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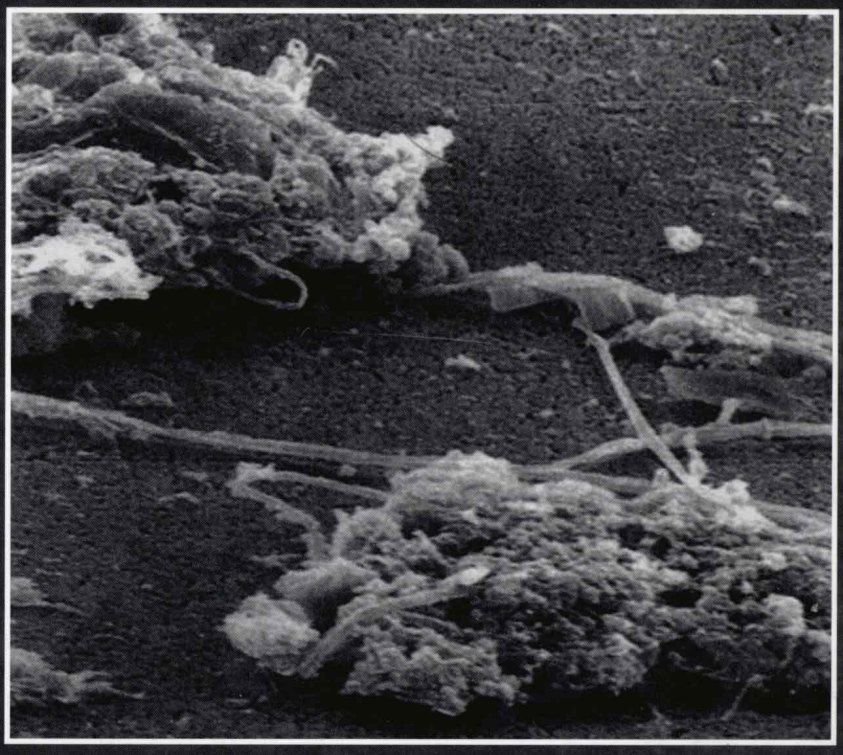
WASTEWATER MICROBIOLOGY

Second Edition



GABRIEL BITTON

WASTEWATER MICROBIOLOGY

Second Edition

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WASTEWATER MICROBIOLOGY
2nd Edition
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Whenever one ceases to study, one forgets.
MAIMONIDES, *Book of Knowledge*

PREFACE

The second edition of *Wastewater Microbiology* incorporates the latest findings in a field covering a wide range of topics.

During the past few years, we have witnessed significant advances in molecular biology, leading to the development of genetic probes, particularly the ribosomal RNA (rRNA) oligonucleotide probes, for the identification of wastewater microorganisms. The road is now open for a better identification of the microbial assemblages in domestic wastewater and their role in wastewater treatment.

The use of genetic tools has also been expanded as regards the detection of pathogens and parasites (Chapter 4), and biotechnological applications for wastewater treatment (Chapter 17). Chapter 4 has been expanded due to the emergence of new pathogens and parasites in water and wastewater. The topic of drinking water microbiology has been expanded, and two chapters are now devoted to this subject. Chapter 15 deals with water treatment and Chapter 16 covers the microbiology of water distribution systems. New methodology that shows the heterogeneous structure of biofilms and their complex biodiversity includes nondestructive confocal laser-scanning microscopy in conjunction with 16S rRNA-targeted oligonucleotide probes (Chapter 16). The topic of wastewater and biosolids disposal on land and in receiving waters has also been expanded and is now covered in two chapters (Chapters 20 and 21).

New figures and tables have been added to further enhance the illustration of the book. Many old figures and graphs were redrawn to improve the visual aspect of the book.

