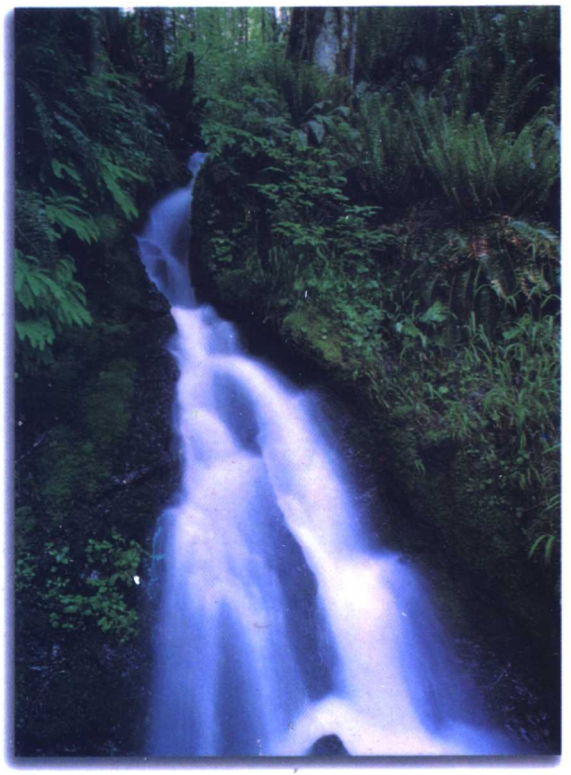
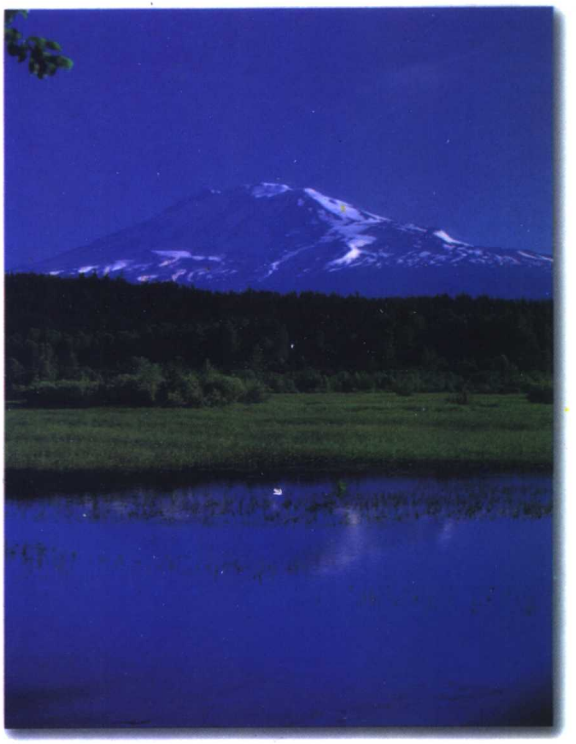
Environmental Biotechnology:

Principles and Applications





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ENVIRONMENTAL BIOTECHNOLOGY: PRINCIPLES AND APPLICATIONS

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PREFACE

Environmental biotechnology utilizes microorganisms to improve environmental quality. These improvements include preventing the discharge of pollutants to the environment, cleaning up contaminated environments, and generating valuable resources for human society. Environmental biotechnology is essential to society and truly unique as a technical discipline.

Environmental biotechnology is historic and eminently modern. Microbiological treatment technologies developed at the beginning of the 20th century, such as activated sludge and anaerobic digestion, remain mainstays today. At the same time, new technologies constantly are introduced to address very contemporary problems, such as detoxification of hazardous chemicals. Important tools used to characterize and control processes in environmental technology also span decades. For example, traditional measures of biomass, such as volatile suspended solids, have not lost their relevance, even though tools from molecular biology allow us to explore the diversity of the microbial communities.

Processes in environmental biotechnology work according to well established principles of microbiology and engineering, but application of those principles normally requires some degree of empiricism. Although not a substitute for principles, empiricism must be embraced, because materials treated with environmental biotechnology are inherently complex and varying in time and space.

