ENVIRONMENTAL BIOTECHNOLOGY

a biosystems approach

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Environmental Biotechnology: A Biosystems Approach

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To Chloe Jayne Randall

PREFACE

Environmental biotechnology is a vital component of the scientific and engineering toolkit needed to address environmental problems. Environmental biotechnology embodies more than an explanation of the biological principles underlying environmental engineering. Environmental biotechnology depends on a systematic view of the myriad factors involved when organisms are used to solve society's problems. Thus, both the title and subtitle of this book are important.

A systems approach to biotechnology requires a modicum of understanding of a number of disciplines, especially environmental engineering, systems biology, environmental microbiology, and ecology. This book introduces all of these fields from the perspective of how to apply them to achieve desired environmental outcomes *and* how to recognize and avoid problems in such applications. This approach means that the treatment of these four disciplines is predominantly focused on biotechnology and is not meant to be an exhaustive treatise on any of the four. This book's principal value lies at the intersection of the four disciplines. However, engineering requires specifics, so my intention is that the reader gain a sufficient grasp of each so as to know when more details are needed and when to consult the references at the end of each chapter to seek out these important details.

BIOTECHNOLOGY AT THE INTERSECTION OF DISCIPLINES

Environmental engineering is a broad field, including both *abiotic* and *biotic* solutions to pollution and environmental problems. This book's primary environmental engineering focus is on the biotic solutions, so the reader should consult general environmental engineering texts and specific chemical and physical treatment resources to find abiotic treatment methods to match the biotic approaches discussed here. For example, after reading a discussion of a particular biotechnology, e.g. Chapter 7's exposition of a biofilter used to treat a specific organic pollutant, the reader may be inclined to look up that pollutant to see what other non-biotechnological methods, e.g. pumping and air sparging, have been used in its treatment. This book certainly includes discussions on abiotic techniques in Chapter 10, but limits the discussion to the treating of those pollutants that may result from biotechnologies (e.g. if a hazardous byproduct is produced, it may need to undergo thermal treatment).

Systems biology and molecular biology are addressed insofar as genetic engineering is an important part of environmental biotechnology. An understanding of genetic material and how it can be manipulated either intentionally or unintentionally is crucial to both applications and implications. As in environmental engineering, the discussion is focused less on a theoretical and comprehensive understanding of DNA and RNA for their own sake than would be found in a systems biology text. Again, if the reader needs more information, the references should be consulted and should lead to more specific information. In addition, the book addresses a number of emerging technologies used in environmental assessment, particularly drawing on systems biology, such as the computational methods associated with genomics, proteomics, and the other "omics" systems.

I recall at least one of my professors at the University of Kansas differentiating microbiologists from engineers. Microbiologists are interested in intrinsic aspects of the "bugs," whereas engineers are interested in what the "bugs" can do [1]. I have been careful with the taxonomy of the organisms, but it is not the intent to exhaustively list every microbe of value to environmental biotechnology. When the reader needs more detail on a particular organism and when trying to find other microbes that may work in a biotechnology, the references and notes should help initiate the quest.

More than a few of my ecologist colleagues may cringe when I say that microbes have instrumental value, not intrinsic value, in many environmental biotechnologies. Engineers, including environmental engineers, are focused on outcomes. They design systems to achieve target outcomes within specified ranges of tolerance and acceptability. As such, they say a bacterium is a means, not an end in itself. Ecologists tend to be more interested in the whole system, i.e. the ecosystem. Thus, the microbes, especially those that have been supercharged genetically, must be seen for how they fit within the whole system, not just the part of the system that needs to be remediated. This book, therefore, includes this ecological perspective, especially when addressing potential implications, such as *gene flow* and *biodiversity.* In fact, one of the themes of this book is that engineers must approach even "slam dunk" biotechnologies with whole systems in mind, with considerations of impact in space and time, i.e. a systems approach to biotechnology.

THE SYSTEMS APPROACH

One way to address environmental biotechnology is to ask whether it is "good" or "bad." Of course, the correct answer is that "it depends." According to my colleague at Duke, Jeff Peirce, this is one of the few universally correct statements in engineering. The tough part of such a statement, of course, is deciding to some degree of satisfaction on just what "it depends."

The same biotechnology can be good or bad. It just depends. It depends on risks versus rewards. It depends on what is valued. It depends on reliability and uncertainty of outcome. It depends on short-term versus long-term perspectives. It depends on the degree of precaution needed in a given situation. Mostly, it depends on whether the outcome is ideal, or at a minimum acceptable, based on the consideration of the myriad relationships of all of the factors. Such factors include not only the physical, chemical, biological aspects of a biotechnology, but also those related to sociological and economic considerations. That is, the same technology is good or bad, depending on the results of a systematic perspective.