I am very grateful to the colleagues who reviewed the book proposal for their valuable suggestions concerning the second edition of *Wastewater Microbiology.* I am particularly grateful to my mentor and friend, Professor Ralph Mitchell, of Harvard University. As editor of the Wiley series in Ecological and Applied Microbiology, he offered me his full support in the undertaking of this project. I thank Dr. Charles Gerba of the University of Arizona for his continuous moral support and enthusiasm. I thank Dr. Robert Harrington, senior editor at Wiley, for enthusiastically endorsing this second edition of *Wastewater Microbiology.*

A picture is worth a thousand words. I thank Dr. Christopher Robinson of the Oak Ridge Institute of Science and Education, and Dr. H.D. Alan Lindquist of the U.S. EPA for promptly and kindly sending me photomicrographs of *Cryptosporidium parvum.* I am grateful to Dr. Rudolf Amann of the Max-Planck Institute for Marine Microbiology, Bremen, Germany, for allowing me to use his excellent color pictures on the use of rRNA probes in wastewater microbiology, and to Dr. Trello Beffa of the Universite de Neufchatel, Switzerland, for his scanning electron micrograph of compost microorganisms. Many thanks to Dr.

Samuel Farrah and his students, Fuha Lu and Jerzy Lukasik, for supplying a scanning electron micrograph of *Zooglea.*

I am grateful to Nancy, Julie, Natalie, my entire family, and friends for their love and moral support.

GABRIEL BITTON
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PREFACE TO THE FIRST EDITION

Numerous colleagues and friends have encouraged me to prepare a second edition of *Introduction to Environmental Virology,* published by Wiley in 1980. Instead, I decided to broaden the topic by writing a text about the role of *all* microorganisms in water and wastewater treatment and the fate of pathogens and parasites in engineered systems.

In the 1960s, the major preoccupation of sanitary engineers was the development of wastewater treatment processes. Since then, new research topics have emerged and emphasis is increasingly placed on the biological treatment of hazardous wastes and the detection and control of new pathogens. The field of wastewater microbiology has blossomed during the last two decades as new modern tools have been developed to study the role of microorganisms in the treatment of water and wastewater. We have also witnessed dramatic advances in the methodology for detection of pathogenic microorganisms and parasites in environmental samples, including wastewater. New genetic probes and monoclonal antibodies are being developed for the detection of pathogens and parasites in water and wastewater. Environmental engineers and microbiologists are increasingly interested in toxicity and the biodegradation of xenobiotics by aerobic and anaerobic biological processes in wastewater treatment plants. Their efforts will fortunately result in effective means of controlling these chemicals. The essence of this book is an exploration of the interface between engineering and microbiology, which will hopefully lead to fruitful interactions between biologists and environmental engineers.

The book is divided into five main sections, which include fundamentals of microbiology, elements of public health microbiology, process microbiology, biotransformations and toxic impact of chemicals in wastewater treatment plants, and the public health aspects of the disposal of wastewater effluents and sludges on land and in the marine environment. In the process microbiology section, each biological treatment process is covered from both the process microbiology and public health viewpoints.

This book provides a useful introduction to students in environmental sciences and environmental engineering programs and a source of information and references to research workers and engineers in the areas of water and wastewater treatment. It should serve as a reference book for practicing environmental engineers and scientists and for public health microbiologists. It is hoped that this information will be a catalyst for scientists and engineers concerned with the improvement of water and wastewater treatment and with the quality of our environment.

I am very grateful to all my colleagues and friends who kindly provided me with illustrations for this book and who encouraged me to write *Wastewater Microbiology.* I will always be indebted to them for their help, moral support, and good wishes. I am indebted to my graduate students who have contributed to my interest and knowledge in the microbi-

ology of engineered systems. Special thanks are due to Dr. Ben Koopman for lending a listening ear to my book project and to Dr. Joseph Delfino for his moral support. I thank Hoa Dang-Vu Dinh for typing the tables for this book. Her attention to details is much appreciated.

Special thanks to my family, Nancy, Julie, and Natalie, for their love, moral support, and patience, and for putting up with me during the preparation of this book.

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CONTENTS

PART A

FUNDAMENTALS OF MICROBIOLOGY

1

THE MICROBIAL WORLD

1.1 INTRODUCTION: THE PROTISTA

Protista are divided into two groups, the *prokaryotes* (bacteria, actinomycetes, cyanobacteria) and *eukaryotes* (fungi, protozoa, algae, plant, and animal cells). Viruses are obligate intracellular parasites that belong to neither of these two groups.

