Wastewater Bacteria

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Water Pollution Biology
Williamsport, Pennsylvania

WILEY-INTERSCIENCE
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WASTEWATER MICROBIOLOGY SERIES

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Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

Gerardi, Michael H.
Wastewater bacteria / Michael H. Gerardi.
p. cm.
Includes bibliographical references and index.
ISBN-13: 978-0-471-20691-0 (cloth : alk. paper)
ISBN-10: 0-471-20691-1 (cloth : alk. paper)
1. Sewage sludge digestion. 2. Anaerobic bacteria. I. Title.
TD769.G48 2006
628.3′54—dc22
2005031921

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

To
Rani Harrison

The author extends his sincere appreciation to
joVanna Gerardi for computer support
and
Cristopher Noviello for artwork used in this text.

Contents

Preface

The basic objectives of wastewater treatment are twofold: (1) Degrade organic wastes to a level where they do not exert a significant, dissolved oxygen demand upon receiving waters and (2) remove nutrients (nitrogen and phosphorus) to levels where photosynthetic organisms in receiving waters are limited in their growth. In order to achieve these objectives, it is essential for plant operators to understand the biological processes and organisms involved in wastewater treatment to ensure that the presence of an adequate, active, and appropriate population of bacteria is present in each process. The bacteria are the organisms of primary concern in all biological processes. However, bacteria in wastewater are not a monoculture but, instead, a diversity of organisms that perform different roles and have different operational conditions that are best for their optimal activity and growth (i.e., wastewater treatment).

The large diversity of bacteria and the roles that they perform in wastewater treatment are represented best in two biological treatment units, namely, the activated sludge process and the anaerobic digester. The bacteria and these two biological treatment units are reviewed in this book. The activated sludge process is the most commonly used aerobic biological treatment unit at municipal wastewater treatment plants. The organisms here consist of procaryotes (bacteria) and eucaryotes (protozoa and metazoa). The biological processes occur in aerobic and anoxic environments and are based on respiration. The anaerobic digester is the most commonly used anaerobic biological treatment unit at municipal wastewater treatment plants. The organisms consist exclusively of procaryotes. The biological processes occur in an anaerobic environment and are based on fermentation. There are significant differences in the microbial communities between the activated sludge process and the anaerobic digester.

This book reviews the significant bacterial groups, the roles they perform in wastewater treatment, and the operational conditions that affect their activity. The roles that are performed by each bacterial group may be beneficial or detrimental to the biological treatment unit and depend upon the operational conditions of the unit. Effective control and proper operation of each biological treatment unit is based upon an understanding of the basic principles of bacterial activity and growth that are presented in this book.

Several of the significant groups of bacteria that are reviewed in this book are denitrifying bacteria, fermentative (acetate-forming and acid-forming) bacteria, filamentous bacteria, floc-forming bacteria, hydrolytic bacteria, methane-forming bacteria, nitrifying bacteria, poly-P bacteria, sulfur-oxidizing bacteria, and sulfur-reducing bacteria. Several of these bacterial groups are presented in comprehensive reviews in other books in the Wastewater Microbiology Series.

Wastewater Bacteria is the fifth book in the Wastewater Microbiology Series by John Wiley & Sons. This series is designed for wastewater personnel, and the series presents a microbiological review of the significant groups of organisms and their roles in wastewater treatment facilities.

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Part I

Bacteria and Their Environment

1

Wastewater Microorganisms

Although most organisms in biological wastewater treatment plants are microscopic in size, there are some organisms such as bristleworms and insect larvae that are macroscopic in size. Macroscopic organisms can be observed with the naked eye—that is, without the use of a light microscope. Microscopic organisms can only be observed with the use of a light microscope. Of the microscopic organisms the bacteria (singular: bacterium) are the most important in wastewater treatment plants and can be seen with the light microscope only under highest magnification. Several groups of microorganisms such as protozoa and some metazoa possess large and more complex cells that can be observed easily with the light microscope without the use of highest magnification. Compared to other organisms, microorganisms have relatively simple structures.

