MICROBIOLOGY FOR ENVIRONMENTAL SCIENTISTS AND ENGINEERS

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PREFACE

This text is written for those whose professional interest is protection of the environment from pollution—environmental engineers and environmental scientists. Pollution control is a rapidly expanding area of interest, which is attracting persons with many different educational backgrounds, including civil engineering, chemical engineering, general biology, zoology, microbiology, chemistry, and perhaps others. All of these areas of knowledge have something to contribute to the development of a relatively new discipline that we prefer to call *bioenvironmental engineering*.

It is the present vogue to attempt to solve problems by the interdisciplinary approach. It is our belief that environmental pollution is a problem of such magnitude and urgency that it demands workers trained specifically in the areas of knowledge that are directly applicable. What is needed is a new type of multidisciplinary individual rather than a team of narrowly trained experts. The field of pollution control needs persons who consider pollution control their profession rather than individuals who consider microbiology or civil engineering, for example, their profession but are willing to apply their training to problems that are peripheral to their major interest and expertise. Everyone who wishes to participate fully in the effort to control environmental pollution should possess a common core of knowledge; that is a basic necessity for people who are to work together and communicate meaningfully with each other.

This core of knowledge contains elements of microbiology, chemistry (including biochemistry), and engineering. Microbiology is important because microbial activity is, in some cases, the result of environmental pollution and, in other circumstances, the means by which pollution is prevented. One cannot hope to control microbial activity without an understanding of microbial physiology. Chemistry is important in two ways. One cannot understand microbial physiology without some knowledge of the biochemical processes that occur in the cell. Some knowledge of chemistry is also required if one is to understand the problems involved in preventing pollution, since pollution may be defined, in most cases, as the addition of inorganic or organic chemicals in potentially harmful amounts to the environment. Finally, engineering is essential because engineers must design and plan the operation of the processes that remove pollutants from wastes that must be recycled to the environment. It is the engineer who will be held accountable for the success or failure of the pollution control strategy. Most processes (both natural and engineered) by which wastes are rendered harmless to the environment are, and probably will remain, dependent on microbial activity. Thus, the engineer designs processes that use microbes. The microbiologist contributes an understanding of the biochemistry of microbial activity, which allows these processes to be designed for optimum effectiveness. The chemist determines the nature of the pollutants and their fate. Biological scientists contribute their knowledge of the effects of environmental stress on organisms of all types, including humans. One could assemble a team composed of individuals expert in each of the areas described above. But if each of them was totally uninformed about the areas of expertise of all the others, meaningful communication would be impossible.

We envision the effective workers in this field as trained as either environmental scientists or environmental engineers. The difference in the two is one of emphasis in training and in practice, and the two must be prepared to work together to accomplish their mutual goal. The environmental scientist has a background in the biological sciences (which implies training also in chemistry and biochemistry) or primarily in chemistry with some knowledge of biology, and is also informed about the engineered processes designed to protect the environment. The environmental engineer is trained in the usual engineering curriculum with its emphasis on mathematics and applied physics, but also must have a sufficiently broad and detailed knowledge of certain aspects of biology, especially microbiology, and chemistry to facilitate intelligent design and operation of pollution control facilities and management of natural processes.

In planning this text, it was our objective to make it as useful as possible to persons with varied backgrounds. It is needed most by engineers and is addressed to their needs in particular, but we have also attempted to make it useful to science students who wish to enter this applied field. With these aims in mind, we felt that the most logical approach would be to assume that the reader needs basic information in each of the areas important to the whole. We have attempted to provide this information in the most readily understandable form and to build upon it the combination of basic and applied microbiology that is essential to the environmental scientist and engineer.