The principles of engineering lead to quantitative tools, while the principles of microbiology often are more observational. Quantification is essential if processes are to be reliable and cost-effective. However, the complexity of the microbial communities involved in environmental biotechnology often is beyond quantitative description; unquantifiable observations are of the utmost value.

In *Environmental Biotechnology: Principles and Applications,* we connect these different facets of environmental biotechnology. Our strategy is to develop the basic concepts and quantitative tools in the first five chapters, which comprise the principles part of the book. We consistently call upon those principles as we describe the applications in Chapters 6 through 15. Our theme is that *all microbiological processes behave in ways that are understandable, predictable, and unified.* At the same time, each application has its own special features that must be understood. The special features do not overturn or sidestep the common principles. Instead, they complement the principles and are most profitably understood in the light of principles.

Environmental Biotechnology: Principles and Applications is targeted for graduate-level courses in curricula that exploit microbiological processes for environmental-quality control. The book also should be appropriate as a text for upper-level undergraduate courses and as a comprehensive resource for those engaged in professional practice and research involving environmental biotechnology.

The material in *Environmental Biotechnology: Principles and Applications* can be used in one or several courses. For students not already having a solid background

in microbiology. Chapter 1 provides a foundation in taxonomy, metabolism, genetics, and microbial ecology. Chapter 1 addresses the microbiology concepts that are most essential for understanding the principles and applications that follow. Chapter 1 can serve as the text for a first course in environmental microbiology, or it can be used as a resource for students who need to refresh their knowledge in preparation for a more process-oriented course, research, or practice.

The "core" of the principles section is contained in Chapters 2, 3, 4 and 5. Chapter 2 develops quantitative tools for describing the stoichiometry and energetics of microbial reactions: what and how much the microorganisms consume and produce. Stoichiometry is the most fundamental of the quantitative tools. Chapters 3 and 4 systematically develop quantitative tools for kinetics: how fast are the materials consumed and produced. Reliability and cost-effectiveness depend on applying kinetics properly. Chapter 5 describes how principles of mass balance are used to apply stoichiometry and kinetics to the range of reactors used in practice.

Chapters 6 through 15 comprise the applications section. Each chapter includes information on the stoichiometry and kinetics of the key microorganisms, as well as features that are not easily captured by the stoichiometric or kinetic parameters. Each chapter explains how processes are configured to achieve treatment objectives and what are the quantitative criteria for a good design. The objective is to link principles to practice as directly as possible.

In one sense, the applications chapters are arranged more or less in order from most traditional to most modern. For example, Chapters 6, 7, and 8 address the aerobic treatment of wastewaters containing biodegradable organic matter, such as the BOD in sewage, while Chapters 14 and 15 address biodegradation of hazardous chemicals. Aerobic treatment of sewage can be traced back to the early 20th century, which makes it quite traditional. Detoxification of hazardous chemicals became a major treatment goal in the 1980s. On the other hand, Chapters 6 to 8 describe newly emerging technologies for attaining the traditional goal. Thus, while a goal may be traditional, the science and technology used to attain it may be very modern.

We prepared a chapter on "Complex Systems" that does not appear in the book in an effort to keep the book to a reasonable length. The website chapter extends principles of Chapters 1 to 5 by systematically treating nonsteady-state systems (suspended and biofilm) and systems having complex multispecies interactions. McGraw-Hill agreed to put this chapter on a web site so that it would be available to those who are interested. Having an official web site for the book provides another advantage: We will now have a convenient location to post corrections to the inevitable errors that remain in the book. Perhaps there will be other book-related items that we may wish to post as times go by; we encourage the reader to occasionally check the web page.

One important feature of *Environmental Biotechnology: Principles and Applications* is that it contains many example problems. These problems illustrate the step-by-step procedures for utilizing the tools in order to understand how microbial systems work or to design a treatment process. In most cases, learning by example is the most effective approach, and we give it strong emphasis.