All living cells can be classified as procaryotic or eucaryotic (Table 1.1). Procaryotic cells lack a nucleus and other membrane-bound structures, while eucaryotic cells possess these structures (Figure 1.1). The nucleus is the primary membrane-bound structure in eucaryotic cells. It regulates cellular activity and contains the genetic information. Examples of membrane-bound structures or organelles found in eucaryotic cells include the golgi apparatus (which regulates cellular metabolism) and lysomes (which contain hydrolytic enzymes).

Based upon cellular structure and function, microorganisms are commonly classified as eucaryotes and procaryotes. The procaryotes consist of (1) eubacteria or "true" bacteria and (2) archaebacteria or "ancient" bacteria (Table 1.2). The eubacteria and archaebacteria are the most important microorganisms in biological, wastewater treatment plants. Together, these two procaryotes commonly are referred to as bacteria.

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TABLE 1.1 Major Differences between Procaryotic Organisms and Eucaryotic Organisms

Feature	Procaryotic Organism	Eucaryotic Organism
Genetic material	Not contained in a membrane	Contained in a membrane
Organelles	None	Many
Structure	Simple	Complex

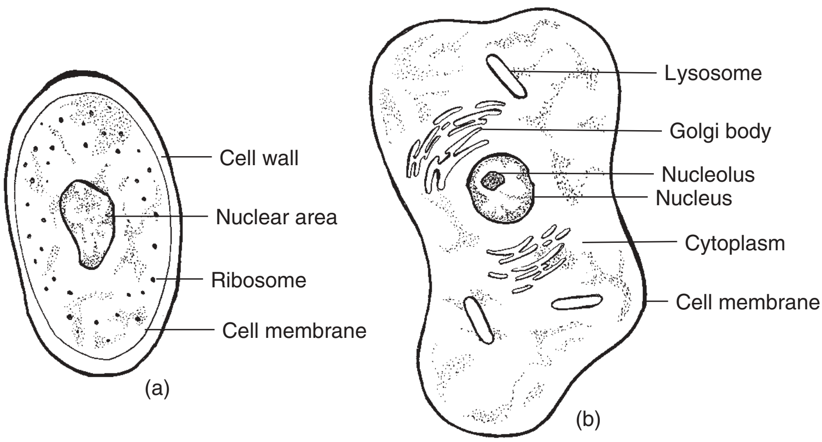

FIGURE 1.1 *Procaryotic and eucaryotic cells. The procaryotic cell (a) contains no membrane-bound organelles such as the nucleus, golgi body, and lysosome that are found in the eucaryotic cell (b).*

TABLE 1.2 Classification of Microorganisms in Wastewater Treatment Plants

Group	Cell Structure	Organization	Representatives
Eucaryotes	Eucaryotic	Multicellular	Bristleworms, flatworms, free-living nematodes, waterbears
Eubacteria	Procaryotic	Unicellular	Bacteria
Archaebacteria	Procaryotic	Unicellular with unique cellular chemistry	Halophiles, methanogens, thermacidophiles

There are four important eucaryotic organisms in the activated sludge process. These organisms are fungi, protozoa, rotifers, and nematodes. These free-living (non-disease-causing) eucaryotes enter wastewater treatment plants through inflow and infiltration (I/I) as soil and water organisms.

FUNGI

Fungi usually are saprophytic organisms and are classified by their mode of reproduction. As saprophytes they obtain their nourishment from the degradation of dead organic matter. Most fungi are free-living and include yeast, molds, and mushrooms.