The first four chapters are designed to provide the basic information upon which the remainder of the text is built. Persons with backgrounds in biology, chemistry, or engineering will find parts of this material already familiar. Chemists and chemical engineers will find the chemistry in Chapter 3 elementary. Microbiologists and other biological scientists already will have seen the material in Chapter 4. Most civil engineers will find that parts of

Chapters 1 and 2 describe processes with which they are familiar. However, all of these individuals should find new information covering areas outside their usual training and new applications of familiar information. Engineers, other than chemical engineers, usually have little chemistry and no biology in the prescribed curriculum. Chemical engineers normally have no training in biology. Biologists learn little about the engineered processes that protect the environment. Thus, the first four chapters are written to accomplish three objectives:

1. To provide a background in unfamiliar areas for persons entering the field of pollution control.
2. To relate basic knowledge to applications of which persons in each field may be unaware.
3. To bring all readers to a point at which they possess the common background that will serve as a basis for understanding the material in Chapters 5 through 14.

While we believe that microbiology is as basic to the study of environmental engineering as are the more traditional tools of engineering, mathematics, and physics, we feel that a textbook on microbiology aimed primarily at the environmental engineer should differ in several ways from the standard microbiology text written for students of microbiology or environmental microbiology. An engineer is, by training and practice, oriented toward the application of basic knowledge and is interested most in those facts that can be useful in professional practice. We have tried to present these facts in a format that will allow the engineer to relate principles to practical applications.

First, we have attempted to limit the topics covered to those that we consider essential to the quantitative expression and mechanistic understanding of the microbial activities that occur in natural environments or in processes engineered for the purpose of exerting useful control over the natural environment. Second, our approach is more process-oriented than species-oriented. Names of genera and species are introduced where their activities are discussed, since it is as necessary to use names for microbes as for any other material in discussing its properties. While it is true that any natural population is composed of individual microbial species, it is at times advantageous to treat the entire heterogeneous population (the *biomass*) as an engineering material, with properties that are definable and controllable within certain limits. In other circumstances, it is necessary to consider the ecological relationships between individual species or metabolic groups of microorganisms. Both approaches are used, each where it is most appropriate or useful. Third, we have attempted to integrate the basic information directly with the appropriate environmental applications in each chapter rather than following the traditional format of presenting basic information followed by chapters covering various applications.

Much of the material in this text has been tried out on the many students who have passed through our courses in the past two decades. We are particularly grateful for their comments and suggestions while they were students and for their continued feedback as they progress through careers in environmental engineering or science. Their successful solving of pollution control and environmental management problems, using the practical microbiological principles we have tried to impart, was a source of encouragement that helped us to keep burning the midnight oil while writing this text during the past three years.

The help of our students goes beyond the encouragement and stimulation that good students provide for professors. It will become apparent to the reader that many of the figures used in this text are based upon experimental results which our graduate students have helped to obtain. In truth, these examples are a small sampling of the investigative efforts of student-professor research teams which have engaged in more than 300 person-years of professional investigation into the microbiological aspects of environmental engineering in the past two decades.

Specific acknowledgments are difficult to write because when we think of all those who should be acknowledged the list becomes endless. For their immediate contributions, we are grateful for the thorough review and criticism of this work by Dr. Ven Te Chow, University of Illinois, Dr. Linda Little, environmental biologist, and Dr. W. W. Umbreit, Rutgers University. Their thoughtful comments and suggestions were very valuable to us. Also special thanks are due to Drs. T. S. Manickam and Crosby Jones and to our graduate students, M. P. Reddy, Mike Hunt, and Brenda Mears for their help in assembling material used in this text. Our grateful appreciation goes to Mrs. Grayce Wynd, a friend of many years, for her expert typing of the manuscript and her helpful suggestions. Dr. D. F. Kincannon deserves special mention. His relationship with both of us, but especially with A. F. Gaudy, has progressed from that of professor and student to that of colleague and friend and we are grateful for his support and encouragement, not only during the writing of this text, but also through the many years of our association.

The development of an investigative team requires more than the vital interest and effort of professor and students. There must be help and encouragement from administrators. We are very grateful for the help given us by Dr. Jan J. Tuma, former head of the School of Civil Engineering, and Dr. Lynn L. Gee, former head of the Department of Microbiology, Oklahoma State University. We appreciate the faith they showed in us early in our professional careers and their friendship through the years.