Each chapter contains many problems that can be assigned as "homework," used as supplemental examples in class, or used as study tools. The problems range

in scope. Some are simple, requiring only a single calculation or a short expository response. At the other extreme are extensive problems requiring many steps and pages. Most problems are of intermediate scope. Thus, the instructor or student can gradually advance from simple, one-concept problems to comprehensive problems that integrate many concepts. Computer spreadsheets are very helpful in some cases, particularly when complex or iterative solutions are needed.

In an effort to promote uniformity in notation, we have elected to adapt the "Recommended Notation for Use in the Description of Biological Wastewater Treatment Processes," agreed upon internationally and as published in *Water Research* **16**, 1501–1505 (1982). We hope this will encourage others to do the same, as it will facilitate much better communication among us.

This text is too brief to do justice to general principles, applications of environmental biotechnology, and the numerous specific mechanical details that one must consider in the overall design of biological systems. We have chosen to focus on the principles and applications. For the specific design details, we suggest other references, such as the two-volume *Design of Municipal Wastewater Treatment Plants*, published jointly by the Water Environment Federation (Manual of Practice No. 8) and the American Society of Civil Engineers (Manual and Report on Engineering Practice No. 76).

We take this opportunity to thank our many wonderful students and colleagues, who have taught us new ideas, inspired us to look farther and deeper, and corrected our frequent errors. The numbers are too many to list by name, but you know who you are. We especially thank all of the students in our environmental biotechnology classes over the past few years. These students were subjected to our chapter first drafts and provided us with much welcomed feedback and many corrections. Thank you for everything.

A few individuals made special contributions that led directly to the book now in print. Viraj deSilva and Matthew Pettis provided the model simulations in the website chapter on "Complex Systems." Drs. Gene F. Parkin and Jeanne M. VanBriesen provided extensive suggestions and corrections. Pablo Pastén and Chrysi Laspidou provided solutions to many of the problems in the Solutions Manual. Janet Soule and Rose Bartosch deciphered BER's handwriting to create the original electronic files for all or parts of Chapters 1, 3, 4, 6, 8, 9, 10, 11, 12, and 15. Dr. Saburo Matsui and the Research Center for Environmental Quality Control (Kyoto University) provided a sabbatical venue for BER so that he could finish all the details of the text and send it to McGraw-Hill on time.

Finally, we thank Marylee and Martha for loving us, even when we became too preoccupied with the "book project."

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CONTENTS

chapter

1

BASICS OF MICROBIOLOGY

Environmental biotechnology applies the principles of microbiology to the solution of environmental problems. Applications in environmental microbiology include

- Treatment of industrial and municipal wastewaters.
- Enhancement of the quality of drinking water.
- Restoration of industrial, commercial, residential, and government sites contaminated with hazardous materials.
- Protection or restoration of rivers, lakes, estuaries, and coastal waters from environmental contaminants.
- Prevention of the spread through water or air of pathogens among humans and other species.
- Production of environmentally benign chemicals.
- Reduction in industrial residuals in order to reduce resource consumption and the production of pollutants requiring disposal.

Although this textbook can cover only some of the numerous topics that can be categorized under environmental biotechnology, the principles of application in one area of the environmental field often apply equally to other environmental problems. What is required in all cases is a linking of the principles of microbiology with engineering fundamentals involving reaction kinetics and the conservation of energy and mass.

The purpose of this first chapter is to review the basic principles of microbiology. Fundamentals of reaction kinetics and mass and energy conservation are addressed in four subsequent chapters, while the last chapters in the text address important applications. Readers desiring more detailed information on microbiology are referred to texts such as Madigan, Martinko, and Parker (1997) and Alcamo (1997).

This chapter summarizes

- How microorganisms are classified (*taxonomy*).
- What they look like (*morphology*).
- How they reproduce so that their functions can be maintained.
- The biochemical reactions that they mediate (*metabolism*).
- The major divisions among microorganisms based upon their function in the environment (*trophic groups*).
- How information about structure and function of organisms is transmitted and changed (*genetics*).
- An aspect of great importance in environmental biotechnology, that is *microbial ecology*, or the interactions among organisms and their environment.