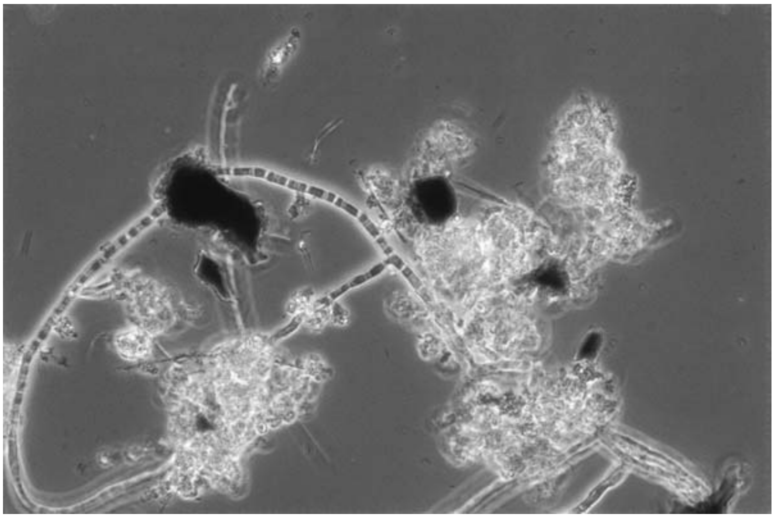

FIGURE 1.2 *Filamentous fungi. Filamentous fungi occasionally bloom in activated sludge processes due to low pH or nutrient deficiency. Filamentous fungi are relatively large in size and display true branching.*

Most fungi are strict aerobes and can tolerate a low pH and a low nitrogen environment. Although fungi grow over a wide range of pH values (2–9), the optimum pH for most species of fungi is 5.6, and their nitrogen nutrient requirement for growth is approximately one-half as much as that for bacteria.

In the activated sludge process filamentous fungi (Figure 1.2) may proliferate and contribute to settleability problems in secondary clarifiers. The proliferation of filamentous fungi is associated with low pH (<6.5) and low nutrients. Although filamentous fungi contribute to settleability problems in the activated sludge process, the presence of a large and diverse population of fungi is desired for the treatment of some industrial wastewaters and composting of organic wastes. Fungi have the ability to degrade cellulose, tolerate low nutrient levels, and grow in the presence of low moisture and low pH conditions.

An example of a unicellular fungus is the yeast (*Saccharomyces*). They reproduce by budding. Budding results in the production of numerous daughter cells (offspring) from one parent cell. Yeast can degrade organic compounds to carbon dioxide and water with the use of free molecular oxygen (O_2), or as facultative anaerobes they can degrade organic compounds such as sugars to ethanol (CH_3CH_2OH) in the absence of free molecular oxygen.

PROTOZOA

Protozoa are unicellular organisms. Most protozoa are free-living and solitary, but some do form colonies. Most protozoa are strict aerobes, but some including amoebae and flagellates can survive anaerobic conditions.

In the activated sludge process, protozoa are placed commonly in five groups according to their means of locomotion. These groups are amoebae (Figure 1.3),

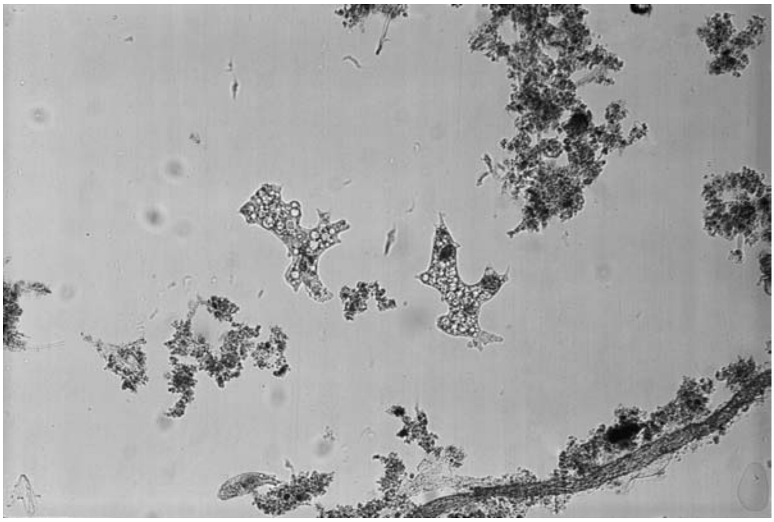

FIGURE 1.3 *Amoeba. The amoeba is a single-celled organism that moves by a pseudopodia ("false-foot") mode of locomotion—that is, the streaming of cytoplasm against the cell membrane.*