Finally, we are grateful for the patience, encouragement and faith in us shown by our mothers and fathers throughout our lives and particularly during the last few years. Their loving interest and questions as to progress were delightfully reminiscent of their concern over our report cards many years ago.

Anthony F. Gaudy, Jr.
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CONTENTS

CHAPTER
ONE

THE LIFE-SUPPORT SYSTEM

Most books on microbiology begin with a statement about the importance of microbiology and its pivotal role in the basic and applied sciences. So, too, will this text, but the point need not be belabored because, as we discuss the life-support system in this chapter, the absolute necessity for the existence (and the control) of microorganisms will become apparent.

Microbes, both the procaryotic, or nonnucleated, cell types such as bacteria and blue-green bacteria* and the eucaryotic, or nucleated, types such as protozoa, fungi, and algae, are found nearly everywhere in nature and therefore are a force in the environment. Anyone aspiring to gain an understanding of the environment with an eye toward enhancing or controlling it must study microbiology. Just as an electrical, civil, or chemical engineer comes to know that learning to use mathematics and physics as tools of the profession is necessary in solving various problems or predicting various outcomes, i.e., in designing processes, so must the environmental engineer be aware that microbiology is equally necessary. In fact, another tool—chemistry, and especially biochemistry, which is peculiar to living cells—is also required. In like manner, an aspiring environmental biologist must become aware that one needs the tools of chemistry, mathematics, and physics in order to understand and use microbiology in solving environmental problems.

Microbiology is not the only area in the biological sciences that is important in environmental technology. However, microbiology permits one

*The organisms previously classified as blue-green algae are now considered to be bacteria and are called *blue-green bacteria* or *cyanobacteria*.

to bring chemistry and biology together in the study of the smallest self-producing living unit, the cell. It is, therefore, a very basic and necessary tool which ranks with mathematics, physics, and chemistry.

THE ENVIRONMENT—THE LIFE-SUPPORT SYSTEM

Before discussing the role of microorganisms in the environment, it is necessary to lay down a ground rule about the meaning of the word *environment* as used in this text. It is a word that implies as many different meanings to as many different people as does *ecology*. While there is a wide array of items that could justifiably be included under the environmental umbrella, the term *environment*, stripped of all rhetoric, simply means the life-support system. All other aspects of the environment are ancillary to the life-support system, which we can define as those items that are absolutely essential to the sustenance of aerobic life: *water*, *air*, and *food*.

Like one type of microorganism to be studied in this text, humans can be considered aerobic organotrophs. Such microorganisms require organic food and oxygen for controlled combustion of organic foodstuff, and they must be bathed in an aqueous environment. The cells of the human body have the same requirements. This life-support system, food, air, and water, the protection and control of which are the basis for the emerging profession of environmental technology, is maintained by two major natural cycles and accompanying mineral cycles. The two major cycles are the hydrologic cycle (water source) and the carbon-oxygen cycle (food and air source). Since the organic food source contains, in addition to carbon, some nitrogen, phosphorus, sulfur, and other elements, the cyclic migrations of these elements in the biosphere (the sphere of living organisms) are also vital to the life-support system. These cycles, so important to the life of humans and microorganisms, are discussed briefly below along with some of the artificial subsystems that our species has inserted into nature.

The Water Cycle

Slightly more than 70 percent of human body weight is water, and water comprises approximately 80 percent of the weight of a microorganism. There is little wonder, then, that it is an essential ingredient in the life-support system. Water is delivered in an unending cycle of evaporation and condensation. This constant water supply has been reused for millions of years. It is well to examine this cycle, not only because it is vital to life, but also because it is responsible in large measure for moderating and maintaining the earth's climate, thus providing our living space. Water is an excellent heat reservoir, and the earth is a gigantic solar still.