I would recommend that the question about the dependencies driving the acceptability of a given environmental biotechnology be asked at the beginning of any environmental biotechnology course. I recognize just how tempting it is in teaching an environmental biotechnology course to jump into *how* to use living things to treat pollution, with little thought as to *whether* to use a biotechnology. Perhaps this is because we expect that other perspectives, such as abiotic treatment, will be addressed in courses specifically addressing these technologies, and after having completed courses in every major treatment category, the student will then be able to select the appropriate method for the contaminant at hand. This is much like the need for a really good course in concrete and another excellent course in steel, as a foundation (literally and figuratively) in structural engineering. Such reductionism has served engineering well. In environmental sciences and engineering, the newer views do not lessen the need for similar specific knowledge in the foundational sciences, but in light of the importance of the connections between living things and their surroundings, newer pedagogies are calling for a more systematic view to put these basics into systems that account for variations in complexity and scale.

Biotechnologists are justifiably tempted to keep doing that which has worked in the past. For those in the fields of biological wastewater treatment and hazardous waste biotechnologies, the art of engineering is to move thoughtfully, with some trepidation, from what is known to the realm of the unknown. This microbe was effective in treating contaminant A, so why not acclimate the microbe to a structurally similar compound, e.g. the same molecule with a methyl group or one with an additional ring? Often this works well under laboratory conditions and even in the field, so long as conditions do not change dramatically. Such acclimation was the precursor to more dramatic and invasive forms of *genetic modification*, especially recombinant DNA techniques. This book explores some of the knowns and unknowns of what happens *systematically* when we manipulate the genetic material of an organism. Perhaps, the system is no more influenced by a genetically modified organism than by those that bioengineers have manipulated by letting the organism adapt on its own to the new food source. But, perhaps not.

When I originally proposed the concept for this book, I thought that I would dedicate it almost exclusively to potential *implications* of environmental biotechnologies. I thought that others had done admirable jobs of writing about the *applications*. After delving into the topic in earnest, I came to the conclusion that I was only half right. Indeed, the previous texts in environmental biotechnologies were thorough and expansive. Some did a really good job of laying out the theory and the techniques of environmental biotechnology. However, most were not all that interested in what may go wrong or what happens outside of the specific application. This is not meant to be a criticism, since the authors state upfront that their goal is to enhance the reader's understanding of these applications. The implication, to me at least, is that their work starts after the decision has been made to destroy a certain chemical compound, using the most suitable technique. In this instance "suitable" may be translated to mean "efficient." How rapidly will microbe X degrade contaminant A? How complete is the degradation (e.g. all the way to carbon dioxide and water)? How does microbe X compare in degradation rates to microbes Y and Z? How efficiently will microbe X degrade contaminant A if we tweak its DNA? How broadly can microbe X's degradation be applied to similar compounds?

These are all extremely important questions. Efficiency is an integral but not an exclusive component of effectiveness. Thus, my original contention was half wrong. I could not discuss implications without also discussing applications. I liken this to the sage advice of a former Duke colleague, Senol Utku. He has been a leader in designing adaptive structures that often follow intricate, nonlinear relationships between energy and matter. His students were therefore often eager to jump into nonlinear mathematical solutions, but he had to pull them back to a more complete understanding of linear solutions. He would tell them that it is much like a banana. How can one understand a "non-banana" without first understanding the "banana"? Thus, my systematic treatment of environmental biotechnology requires the explanation of both applications (bananas) and implications (non-bananas).

The term "systems" has become an adjective. For decades, engineers have had systems engineering. We now have systems biology, systems medicine, and even systems chemistry. Early on, systems simply meant a comprehensive approach, such as a life cycle or critical path view. Later, another connotation was that it provided a distinction from compartmental or reductionist perspectives. Now, the systems moniker conveys a computational approach. Lately, subdivisions of the basic sciences have also become systematic in perspective. For example, systems microbiology approaches microorganisms or microbial communities comprehensively by integrating fundamental biological knowledge with genomics and other data to give an integrated representation of how a microbial cell or community operates. This text attempts to address all of these perspectives and more, but all through the lens of the environment.