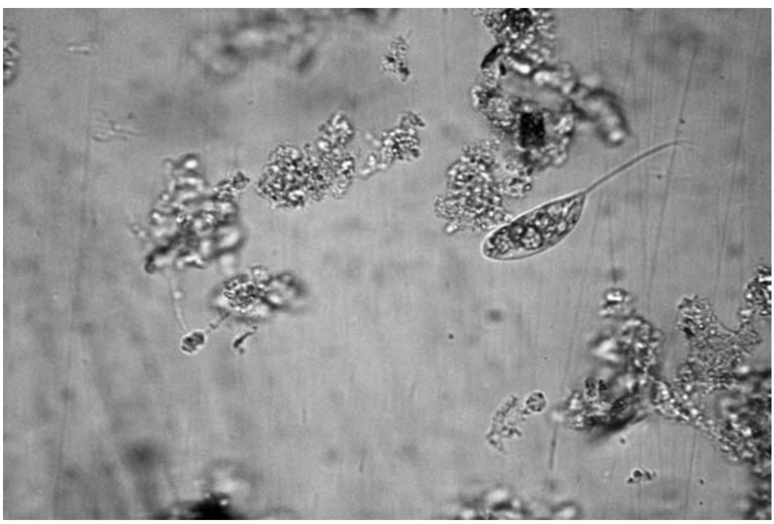

FIGURE 1.4 *Flagellate. The flagellate is a single-celled organism that moves by the beating action of one (flagellum) or more (flagella) whip-like structures.*

flagellates (Figure 1.4), free-swimming ciliates (Figure 1.5), crawling ciliates (Figure 1.6), and stalked ciliates (Figure 1.7).

Ciliated protozoa are the most important groups of protozoa in the activated sludge process. They possess short hair-like structures or cilia that beat in unison to produce a water current for locomotion and food gathering—that is, to bring bacteria into their mouth opening (Figure 1.8). Ciliated protozoa provide the following benefits to the activated sludge process:

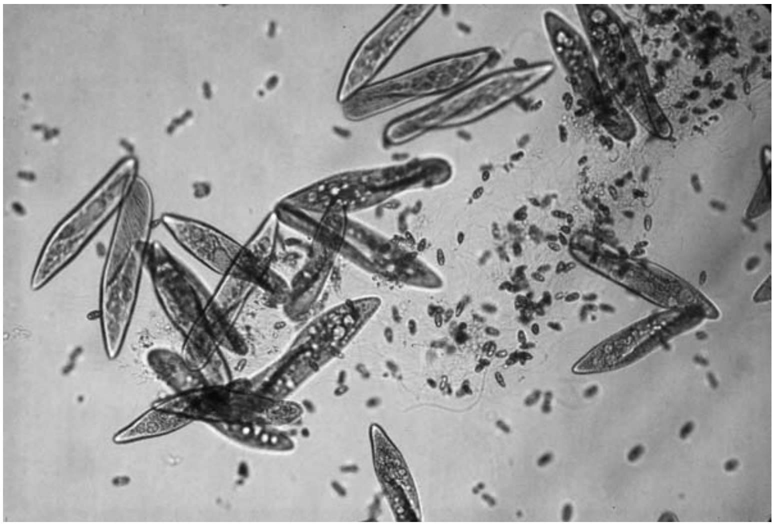

FIGURE 1.5 *Free-swimming ciliate. The free-swimming ciliate is a single-celled organism that moves by the beating action of hair-like structures or cilia that are found in rows that cover the entire surface of the organism.*

FIGURE 1.6 *Crawling ciliate. The crawling ciliate is a single-celled organism that moves by the beating action of hair-like structures or cilia that are found in rows that cover only the ventral or "belly" surface of the organism.*

- Add weight to floc particles and improve their settleability.
- Consume dispersed cells and cleanse the waste stream.
- Produce and release secretions that coat and remove fine solids (colloids, dispersed cells, and particulate material) from the bulk solution to the surface of floc particles.
- Recycle nutrients (nitrogen and phosphorus) through their excretions.