The main characteristics that distinguish prokaryotes from eukaryotes are the following (Fig. 1.1):

1. Eukaryotic cells are generally more complex than prokaryotic cells.
2. DNA is enclosed in a nuclear membrane and is associated with histones and other proteins only in eukaryotes.
3. Organelles are membrane-bound in eukaryotes.
4. Prokaryotes divide by binary fission, whereas eukaryotes divide by mitosis.
5. Some structures are absent in prokaryotes, e.g., Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts.

Other differences between prokaryotes and eukaryotes are shown in Table 1.1.

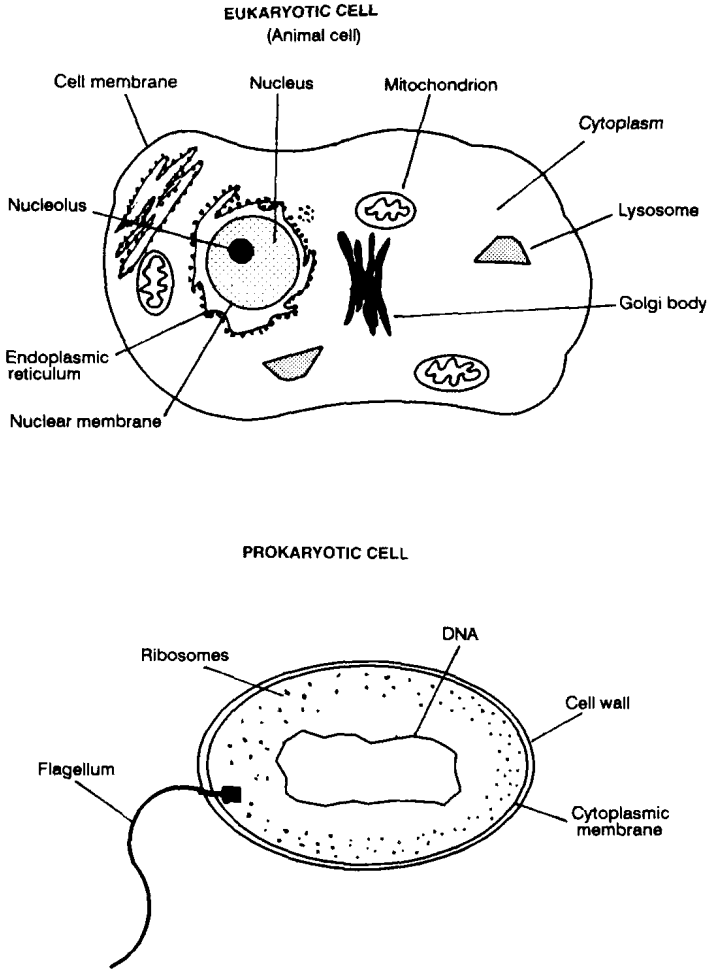

Figure 1.1 Prokaryotic and eukaryotic cells.

We will now review the main characteristics of prokaryotic and eukaryotic microorganisms that are important from a process microbiology and public health viewpoints. We will also introduce the reader to environmental virology and parasitology, the study of the fate of viruses, and protozoan and helminth parasites of public health significance in wastewater and other fecally contaminated environments.

1.2 CELL STRUCTURE

1.2.1 Cell Size

Except for filamentous bacteria, prokaryotic cells are generally smaller than eukaryotic cells. Small cells have a higher growth rate than that of larger cells. This may be explained by the fact that small cells have a higher surface-to-volume ratio than larger cells. Thus, the higher metabolic activity of small cells is due to additional membrane surface available for transport of nutrients into and waste products out of the cell.

1.2.2 Cytoplasmic Membrane (Plasma Membrane)

The cytoplasmic membrane is a 40- to 80-A-thick semipermeable membrane that contains a phospholipid bilayer with proteins embedded within the bilayer (fluid mosaic model) (Fig. 1.2). The phospholipid bilayer is made of hydrophobic fatty acids oriented toward the inside of the bilayer and hydrophilic glycerol moieties oriented towards the outside of the bilayer. Certain cations, such as Ca^{2+} and Mg^{2+} help stabilize the membrane structure. Sterols are other lipids that enter into the composition of plasma membranes of eukaryotic cells as well as some prokaryotes, such as mycoplasmas (these bacteria lack a cell wall).