Along the way, I became aware that there was not a good term that included all of these perspectives. Pioneers in environmental modeling, such as Donald MacKay and Panos Georgopoulos, advanced the field of chemodynamics. In fact, I have drawn heavily from their work. The challenge is how to insert biology into such chemodynamic frameworks.

For many in the environmental sciences and engineering fields, environmental biotechnologies that most readily come to mind are various waste treatment processes, those that often begin with the suffix "bio." Thus, I decided to use the term *biochemodynamics* to refer to the myriad bio-chemo-physical processes and mechanisms at work in environmental biotechnologies. At one point, I even suggested calling this book *Environmental Biochemodynamics*. However, while such a title would distinguish the focus away from abiotic processes, it would leave out some of the important topics covered, such as the societal and feasibility considerations needed in biotechnological decisions.

Environmental biotechnology is all about optimization, so it requires a systematic perspective, at least in its thermodynamic and comprehensive connotations. In particular, biotechnologists are keenly interested in bioremediation of existing contaminants.

To optimize, we must get the most benefit and the least risk by using biology to solve an important problem or fill a vital need. In my research, I discovered a very interesting workshop that took place in 1986 [2]. The workshop was interesting for many reasons. It was held by a regulatory agency, the US Environmental Protection Agency, but predominantly addressed ways to advance environmental biotechnology. In other words, the entity that was chastising polluters was simultaneously looking for ways to support these same polluters financially and scientifically so as to become non-polluters!

Such an approach is not uncommon in its own right, since in the previous decade the same agency had funded research and paid to build wastewater treatment plants to help the same facilities being fined and otherwise reproved for not meeting water quality guidelines and limits. This is a case of the "stick" being followed by the "carrot." The 1986 workshop was actually refreshing, since it was an effort to help scientists come up with ways to push the envelope of technology to complement the growing arsenal of rules and standards for toxic chemicals in the environment.

One of the challenges posed in the mid-1980s was that the National Academy of Sciences had just sketched a schematic to address risks posed by chemicals. It followed a physicochemical structure that consisted of identifying chemical *hazards* and seeing how people may come into contact with these hazards, i.e. *exposure*. The combination of these factors led to what the academy called *risk assessment*. This seemed to work adequately for chemical hazards to one species (*Homo sapiens*), but did not fit quite well with hazards that behave differently than pharmaceuticals, pesticides or other chemical agents, i.e. physical (e.g. UV light) or biological (e.g. microorganisms) hazards. The Academy recently has proposed new schema that may better fit biotechnological risks.

So, indeed, it was good that experts were getting together in 1986 to find new applications of biotechnology to treat and control pollution. However, it appears that even after almost a quarter century some of the challenges have not been addressed, at least not fully. Some of the concerns expressed in 1986 are no longer being expressed widely. The proceedings of the meeting state:

> Federal, State and local regulatory policies pose barriers to field-testing and thereby the development of commercial genetically engineered biotechnology products. Permitting and reporting requirements and the uncertain regulatory climate were identified as additional barriers to the development of the biotechnology control technology [3].

Other concerns persist, as evidenced when the proceedings mention that:

> The public has vague concerns about the risks that may be presented by the use of biotechnology products. The Panelists felt that the public does not usually perceive a distinction between engineered and nonengineered microorganisms and that the public does not understand the scientific basis or applications of biotechnology. These deficiencies pose a barrier to the public's ability to evaluate the issues raised by and the risks associated with biotechnology. ... The

> concerns involve the credibility and capabilities of industry and regulatory agencies to identify and assess potential risks presented by biotechnology and how risks and benefits are balanced in the decision-making process. [4]

A number of biotechnologies still have these credibility problems, most notably those related to food supplies. However, and I am not sure when it happened, at some point in time in the last few decades, environmental biotechnology passed the initial risk test. At least in the United States, there has been some tacit consensus that the environmental advantages of manipulating genetic material in microorganisms to clean up wastes override any environmental and other risks that may result from such modifications. My research did not uncover a specific declaration of this consensus, but it becomes obvious if one compares the uncertainties and questions asked in the 1980s to the research and regulatory agendas today. Interestingly, such a scientific consensus is not universal. For example, some European scientists look at genetically modified organisms (GMOs) of all types with a healthy skepticism.