The major difference between environmental biotechnology and other disciplines that feature biotechnology is that environmental applications almost always are concerned with mixed cultures and open, nonsterile systems. Success depends on how individual microorganisms with desired characteristics can survive in competition with other organisms, how desired functions can be maintained in complex ecosystems, and how the survival and proliferation of undesired microorganisms can be prevented.

Anyone interested in environmental biotechnology needs to be familiar with organism interactions and the principles of mixed culture development and maintenance in order to obtain sound solutions to environmental problems. For example, creating novel organisms that can carry out specific reactions of interest seems like a wonderful way to solve difficult environmental problems. The question of importance then is: How can such organisms survive in competition with the thousands of other organisms in the environment that are also fighting for survival in situations that can be quite hostile to them? Developing robust microbiological systems that can carry out intended functions over time is the major challenge before those seeking to apply principals of biotechnology to the solution of environmental problems.

1.1 THE CELL

The *cell* is the fundamental building block of life. A cell is an entity that is separate from other cells and its environment. As a living entity, a cell is a complex chemical system that can be distinguished from nonliving entities in four critical ways.

1. Cells are capable of growth and reproduction; that is, they can self-produce another entity essentially identical to themselves.
2. Cells are highly organized and selectively restrict what crosses their boundaries. Thus, cells are at low entropy compared to their environment.
3. Cells are composed of major elements (C, N, O, and S, in particular) that are chemically reduced.
4. Cells are self-feeding. They take up necessary elements, electrons, and energy from their external environment to create and maintain themselves as

reproducing, organized, and reduced entities. They require sources of the elemental building blocks that they use to reproduce themselves. They require a source of energy to fuel the chemical processes leading to all three properties. In addition, they require a source of electrons to reduce their major elements. How the cells obtain elements, energy, and electrons is called *metabolism,* and it is one essential way in which we characterize cells. Understanding metabolism is a theme that runs throughout this book.

Cells are physically organized so that they can carry out the processes that make them living entities. Later in this chapter, the basic components of cells are described in more detail. At this point, the essential components of cells are identified and connected to the distinguishing features of what makes a living cell.

- The *cell membrane* is a barrier between the cell and its environment. It is the vehicle for restricting what crosses its boundaries, and it is the location of reactions that the cell needs to conduct just outside itself.
- The *cell wall* is a structural member that confers rigidity to the cell and protects the membrane.
- The *cytoplasm* comprises most of the inside of the cell. It contains water and the macromolecules that the cell needs to function.
- The *chromosome* stores the genetic code for the cell's heredity and biochemical functions.
- The *ribosomes* convert the genetic code into working catalysts that carry out the cell's reactions.
- The *enzymes* are the catalysts that carry out the desired biochemical reactions.

Cells may have other components, but these are the essential ones that define them as living entities.

Figure 1.1 shows that three major *domains* comprise all organisms. The *Bacteria* and the *Archaea* domains contain the *prokaryotes,* or cells that do not contain their chromosome inside a nucleus. The organisms within these two major domains are single cellular, because they are complete living entities that consist of only one cell. The other major domain is the *Eukarya,* which comprise organisms that may be single cellular or multicellular and have their chromosomes inside a nucleus. All higher plants and animals belong to the Eukarya domain.

All prokaryotes are microorganisms, or organisms that can only be seen with the aid of a microscope. Some of the eukaryotic life forms are microorganisms, and some are not. Eukarya range from single cellular microscopic algae and protozoa (*protista*) up to large multicellular mammals, such as the whales, and plants, such as the redwood trees. Organisms from all three domains are of importance in environmental microbiology, and thus the structure and function of all are of interest.