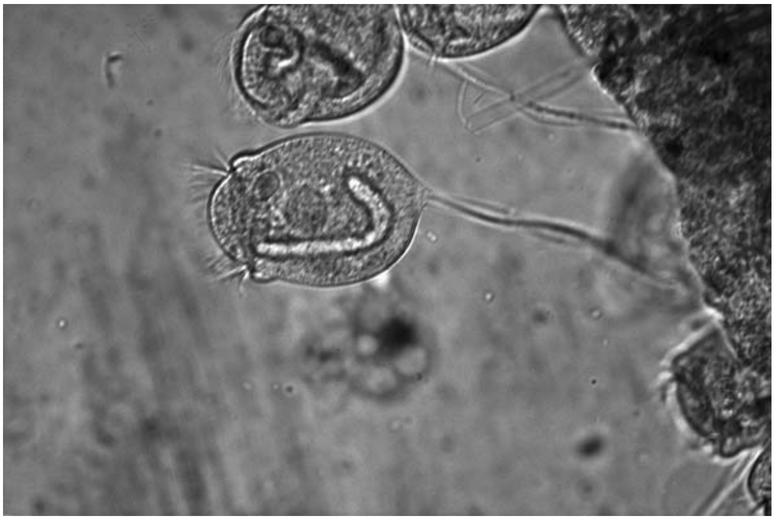

FIGURE 1.7 *Stalked ciliate. The stalked ciliate is a single-celled organisms that moves by the beating action of hair-like structures or cilia that are found in rows that surround only the mouth opening of the organism. Some stalked ciliates may grow in a colony, and some by "spring" by means of a contractile filament or myoneme in the posterior portion or "stalk" of the organism.*

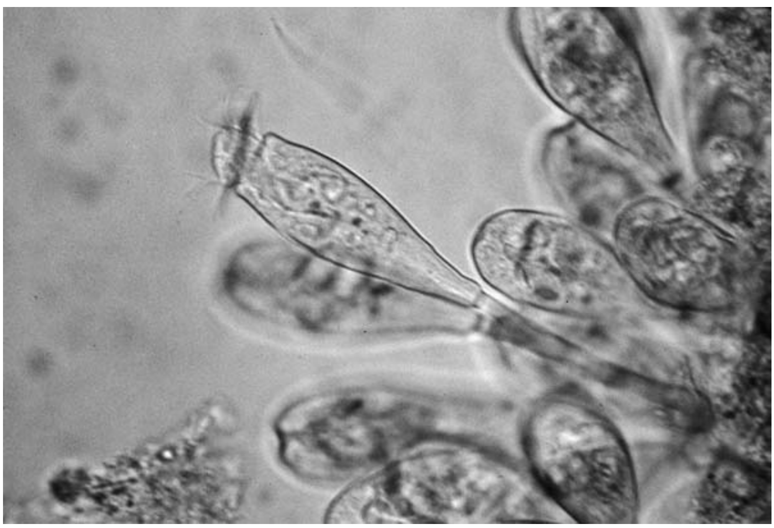

FIGURE 1.8 *Cilia surrounding the mouth opening of a stalk ciliated protozoa.*

ROTIFERS AND NEMATODES

Rotifers (Figure 1.9) and nematodes (Figure 1.10) are multicellular microscopic animals (metazoa) that also provide numerous benefits to the activated sludge process. In addition to these benefits provided by the ciliated protozoa, the metazoa burrow into floc particles. The burrowing action promotes acceptable bacterial activ-