At least some of the reason for less skepticism toward environmental biotechnology may be the result of the environment in which it emerged. The reader is reminded that in the early 1980s, hazardous waste sites seemed to be cropping up all over the nation. In fact, in the letter to the EPA Administration that transmitted the proceedings mentioned above, the chair, G.E. Omenn, Dean of the School of Public Health and Community Medicine at the University of Washington, stated that:

> The Nation needs alternative technologies to complement present "burn or bury" approaches to chemical pollutants ... Within the microbial treatment arena, improvements are needed, some of which might draw upon genetic engineering methods. [5] The United States does indeed continue to worry a great deal about research that involves genetic manipulation to produce medical and warfare agents, sometimes involving near relatives to the microbes being used in other biotechnologies, including remediation. In fact, the National Institutes of Health have comprehensive guidelines to address physical containment of GMOs and their genetic material. But that is addressed only at research. This begs the question of when is the introduction of genetic material no longer research. History has shown us that when something is introduced into a different, less controlled system, unexpected outcomes are almost always assured. That is, to some extent all environmental biotechnologies can be considered "research."

SEMINAR DISCUSSIONS

These uncertainties and differences in perspective led to my recognition of the need to approach all biotechnologies with a large degree of humility. So, this book includes a "seminar" at the end of each chapter. The seminar addresses a topic about which there is no consensus or where the understanding of the potential outcomes is only now emerging. The topics are those of public concern and of scientific importance. As such, there are many right and wrong answers to the questions posed at the end of each seminar. The seminars are designed for open discussion, so I recommend that a three-step process be used in the classroom or breakout group, depending on the learning environment.

First, the seminar should be read and the references consulted. Second, the students and/or discussion group members write their individual answers to the seminar questions. Third, the class/group openly shares their answers with the whole group with the facilitator ensuring each perspective is shared and with the main points written on a whiteboard or flipchart. Perhaps the major points could be grouped into natural categories (e.g. social concerns, scientific uncertainties, unacceptable risks, etc.), with each member given two votes on which are most

Table P.1 **Comparison of benefits and risk from transgenic herbicide-resistant plant**

Potential benefits	Potential risks
Simpler weed management based on fewer herbicides	Greater reliance on herbicides for weed control
Decrease in herbicide use	Increase in herbicide use
Less contamination of the ecosystem	More contamination of the water, soil, and air and shift in exposure patterns
Use of environmentally more benign herbicides	Development of resistance in weed species by introgression of the transgenes
Reduction of the need for mechanical soil treatment	Shifts in population of weeds towards more tolerant species
Less crop injury	Increase in volunteer problems in agricultural rotation systems
Improved weed control	Negative effects of herbicides on non-target species

Source: H.A. Kuiper, G.A. Kleter and M.Y. Noordam (2000). Risks of the release of transgenic herbicide-resistant plants with respect to humans, animals, and the environment. *Crop Protection* 19 (8-10): 773–778.

important. The top few problems could then be discussed with regard to possible solutions, including needed research.

For example, the United States has had a fairly strong consensus in support of many biotechnological applications in drug development, industry, and environmental cleanup, but there remains a comparative uneasiness about certain biotechnologies. In the case of food supplies, this may be recognition that the final product may find its way to our kitchen table. It may also be because agriculture systems are very complex, with many steps from seed to table, and are vulnerable to mistakes. Kuiper *et al.*, for example, indicated that humans, animals, and the environment are at some level of risk whenever a GMO, in this case an herbicide-resistant plant, is used. In fact, every decision is a balance between potential benefits and potential risks (see Table P.1) [6]. A key question is why is there a difference between such biotechnologies and the seeming lack of concern about environmental biotechnologies.

REDUCTIONISM VERSUS THE SYSTEMS APPROACH

In times of specialization among and within the sciences, we tend to sharpen our focus, which is usually a good thing. For instance, bioscientists, biotechnologists, and bioengineers often pursue and apply information that meets a particular need. We often isolate our research and interest so tightly that we cannot worry about what is going on in the rest of our own discipline, let alone other disciplines. This baby is usually only well understood by a small cadre of fellow sojourners with a common expertise in highly esoteric subject matter.

I recently discussed with a fellow "seasoned" researcher, who happens to be a world-class microbiologist, the safety and risk of using genetically modified organisms for bioremediation. We both expressed concern that some of the questions that were asked in the late 1970s were still not completely answered. As mentioned, it appears that somewhere along the way, the engineering community dropped these questions, but neither of us could find a clear point in time for such a decision.

Thus, those who apply the physical and biological sciences must decide how they go about using data, making those data into information, and, hopefully add knowledge on how this information, evidence if you will, can best be used to solve the big and mounting problems. Biotechnology provides an excellent illustration of such optimization schemes.