Consider the simplified diagram of the hydrologic cycle shown in Fig. 1-1. Solar energy is absorbed into liquid water and causes it to vaporize. In the

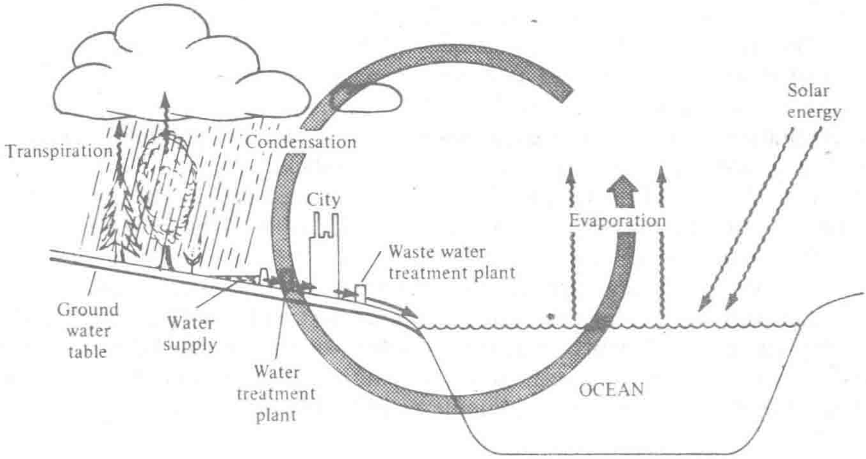

Figure 1-1 The water cycle.

diagram, the liquid bodies are designated as oceans and, while they form the major surfaces, there are, of course, others such as lakes, rivers, and ponds. Also, it must be realized that not all the water vapor is produced by evaporation from liquid surfaces. Transpiration (evaporative loss of water from macroscopic plants) accounts for some. Since the earth receives more direct sunlight in the equatorial region, this region absorbs more heat, so more water evaporates near the equator. This moisture-laden ring of heated air rises and diffuses toward the poles, and as it cools, the water vapor condenses and provides two environmental benefits. It precipitates to deliver a pure water supply, and it releases the heat absorbed during the vaporization process to warm the atmosphere. This phenomenon helps create the temperate zones in which much of the earth's human population lives.

Some of the precipitation seeps into the ground; some drains over the surface. Some of the water is used by plants and animals. Since the use of water by humans is essentially nonconsumptive, the water is generally returned to the surface water supply as waste-bearing "used" water. The waste materials, along with dissolved and suspended material eroded or leached from the earth, are carried to the oceans. There water is again purified by solar distillation. This purification and delivery process does not suffice for human needs because the water is used repeatedly in transit as surface and groundwater before it undergoes the physical purification process of evaporation. A number of biological processes intervene while the water is in transit; these can both purify and further contaminate the water. These processes involve the carbon-oxygen cycle, to be discussed subsequently. Also, we will see later that it is necessary to insert into this natural water supply–wastewater system some humanly devised technological subsystems.

These will also be described later, since they too involve microorganisms.

The tremendous heat sink function of the hydrologic cycle can be appreciated when it is realized that approximately 5×10^5 km^3 (120,000 mi^3) of water is evaporated from the land and oceans and subsequently falls as precipitation each year. For each pound of water evaporated, approximately 600 cal/g (1000 Btu) is absorbed. This amounts to slightly less than 3×10^{20} kg·cal per year. An idea of the magnitude of this energy transfer may be obtained by comparing it with the yearly energy consumption of the United States, which in 1974 amounted to nearly 2×10^{16} kg·cal. Thus, each year the hydrologic cycle alone distributes approximately 10,000 times the energy consumed in the United States. In fact, the amount of energy stored and distributed by the hydrologic cycle amounts to nearly a quarter of the energy beamed to the earth from the sun. The tremendous amount of water evaporated and condensed in the water cycle is a minute fraction of the 1.5×10^9 km^3 of water estimated to be on earth. Approximately 97 percent of this water is liquid salt water in the oceans. Of the 3 percent fresh water, approximately 75 percent exists in solid form in the polar ice caps and in glaciers. At least 90 percent of the less than 1 percent remaining exists as groundwater, and the remainder as fresh surface water mainly in lakes and rivers. A minute fraction is the water vapor that stores, transports, and delivers heat and fresh water. About a quarter of the 5×10^5 km^3 of fresh water that is delivered falls on the land surfaces, where some of it can be used for human and plant life, as well as for the other uses to which this multipurpose resource is put.