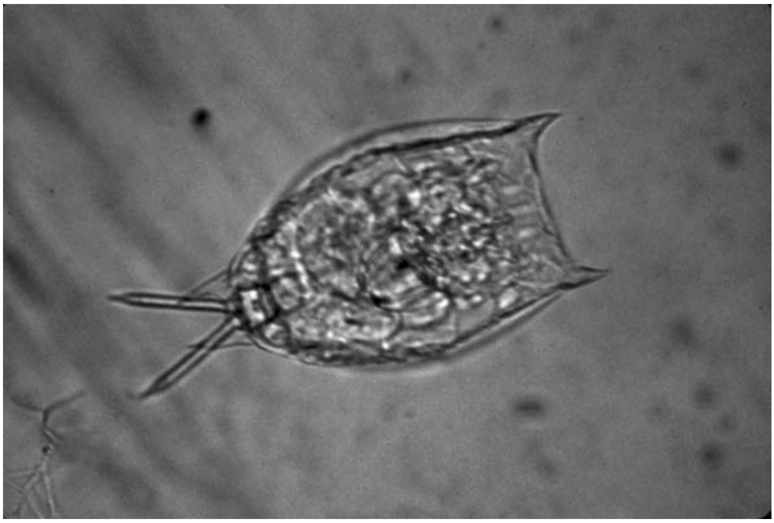

FIGURE 1.9 *Rotifer in free-swimming mode.*

FIGURE 1.10 *Free-living nematode.*

ity for the degradation of substrates in the core of the floc particle by permitting the penetration of dissolved oxygen, nitrate (NO_3^-), substrates, and nutrients. Substrates are the carbon and energy sources used by bacteria for cellular growth and activity. With exceptions, substrates consist of carbonaceous, biochemical oxygen demand (cBOD) compounds and nitrogenous, biochemical oxygen demand (nBOD) compounds.

BACTERIA

The most important organisms in biological, wastewater treatment plants are the bacteria—eubacteria and archaebacteria. Recognition of the distinction between these two groups of organisms or domains (Bacteria and Archaea) is relatively recent, and it is common for species of both groups to be referred to as bacteria. Bacteria enter wastewater treatment plants through fecal waste and I/I as soil and water organisms.

The archaebacteria consist of the halophiles, thermacidophiles, and methanogens. Only the methanogens or methane-forming bacteria are of importance in wastewater treatment plants. Methane-forming bacteria stabilize wastes through their conversion to methane (CH_4).

Halophiles (salt-loving) or halophilic bacteria are found in saltwater where the salt concentration (3.5%) is optimum for their growth. These marine organisms need an elevated sodium ion (Na^+) concentration in their environment in order to maintain the integrity of their cell wall and an elevated potassium ion (K^+) concentration in their cells for proper enzymatic activity. Halophilic bacteria along with cyanobacteria and photosynthetic bacteria produce gas vacuoles. These vacuoles are used to regulate cell buoyancy; that is, they are a cellular floatation device.

Thermacidophiles (high-temperature-loving and low-pH-loving) or thermacidophilic bacteria perform no role in wastewater treatment plants. These organisms live in hot acidic environments such as volcanic vents on the ocean floor.

2

Microbial Ecology

Microbial ecology as applied to the activated sludge process and the anaerobic digester is the review of the significant groups of wastewater organisms and the operational conditions in each biological treatment unit. This review includes the effects of abiotic and biotic factors upon the organism including their activity and growth—that is, wastewater treatment efficiency. Biological treatment units are simply biological amplifiers—that is, the removal or degradation of waste results in an increase in the number of organisms (sludge). Therefore, acceptable activity and growth of the organisms or biomass is acceptable wastewater treatment.

Collectively, all organisms and operational conditions are interrelated by the transfer of carbon and energy through a food chain (Figure 2.1) or more appropriately a food web (Figure 2.2). Within the food web there are numerous habitats, niches, and relationships (symbiotic and predator–prey) that determine the success or failure of the biological treatment unit to treat wastewater.