Some cells may undergo change in form or function through the process of *differentiation.* For example, cells within the human body act differently depending upon whether they form part of an eye, a muscle, or a strand of hair. As part of differentiation, cells can often interact with one another through various chemical

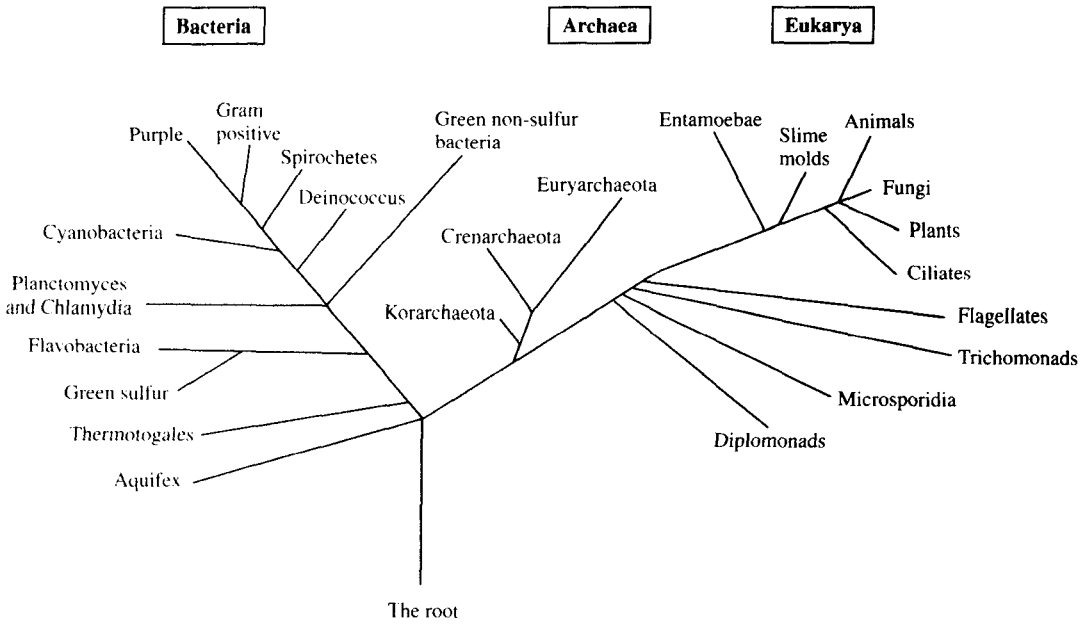

Figure 1.1 Phylogenetic tree of life as determined from comparative ribosomal RNA sequencing. SOURCE: Based on data from Carl R. Woese and Gary J. Olsen.

signals in ways that can change their form or function. It is significant that cells also can evolve into organisms that are markedly different from the parent, a process that usually is quite slow, but nevertheless of great importance to the formation of new organisms or to the development of new capabilities that may aid in organism survival.

Our interest in environmental biotechnology centers primarily on the single-celled organisms, which include the microorganisms in the Bacteria and Archaea domains. In the Eukarya domain, microorganisms of particular interest are algae and protozoa. Recently, environmental biotechnology has begun to focus on plants, too. *Phytoremediation* is a process in which plants help to bring about the destruction of toxic chemicals in soils and groundwater. Here, trees such as the poplar may uptake toxic chemicals along with water. In some cases, the plants or microorganisms associated with the plant roots transform the toxic compounds into nonharmful products. Phytoremediation is an evolving field with much yet to be learned. For the most part, however, the primary focus of this text will be with the single-celled microorganisms.

1.2 TAXONOMY AND PHYLOGENY

Taxonomy is the science of classification. Microbiologists and engineers need to classify microorganisms for many practical reasons: for example, to determine the possible presence of disease-related species in drinking water. Taxonomy relies on the

observable physical properties of organisms to group microorganisms. Observable properties are called a cell's *phenotype* and may involve its appearance (*morphology*), the manner in which it interacts with dyes or staining, and its ability to use or convert a given chemical into another one (*transformation*).

Phylogeny is a different and newer method of classification that detects differences in microorganisms based upon genetic characteristics. Such characteristics are encoded in the organism's DNA (deoxyribonucleic acid), which contains the hereditary material of cells, and RNA (ribonucleic acid), which is involved in protein synthesis. Of particular importance here are the sequences of base pairs in an organism's 16S ribosomal RNA (rRNA), one of the three major categories of RNA. Through the analysis of 16S rRNA, Carl R. Woese discovered that the large group of single-celled microorganisms once collectively termed bacteria was actually comprised of two very distinct domains, the Bacteria and the Archaea.