Thus far it can be seen that the hydrologic cycle is in the main a physical phenomenon. The following information will emphasize the biological role of water, in particular the role it plays in tying together the remainder of the life-support system.

The Carbon-Oxygen Cycle

The carbon-oxygen cycle provides the major portion of the remainder of the life-support system. Figure 1-2 is a very simplified representation of this cycle. It is seen that there are two divisions or "legs"—photosynthesis and aerobic decay. Photosynthesis is shown on top because, thermodynamically speaking, it is an "uphill" process. Inorganic compounds, carbon dioxide and water, are converted to a compound consisting of carbon and water, which appropriately is called *carbohydrate.* Although the diagram shows the fixation of only one carbon atom, the organic compounds synthesized contain more than one carbon, and these are linked in carbon–carbon bonds. This type of bond is a distinguishing characteristic of organic matter. Thus, in the energy-requiring (endergonic) reaction, organic matter is synthesized. In general, syntheses require energy. In this overall reaction, water has been split and oxygen has been produced as a by-product. It is this process that permits us to live, since the reaction is responsible for the oxygen in our atmosphere. The synthesis of organic matter is, of course, the photosynthetic phenomenon

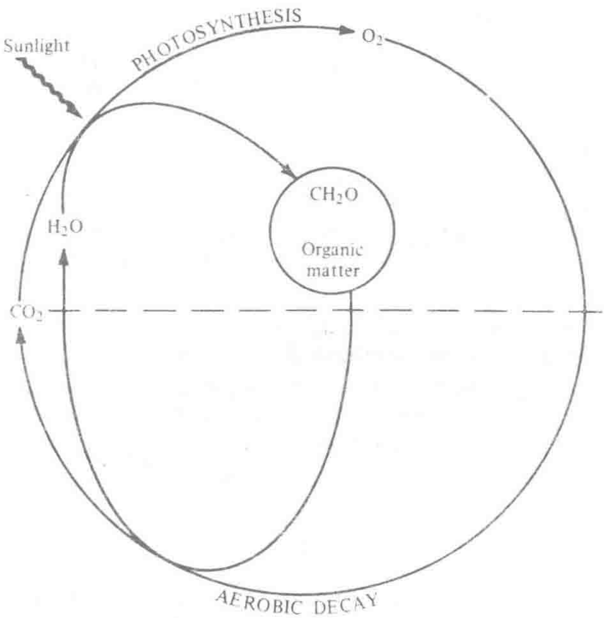

Figure 1-2 The carbon-oxygen cycle.

that creates our food supply. The source of energy that drives this biochemical process, the sun, also drives the physical process, the hydrologic cycle. How the sun's energy is captured and stored in organic compounds and is subsequently used by microorganisms is one of the subjects to be dealt with later.

The bottom leg of the cycle is labeled "aerobic decay." Here, the organic foodstuff is oxidized. The endergonic reaction (synthesis) is reversed, and in the exergonic aerobic decay reaction, energy is released. Aerobic organotrophs (such as humans) can perform this reaction, obtaining energy that can be used to synthesize organic body substances for growth and replication. Thus, solar energy flows through the living world, proceeding through photosynthesis to decay, and water, the universal solvent, is in a way the "glue" that holds it all together. The human species is part of the decay cycle and has become a potent force in it. Many of the technological processes set into the natural decay cycle (e.g., wastewater treatment plants) are engineered subsystems placed there for the purpose of providing localized control of it.

The decay reaction occurs in numerous biochemical steps and through various ecological (food) chains. Consider two general food chains: the organic matter stored in plants through photosynthesis is used as food material by various plant-eating animals (herbivores), and some of it is subject to microbial decay. Some of the herbivores die and are subject to microbial