At one end of the spectrum is the total devotion to the application of living things to solve problems; doing whatever gets us to the levels of thermodynamic efficiency we have defined as a performance standard. This means that we can go about unchallenged in modifying genetic material, moving massive amounts of soil and water to bioreactors, and tightly controlling the conditions that give us some predefined metric for efficiency. At the other end is stifling caution that keeps us from designing and using tools based on the state-of-the-science.

Bioremediation, for example, has been greatly improved by understanding the environmental conditions and the microbial processes that lead to more efficiency degradation of some very recalcitrant compounds. As has been standard practice of biological treatment for over a century, we put the microbes to work and use their needs for carbon and energy to do things they would not do with the prodding of an engineer. This logically led to the innate and learned creativity of the bioengineer who began to ask whether we could do something to the "bugs" to make them even more efficient. This gave birth to the bioreactor (first the common tricking filters and their ilk) where we chose the right bugs from their natural habitats, observed how they broke down similar organic material, withheld their natural sources of carbon, exposed them to some new food (our wastes), and patiently and incrementally added enough of the new food so that the endogenous processes found new ways of donation and acceptance of electrons (energy).

In the process, where before a few bugs would take many days to break down such organic matter, our bioreactors could now process millions of gallons of waste per day and release effluent that met what were before thought to be unreachable standards of purity. In 1976, when I started in this business, the gold standard was 20 parts per million (ppm) total suspended solids and 20 ppm biochemical oxygen demand for effluent discharges to the waters of the United States. To my young colleagues, this is like saying that my first PC had 128 kilobytes of random access memory (which it did). These were nevertheless profoundly difficult measures of success.

The next logical step was to treat substances heretofore not considered amenable to biological treatment. The microbes rarely had to rely on these compounds as sources of carbon and energy. There simply were always enough other food sources that were easier to digest; with no need to remove chlorine or to break aromatic rings. So, some time in the late 1970s biological treatment began to emerge as a very viable hazardous waste treatment processes. But, the recalcitrance and variability of chemical composition, as well as the arrival of new DNA techniques made for a logical arranged marriage between the microbes and synthetic organic contaminants.

The need to reconcile reductionist and systematic thinking is ongoing in numerous scientific and design disciplines. For instance, there is an ongoing debate within engineering and design professions concerning the role of evidence in support of the often-stated "form follows function." The postulation is that designers must not only gather physical data, but must add social scientific information and human factors to the mix. This requires asking questions of past users (e.g. clients, patients, subjects, consumers, visitors, policy makers, taxpayers, etc.). From that, a better design will emerge.

The *bioinformatics* challenge is two-fold, however. First, how can reliable information be gathered to address the needs problem at hand? For example, in designing a genetic laboratory, how much of it follows the traditional lab needs for good lab practice (e.g. bench surface area, chemical segregation, storage of hazardous materials, hood design, clean areas, etc.) versus what is specific to the type of genetic research that will be taking place (e.g. tissue preparation, other media needs such as soil, water, and biota handling, genetic material identification apparatus, etc.)? The delta between these two paradigms according to the evidence-based designers, cannot follow the old paradigms, but needs reliable information.

The book attempts to find a balance between rigorous reductionism and the systems approach. The engineering community must avoid being overly myopic in its general acceptance of technologies and designs that work (e.g. bioremediation of oil spills using genetically modified bacteria), while being sufficiently cautious in taking a systematic view (e.g. considering the possible impacts of these modified bacteria in the whole ecosystem, including gene flow and changes in the chemical compounds in the oil that may change their affinity for certain media and compartments in the environment).

STRUCTURE AND PEDAGOGY

This book consists of 12 chapters. They have been designed to provide a primary text for two full semesters of undergraduate study (e.g. Introduction to Environmental Biotechnology; Advanced Environmental Biotechnology). It is also designed to be a resource text for a graduate level seminar in environmental biotechnology (e.g. Environmental Implications of Biotechnology).

Chapter 1 introduces the science that underpins both the applications and implications of environmental biotechnology. It provides the background and historical context of contemporary issues in biotechnology, using the environmental impact assessment process as a teaching and learning vehicle. In particular, the chapter attempts to enhance the chaotic nature of environmental outcomes, i.e. how initial conditions can lead to various outcomes as demonstrated by event and decision trees. The seminar, Antibiotic Resistance and Dual Use, expands the reader's perspectives on the science (e.g. aerosol science and biology) and societal issues associated with current environmental and security issues.