TABLE 1.1. Comparison of Prokaryotes and Eukaryotes

Feature	Prokaryotes (Bacteria)	Eukaryotes (Fungi, Protozoa, Algae, Plants, Animals)
Cell wall	Present in most prokaryotes (absent in Mycoplasma); made of peptidoglycan	Absent in animal; present in plants, algae, and fungi
Cell membrane	Phospholipid bilayer	Phospholipid bilayer + sterols
Ribosomes	70S in size	80S in size
Chloroplasts	Absent	Present
Mitochondria	Absent; respiration associated with plasma membrane	Present
Golgi complex	Absent	Present
Endoplasmic reticulum	Absent	Present
Gas vacuoles	Present in some species	Absent
Endospores	Present in some species	Absent
Locomotion	Flagella composed of one fiber	Flagella or cilia composed of microtubules; amoeboid movement
Nuclear membrane	Absent	Present
DNA	1 single molecule	Several chromosomes where DNA is associated with histones
Cell division	Binary fission	Mitosis

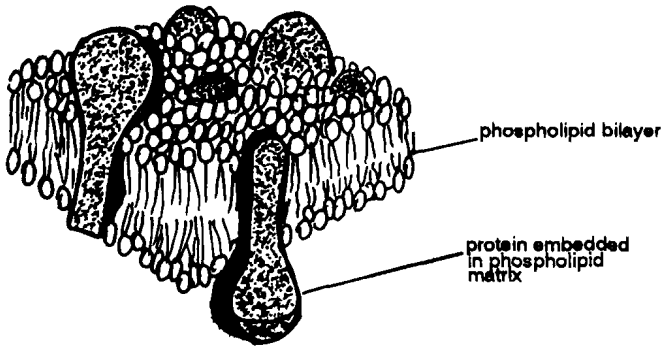

Figure 1.2 Structure cytoplasmic membrane. Adapted from Alberts et al. (1989).

Chemicals cross biological membranes by diffusion, active transport, and endo cytosis.

- *Diffusion:* Because of the hydrophobic nature of the plasma membrane, lipophilic compounds diffuse better through the membrane than do ionized compounds. The rate of diffusion across cell membranes depends on their lipid solubility and concentration gradient across the membrane.
- *Active transport:* Hydrophilic compounds (i.e., lipid insoluble) may be transferred through the membrane by active transport. This transport involves highly specific *carrier proteins,* requires energy in the form of adenosine triphosphate (ATP) or phosphoenol-pyruvate (PEP) and allows cells to accumulate chemicals against a concentration gradient.

  There are specific active transport systems for sugars, amino acids and ions. Toxic chemicals gain entry into cells mainly by diffusion and some may use active transport systems similar to those used for nutrients.
- *Endocytosis:* In eukaryotes cells, substances can cross the cytoplasmic membranes by endocytosis, in addition to diffusion and active transport. Endocytosis includes *phagocytosis* (uptake of particles) and *pynocytosis* (uptake of dissolved substances).

1.2.3 Cell Wall

All bacteria, except *Mycoplasma,* have a cell wall. This structure confers rigidity to cells and maintains their characteristic shape, and it protects them from high osmotic pressures. It is composed of a mucopolysaccharide called peptidoglycan or murein (glycan strands cross-linked by peptide chains). Peptidoglycan is composed of N-acetylglucosamine and N-acetylmuramic acid and amino acids. A cell wall stain, called the Gram stain, differentiates between *gram-negative* and *gram-positive* bacteria on the basis of cell wall composition. Peptidoglycan layers are thicker in gram-positive bacteria than in gram-negative bacteria. In addition to peptidoglycan, gram-positive bacteria contain teichoic acids made of alcohol and phosphate groups.

