	
$C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$			
Bioelectricity with an MFC	−2870	~65% (Direct MFC [10])	−1870
$C_6H_{12}O_6 + 6H_2O \rightarrow 24H^+ + 24e^- + 6CO_2$			
$\underline{24H+ \;\; + 24e- \;\; + 6O_2 \rightarrow 12H_2O}$			
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$			

[a] Organic matter is represented as Glucose ($C_6H_{12}O_6$). Standard free energy and net energy yield are in kJ/mol glucose. The analysis shows that the overall reaction (the last reaction for each energy carrier) is the same in each case. However, routing the electrons to methane (CH_4), ethanol (C_2H_5O), hydrogen (H_2), or directly to electricity in a microbial fuel cell (MFC) makes a noticeable difference in the net energy yield, mainly because the energies of energy capture differ.

and perchlorate (ClO_4^-; Equation 8):

$$NO_3^- + 6H^+ + 5e^- \rightarrow 0.5N_2 + 3H_2O \qquad \text{[Eqn 7]}$$

$$ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O \qquad \text{[Eqn 8]}$$

The key to detoxifying oxidized contaminants is supplying a bioavailable electron donor, and hydrogen is the most universal donor for microbial reductions [6–8].

Because electrons carry the energy value of organic matter, transferring them from complex organic matter to CH_4 is a classic means for recovering the energy value of BOD. Each 64 g of BOD contains eight electron equivalents, which is the requirement to generate one mole of CH_4. Combusting CH_4 to generate electricity captures ~33% of the energy content of the original BOD.

Routing the electrons to bioethanol or biohydrogen is of great interest: the bioethanol can be used as a gasoline fuel additive to reduce emissions that cause smog, and the biohydrogen can be used to power a conventional fuel cell [25] – 64 g BOD can generate four moles of hydrogen because each hydrogen molecule has two electron equivalents (Equation 9):

$$H_2 \rightarrow 2H^+ + 2e^- \qquad \text{[Eqn 9]}$$

A conventional fuel cell can capture up to 55% of the energy content of the BOD, which is routed first to hydrogen and then to the anode [25].

The newest option for energy capture from BOD is the microbial fuel cell, which offers the potential to generate electricity directly from the oxidation of organic compounds, provided the bacteria transfer the electrons to an anode and not directly to a traditional electron acceptor [10,11,25]. Table 2, which compares the net electrical-energy yield from the various energy outlets, shows that the fuel cell options have the potential to capture the greatest energy value of BOD because they avoid combustion. They also avoid the air pollution that is associated with combustion. Figure 3 illustrates how the microbial fuel cell works and why it offers the greatest potential for renewable fuels.

Retain slow-growing biomass

Most processes in environmental biotechnology use specific growth rates (μ) much slower than the rates employed in a microbiology laboratory [1,2]. Aerobic heterotrophs, the fastest growing bacteria in treatment technology, in practice have a specific growth rate smaller than 0.25/day. Processes that exploit slow-growing bacteria, such as nitrifiers and methanogens, slow the specific growth rate down to less than ~0.07/day.

Why do engineers use such slow specific growth rates? Often, they have no choice. Slow growers, such as methanogens and nitrifiers, cannot grow quickly, even under optimal conditions. For example, the maximum specific growth rate of a methanogen or nitrifier is only around 1/day [1]. Furthermore, reliability and stability demand that the actual growth rate is substantially smaller (between 5- and 100-times smaller) than the maximum possible [1,2].

One of the outcomes of using a slow growth rate is that biomass decay becomes important. Often, decay can be overlooked in batch and chemostat experiments, but it is usually dominant in 'real-world applications'. Biomass decay lowers the concentration of active biomass in the system, often making the metabolically active biomass only a small fraction (<50%) of the total dry weight [1]. This reality accentuates the need to have tools to identify and quantify metabolically active microorganisms.

Integrate, integrate, integrate

It is essential for creating successful processes in environmental biotechnology to integrate knowledge of the microbial community with several other factors:

- Modern materials – membranes, ceramics, semi-conductors, conductors, nano-materials – enable us to create technological 'niches' that work for the microorganism, so that they work for us. In many cases, the materials used provide special 'biofilm niches', the ultimate integration of the microbial community with the material.
- Modeling and other forms of quantification (e.g. follow the electrons) link microbiological understanding with mass transport, kinetics, and other engineering principles.
- Outstanding engineering creates systems that are reliable and cost-effective.

In the long run, integration will be the most important component, because it constitutes the 'doing'step. Research and development activities need to be oriented towards integration from their inception to meet the criteria for success using environmental biotechnology.