Chapter 2 addresses the various scientific principles involved in environmental biotechnologies. That is, it introduces biochemodynamics. In fact, Table 2.9 is a digest of much of the subject matter addressed in Chapters 3 through 7, so it can be a good resource for exam preparation and review. The seminar discussion, GMOs and Global Climate Change, addresses the pros and cons of whether and how genetic manipulations are a needed tool to address a major environmental problem. The seminar is the book's major discussion of algae, which are becoming increasingly important to biotechnologies.

Chapter 3 provides detailed discussion of each of the processes described in Table 2.9, i.e. the underpinning biochemodynamic processes. This is also the first place where microbial metabolism and growth are discussed in detail. Thus, Chapter 3 may be used as a standalone source to introduce the science of a graduate seminar, or for professors designing their own "coursebook" who need a chapter on the fundamentals of environmental transport and fate. However, I would strongly recommend that such a coursebook include Chapters 4 and 5, since these go into much greater detail on biotransformation and risk, respectively. The seminar topic addresses how well models can predict the transfer of genetic materials. I must admit, I have more questions than answers regarding this topic, so the questions at the end should reveal some real weaknesses in currently available models. As such, I would greatly appreciate the reader's ideas. Please email them to me at dav1@duke.edu.

Chapter 4 is a pivotal chapter. It suggests the need for a systematic perspective. Up to this point, the science being discussed can be seen from numerous perspectives, e.g. how the principles can be applied to clean up a waste site or how these same principles can be used to avoid problems in such a clean up. Chapter 4, however, imposes an onus on the reader to appreciate the chaos. That is why I begin with the lyrics from Sting's song. (My grammar checker hated this quote, incidentally, due to the double negative, but I believe it captures the peril of single-mindedness that our proposed solution is the best solution.) Too often, we exaggerate the expected benefits and ignore the potential risks and downsides of our decisions. As such, Chapter 4 draws from proven tools, e.g. the fugacity models, industrial ecology, and life cycle analysis, and extends them to biotechnologies. Such extensions require a large helping of humility. The seminar topic deals with comparisons of biological agents used for good and ill, asking questions related to when a biological cleanup is successful and whether the introduction of a species to the environment is worth the risks. The comparison of two species of *Bacillus* points to the need to ask whether genetic manipulations are sufficiently understood before introducing new strains to the environment, even for noble causes like bioremediation.

Chapter 5 introduces environmental risk assessment, especially as it relates to biotechnologies. The problem and challenge in writing this chapter is that the lion's share of risk literature addresses chemical risks, rather than biological risks. The scientific community is increasingly aware that microbial risks do not necessarily follow the traditional hazard identification/dose-response, exposure and effects cascade. However, some biotechnological risk indeed is chemical (e.g. the production of toxin). Thus, Chapter 5 introduces the basics of risk assessment (e.g. thresholds, dose-response curves, exposure assessment techniques), but also introduces nuances that may help tie environmental microbiology to environmental engineering risk concepts. The seminar addresses risk tradeoffs, especially when it comes to manipulating genetic material for environmental results.

Chapter 6 addresses ways to reduce and manage risks. In following the risk assessment discussions in Chapter 5, a number of environmental problems are considered with an eye toward ways to address them (e.g. addressing release of antibiotics and microbial resistance, destruction of endocrine disruptors). Managing risks requires an understanding of possible outcomes, so the chapter includes some expansive thinking on what could happen once a microbe enters the environment.

With the help of Drew Gronewold of the US Environmental Protection Agency, Chapter 6 includes a hypothetical scenario using Bayesian techniques. In the interest of full disclosure, one of the great frustrations in writing this book is the lack of reliable quantitative tools to predict outcomes. Unlike risk assessments in the nuclear industry, for example, few decision trees in biotechnology can produce probabilities of outcomes. This is partially because there are so many variables in the ambient environment compared to the controlled conditions of a nuclear power plant. In addition, nuclear power plants are data-rich. Everyone who is potentially exposed to radiation wears a monitoring device that records values that can be aggregated and compared to reliable radiation health effects data (e.g. cancer). In environmental studies, data are scarce and the outcomes are numerous (human health outcomes, ecosystem damage, etc.). The hypothetical scenario at least gives us an opportunity to consider the changes that could occur. Again, I welcome the reader's ideas on how useful this is and how it can be improved.

The Chapter 6 seminar addresses biomimicry. Is it universally acceptable to mimic nature, or does it introduce unexpected risks under certain conditions? The consideration of the botanical pesticides and their derivatives provides an interesting discussion of the often erroneous assumption that *natural* means *safe*. After all, some of the most toxic substances are natural, e.g. the botulinum toxin and aflatoxins. In addition, many of the pyrethroids have been altered chemically so as not to resemble the original botanical.