Phylogeny relates organisms based on their evolutionary history, while taxonomy relates organisms based on observable characteristics of the cells. Both are powerful methods of classification, and they yield different kinds of information. Despite fundamental differences between taxonomic and phylogenetic classifications, some important phenotypic characteristics consistently differentiate among the three basic domains of organisms from one another. For example, Table 1.1 illustrates that only the Eukarya have a membrane-enclosed nucleus, while only the Archaea are capable of generating methane gas (*methanogenesis*).

The basic taxonomic unit is the *species*, which is the collection of strains having sufficiently similar characteristics to warrant being grouped together. Such a loose definition often makes it difficult to determine the difference between strains (members of a given species that have measurable differences between themselves) and species. Groups of species with major similarities are placed in collections called

Table 1.1 Differing features among Bacteria, Archaea, and Eukarya

Characteristic	Bacteria	Archaea	Eukarya
Membrane-enclosed nucleus	Absent	Absent	Present
Cell wall	Muramic acid present	Muramic acid absent	Muramic acid absent
Chlorophyll-based photosynthesis	Yes	No	Yes
Methanogenesis	No	Yes	No
Reduction of S to H_2S	Yes	Yes	No
Nitrification	Yes	No	No
Denitrification	Yes	Yes	No
Nitrogen fixation	Yes	Yes	No
Synthesis of poly-β-hydroxyalkanoate carbon storage granules	Yes	Yes	No
Sensitivity to chloramphenicol, streptomycin, and kanamycin	Yes	No	No
Ribosome sensitivity to diphtheria toxin	No	Yes	Yes

SOURCE: Madigan, Martinko, and Parker, 1997.

genera (or *genus* for singular), and groups of genera with sufficient similarity are collected into *families*. Microorganisms are generally given a genus and species name. For example, *Escherichia coli* is an indicator organism of fecal pollution of drinking water. The genus name, *Escherichia*, is always capitalized and placed before the species name (*coli*), which is never capitalized. Note that the entire organism name is always written in italics (or underlined if writing in italics is not possible). A common practice once the genus name is identified is to abbreviate the genus name by using only the first capitalized letter (in this case, *E. coli*), but the species name is never abbreviated. These are rules as set out in *The International Code of Nomenclature of Bacteria*, which apply to Archaea as well. Individual differences among the major groupings of microorganisms are now described in some detail.

1.3 PROKARYOTES

In discussing the microorganisms of most general importance to environmental biotechnology, the prokaryotes, little is gained by dividing their descriptions between the Bacteria and Archaea since, functionally, their similarities are greater than their differences. Indeed, until the development of genetic phylogeny, the collection of microorganisms within these two domains was simply called bacteria. Also, the Bacteria and Archaea generally are found together and often participate together to bring about the destruction or mineralization of complex organic materials, such as in the formation of methane from the decay of dead plants and animals. In this example, the Bacteria ferment and convert complex organic materials into acetic acid and hydrogen, and the Archaea convert the acetic acid and hydrogen into methane gas. The organisms must work closely together, as in an assembly line, in order to bring about the destruction of the organic matter.

Another distinction that became clearer with the development of genetic phylogeny is among the photosynthetic microorganisms, which are of great importance in natural and engineered systems. Formerly, *algae* was a term used to describe the group of single-celled organisms that behaved like plants: that is, they contain chlorophyll and derive energy for growth from sunlight. However, a group of photosynthetic prokaryotes formerly called *blue-green algae* has no nucleus, a property of bacteria, not plants. Now, they are classified within a bacterial grouping called *cyanobacteria*. Cyanobacteria and algae are commonly found together in natural waters and tend to compete for the same energy and carbon resources. Cyanobacteria are a pesky group of phototrophs that cause many water quality problems, from tastes and odors in drinking water to the production of toxins that kill cows and other ruminants that may consume them while drinking from highly infested surface waters. Despite important differences between algae and cyanobacteria, grouping together makes good practical sense when such differences are not of interest. Here, we are reminded that nature does not strive to classify things; humans do. Despite many gray areas where classification of organisms is not easy (and sometimes does not seem to make much sense), classification is essential for our organization of knowledge and for communication among scientists, practitioners, and others.