Animal cells do not have cell walls but, in other eukaryotic cells, the cell walls may be composed of cellulose (e.g., plant cells, algae, fungi), chitin (e.g., fungi), silica (e.g., diatoms), or polysaccharides such as glucan and mannan (e.g., yeasts).

1.2.4 Outer Membrane

The outermost layer of gram-negative bacteria contains phospholipids, lipopolysaccharides (LPS) and proteins (Fig. 1.3). The LPS constitute about 20% of the outer membrane by weight and consist of a hydrophobic region bound to an oligosaccharide core. The LPS molecules are held together with divalent cations. Proteins constitute about 60% of the outer membrane weight and are partially exposed to the outside. Some of the proteins form water-filled pores, *porins,* for the passage of hydrophilic compounds. Other proteins have a structural role, as they help anchor the outer membrane to the cell wall. The outer membrane of gram-negative bacteria is an efficient barrier against hydrophobic chemicals, namely some antibiotics and xenobiotics, but is permeable to hydrophilic compounds, some of which are essential nutrients.

Chemicals (e.g., EDTA, polycations) and physical (e.g., heating, freeze-thawing, drying and freeze-drying) treatments, as well as genetic alterations, can increase the permeability of outer membranes to hydrophobic compounds.

1.2.5 Glycocalyx

The *glycocalyx* is made of extracellular polymeric substance (EPS), which surrounds some microbial cells and is composed mainly of polysaccharides. In some cells, the glycocalyx is organized as a capsule (Fig. 1.4). Other cells produce loose polymeric materials, which are dispersed in the growth medium.

Extracellular polymeric substances are important from medical and environmental viewpoints: (1) capsules contribute to pathogen virulence; (2) encapsulated cells are protected from phagocytosis in the body and in the environment; (3) EPS helps bacteria adsorb to surfaces such as teeth, mucosal surfaces, and environmentally important surfaces such as wa-

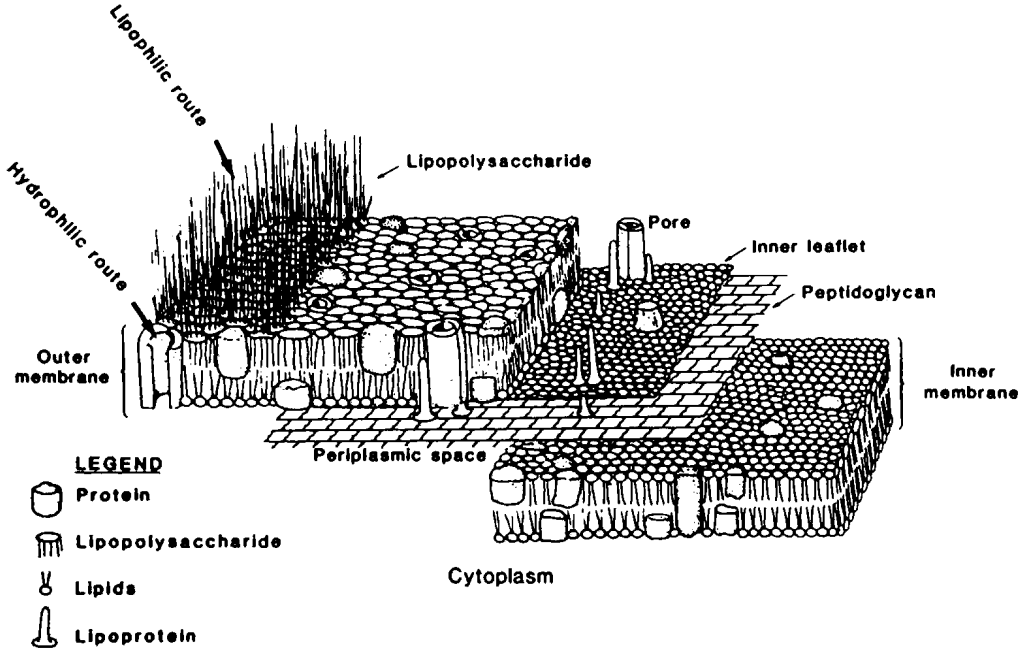

Figure 1.3 Outer membrane of gram-negative bacteria. From Godfrey and Bryan (1984).