Chapter 7 most closely resembles traditional environmental biotechnology texts. It is mainly devoted to the application of microbial systems to clean up pollution. Thus, it can be extracted in its entirety for professors and facilitators needing a summary of biological treatment mechanisms and processes. The seminar discussion addresses a currently important topic: how can the disciplines of environmental microbiology be reconciled with bioremediation? In particular, the seminar goes into detail on previous attempts at providing semi-quantitative tools to predict important factors like biodegradation rates. This is a currently important topic, since regulatory agencies around the world are looking for better ways to predict environmental harm *before* a chemical reaches the marketplace. In fact, it appears that the Toxic Substances Control Act may soon be amended to improve such risk prioritization.

Chapter 8 is the mirror image of Chapter 7, as it presents the implications of environmental biotechnologies. The chapter recognizes the value of those applications considered in

Chapter 7, but encourages systematic thinking that must include proactive measures to prevent negative impacts. The seminar discussion addresses the scary problem of long-term transport of microbes and their possible impacts on coral reefs. I chose this seminar for two major reasons. First, coral reefs are complex biological systems that demonstrate how a slight change can substantially alter their condition. Second, the case demonstrates a global scale transport associated with a micro-scale problem. Thus, it is an ideal "teachable moment" to consider scale and complexity involved in a real-world environmental problem.

Chapter 9 is arguably the most expansive part of the book. It addresses the environmental implications of all non-environmental biotechnologies. In fact, many concerns remain about industrial, medical, and especially agricultural biotechnologies. In addition, considering the specific environmental impacts of the technologies, they also provided some lessons for environmental biotechnologists (see for example the discussion box on Hormonally Active Agents, and the case discussion, King Corn or Frankencorn). Also, the discussion of enzymes ties very closely to environmental bioreactors. The seminar topic on vaccines is particularly timely at this writing, since the H1N1 influenza outbreak has dramatically heightened awareness of the risks and benefits associated with vaccines.

Chapter 10 was written with recognition that biotechnologies, just like all technologies, generate pollutants that must be treated. The biodegradable fraction of these pollutants can be treated using those approaches in Chapter 7. However, other abiotic techniques must at times also be deployed. Thus, the chapter includes study designs and assessment approaches that may need to be used to address pollutants generated during biotechnological operations. The seminar topic, in fact, compares and contrasts traditional environmental study designs to those needed for a specific biotechnological project (i.e. gene flow from crops).

Chapters 11 and 12 address the professionalism needed in environmental biotechnological enterprises. This includes ethical and practice considerations. The chapter seminars address the challenges associated with the first canon of all engineering professions, i.e. to hold paramount the safety, health, and welfare of the public. The Chapter 11 seminar explores ways to be inclusive of the public's input and the Chapter 12 seminar delves into ways to approach risk tradeoffs based on a case involving TNT-laden soil.

This book covers a wide range of scientific disciplines, so some terminology may be new or at least used in ways not familiar to most readers. In fact, a number of terms have multiple definitions, depending on the particular subject matter. Thus, readers are encouraged to turn to the Glossary at the end of the book when encountering any term with which they are not fully familiar. Important terms occurring in the Glossary are signaled by the use of italic in the text. The Glossary is quite expansive, since it includes terms used by numerous professions and disciplines involved in environmental biotechnologies. These terms have been gathered from numerous sources, including my own lexicon. A number of sources are mentioned in the endnotes, but some sources have long been forgotten (e.g. past and present colleagues, former mentors, forgotten articles, etc.).

THE CHALLENGE

My first discussions of the idea for this book with the gifted Elsevier editor Christine Minihane included a fear that no single text could capture the entirety of the applications and implications of environmental biotechnologies. Upon its completion, I am even more convinced of this. Early in our discussions, I offered the possibility that we might be able to create an electronic community where the various elements of environmental biotechnology reside on a website where people could update and correct the material in this book, could expand on topics, and add new topics. In addition, new teaching and learning tools, as well as actual case studies could be added and updated as they change [see Discussion Box: Bioreactors to the Rescue]. Finally, community members could share new analytical and quantitative techniques, such as successful uses of decision trees, Bayesian approaches, models, root cause and failure analyses, and other approaches used within and outside of the environmental biotechnology community. If you believe this is a worthwhile endeavor, and especially if you would like to participate, please let me know.