A look to the future

A look to the future of environmental biotechnology and microbial ecology should focus on scientific advances that open new possibilities and societal needs that pull them towards practical goals. Looking first at the science side, the capabilities of molecular methods to illuminate how microbial communities function and self-assemble will continue to expand at a rapid rate. They will be faster, will generate much larger quantities of information, and will integrate information from genomics, proteomics, metabolomics, and other areas. A big challenge is knowing what to do with all the data. We must understand it using theoretical frameworks and modeling tools – approaches not common in environmental microbial ecology. On the needs side, an environmental biotechnologist must understand that we have already picked the 'low-hanging fruit' – the technologies that almost invent and run themselves. For those applications, the challenge will be to improve reliability, particularly for use in the developing world. Even more challenging, but equally as rewarding to society, will be tackling new goals, particularly those not attempted on a large scale or even any scale. This will include converting all organic wastes into renewable energy instead of pollutants that demand energy-consuming treatment; reliably biodegrading trace organic pollutants that affect the hormone systems of mammals; and preventing or remediating pollution from huge non-point sources such as fertilizer pollution in agricultural run-off or drainage. It is for these giant new challenges that we need to martial all the steps needed for success:

- Aim for the big benefits.
- Use powerful tools.
- Follow the electrons.
- Apply modern materials.
- Retain the biomass.
- Quantify.
- Apply good engineering.

References

1 Rittmann, B.E. and McCarty, P.L. (2001) *Environmental Biotechnology: Principles and Applications*, McGraw-Hill Book Co
2 Grady, C.P.L., Jr *et al.* (1999) *Biological Wastewater Treatment*. (2nd ed), Marcel Dekker, Inc.
3 Metcalf & Eddy. (1979) *Wastewater Engineering: Treatment/Disposal/Reuse*. McGraw-Hill Book Co.
4 Stephenson, T. *et al.* (2000) *Membrane Bioreactors for Wastewater Treatment*, IWA Publishing
5 Daigger, G.T. *et al.* (2005) Are membrane bioreactors ready for widespread application? *Environ. Sci. Technol.* 39, 399A–406A
6 Nerenberg, R. *et al.* (2002) Perchlorate reduction in a hydrogen-based membrane-biofilm reactor. *J. Am. Water Works Assn* 94, 103–114
7 Nerenberg, R. and Rittmann, B.E. (2004) Reduction of oxidized contaminants with a hydrogen-based, hollow-fiber membrane biofilm reactor. *Water Sci. Technol.* 49, 223–230
8 Rittmann, B.E. *et al.* (2004) The hydrogen-based hollow-fiber membrane biofilm reactor (HFMBfR) for reducing oxidized contaminants. *Water Science and Technology: Water Supply* 4, 127–133
9 Rittmann, B.E. (2006) The membrane biofilm reactor: the natural partnership of membranes and biofilm. *Water Sci. Technol.* 58, 219–225
10 Rabaey, K. *et al.* (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.* 25, 1531–1535
11 Liu, H. *et al.* (2004) Production of electricity during wastewater treatment using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* 38, 2281–2285
12 Kim, H.J. *et al.* (2002) A mediator-less microbial fuel cell using a metal-reducing bacterium, *Shewanella putrefaciens*. *Enzyme Microb. Technol.* 30, 145–152
13 Alexander, M. (1964) Biochemical ecology of soil microorganisms. *Annu. Rev. Microbiol.* 18, 217–252
14 Alexander, M. (1971) *Microbial Ecology*, John Wiley & Sons, Inc.
15 Winogradsky, S. (1949) *Microbiologie du Sol*, Masson
16 Blum, J.L. (1956) The ecology of river algae. *Botan. Rev.* 22, 291–341
17 Lane, D.J. *et al.* (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. *Proc. Natl. Acad. Sci. U. S. A.* 82, 6955–6959
18 Stahl, D.A. (1986) Evolution, ecology, and diagnosis. *Biol. Technology* 4, 623–628
19 Marsh, T. (1999) Terminal restriction fragment length polymorphism (T-RFLP): an emerging method for characterizing diversity among homologous populations of amplification products. *Curr. Opin. Microbiol.* 2, 323–327
20 Juretschko, S. *et al.* (2002) The microbial community composition of a nitrifying–denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle 16S rRNA approach. *Syst. Appl. Microbiol.* 25, 84–99
21 Muyzer, G. (1999) DGGE/TGGE, a method for identifying genes from natural ecosystems. *Curr. Opin. Microbiol.* 2, 317–322
22 Freeman, W.M. *et al.* (1999) Quantitative RT-PCR: pitfalls and potential. *Biotechniques* 26, 112–125
23 Amann, R. *et al.* (2001) The identification of microorganisms by fluorescence *in situ* hybridisation. *Curr. Opin. Biotechnol.* 12, 231–236
24 Amann, R.I. *et al.* (1990) Fluorescent oligonucleotides probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J. Bacteriol.* 172, 762–770
25 Logan, B.E. (2004) Extracting hydrogen and electricity from renewable resources. *Environ. Sci. Technol.* 38, 160A–167A
26 Mackay, I. (2004) Real-time PCR in the microbiology laboratory. *Clin. Microb. Infect.* 10, 190–212