Daniel A. Vallero, PhD

Discussion Box: Bioreactors to the Rescue

There is ample evidence that such biological systems can provide cutting edge solutions needed to protect the environment and public health. A case in point is the U.S. Army's Deployable Aqueous Aerobic Bioreactor (DAAB), which is a portable wastewater treatment system being developed to provide: on-site treatment of wastewater at forward operating bases, rapid response to failures (such as during natural disasters) of treatment works, and a rapidly and readily deployed wastewater treatment system for humanitarian needs during crises [7].

Consider two of the most intractable global challenges: natural disasters and war. As this book goes to final printing, engineers, physicians, and first responders from myriad fields are working feverishly against the devastating and truly tragic tolls taken by the earthquake and aftershocks in Haiti. In addition to the hundreds of thousands who perished during and immediately after the earthquake, millions are and will continue to be at risk of waterborne diseases. As discussed in Chapter 7, environmental biotechnologies must be part of the solution to the aftermath of disasters. In the case of Haiti and in war zones, for example, sustainable and low maintenance systems are being employed. As evidence, the U.S. Army has contracted with Sam Houston State University (SHSU) in Texas to develop a bioreactor that can clean water without the need for external sources of energy or chemical compounds.

The bioreactor uses indigenous soil bacteria which have been collected by scientists at SHSU, who describe the process as consisting of a subset of these bacteria whose genetic material is modified to produce "biofilm that is self-regulating and highly efficient at cleaning wastewater" (See Chapter 7). According to the researchers the process is rapid, "cleaning influent wastewater within 24 hours after set-up to discharge levels that exceed the standards established by the Environmental Protection Agency for municipal wastewater." The sludge production is also manageable, i.e. the original waste volume is decreased by over 90%. This compares to about a month needed for a typical septic tank, which often can only decrease volume by 50% of less [8].

Another important feature of any portable waste system is that it be "scalable." The SHSU developers claim that this system can be used to treat wastes from a single residence to larger scales, such as neighborhoods in Haiti or for an army base in Afghanistan. The keys to sustainable biotechnologies are that they not require intricate operations, that they not depend on scarce materials and energy sources that are difficult to obtain and maintain. Biotechnologies can meet these criteria.

Benefits, as discussed in Chapter 11, can be indirect and difficult to quantify. In this instance, one of indirect but crucial benefits of such of an adaptive biotechnology is an improvement in troop safety. In Afghanistan, for example, clean water has to be trucked precariously due to lack of potable local water supplies. The U.S. Marine Corps' Marine and Energy Assessment Team estimates each soldier requires about 22 gallons of clean water daily, so if the prototypes of sustainable, *in situ* biotechnologies work out, they could translate into 50 fewer military trucks needing to traverse the dangerous terrain [9].

Other applications are also possible, such on tankers and cruise ships, as well as temporary conditions, such as during power outages.

NOTES

1. I have actually softened this view in my paraphrasing. If memory serves, it was closer to "microbiologists like to name the bugs, while we don't care what they are called so much as what they do."
2. US Environmental Protection Agency (1986). The Proceedings of the United States Environmental Protection Agency Workshop on Biotechnology and Pollution Control. Bethesda, Maryland, March 20–21, 1986.

3. Ibid., VIII-2.
4. Ibid., VIII-3.
5. G.E. Omenn (1986). Letter to the Honorable Lee M. Thomas, Administrator, US Environmental Protection Agency. March 25, 1986.
6. H.A. Kuiper, G.A. Kleter and M.Y. Noordam (2000). Risks of the release of transgenic herbicide-resistant plants with respect to humans, animals, and the environment. *Crop Protection* 19 (8-10): 773–778.
7. U.S. Army Corps of Engineers (2010). "Deployable Aqueous Aerobic Bioreactor." Environmental Laboratory. *EL Newsroom;* http://el.erdc.usace.army.mil/news.cfm?List=24; accessed on February 11, 2010.
8. S. Holland (2010). Quoted in "'Revolutionary' Water Treatment Units on their Way to Afghanistan." *Today@Sam.* http://www.shsu.edu/~pin_www/T%40S/2010/RevolutionaryWaterTreatmentUnitsQnTheirWay ToAfghanistan.html; accessed on February 11, 2010.
9. K. Drummond (2010). "Pure Water for Haiti, Afghanistan: Just Add Bacteria." *Wired.Com.* February 10, 2010; http://www.wired.com/dangerroom/2010/02/bacteria-based-water-treatment-headed-to-afghanistan-haiti-next/#ixzzOfFIUsYhH; accessed on February 10, 2010.