Abiotic factors are the nonliving components or operational conditions in a biological treatment unit that affect the activity and growth of the biomass. For example, a decrease in pH of the activated sludge process favors the proliferation of filamentous fungi and disfavors the growth of bacteria, and a decrease in pH in the anaerobic digester favors the growth of fermentative bacteria and disfavors the growth of methane-forming bacteria. Biotic factors are the living components or organisms in a biological treatment unit. Each organism has an effect upon other organisms (predator–prey and symbiotic relationships) and abiotic factors in the biological treatment unit. For example, free-swimming ciliated protozoa increase in number in the presence of large numbers of dispersed bacterial cells. However, during floc formation the number of dispersed bacterial cells decreases and, consequently, the number of free-swimming ciliated protozoa decrease in number. In the

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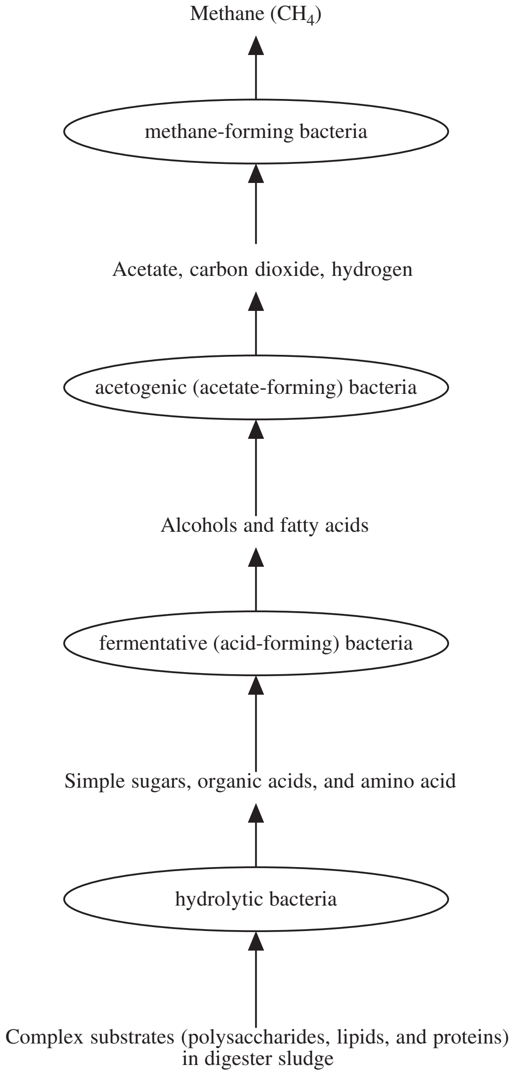

FIGURE 2.1 *Transfer of carbon and energy through an anaerobic digester food chain. Carbon and energy enter the anaerobic digester in the form of large, complex organic molecules such as polysaccharides, lipids, and proteins. These compounds are degraded to smaller and simpler compounds through step-by-step biochemical reactions by a diversity of bacterial groups to methane. Through each biochemical reaction, bacteria are produced.*

activated sludge process, nitrifying bacteria decrease alkalinity and pH, while denitrifying bacteria increase alkalinity and pH.

In the anaerobic digester, four different groups of bacteria have a symbiotic relationship (Figure 2.3). Fermentative bacteria increase the quantities of carbon dioxide and hydrogen (H_2), while hydrogenotrophic methane-forming bacteria decrease the quantities of carbon dioxide and hydrogen. Hydrogenotrophic methane-forming bacteria combine carbon dioxide and hydrogen to form methane. By using

FIGURE 2.2 *Transfer of carbon and energy through an activated sludge food web. Carbon and energy enter the activated sludge process in the form of cBOD and nBOD and alkalinity. These carbon and energy substrates are used by a variety of organisms in the activated sludge process, and many of the organisms that grow from these substrates in turn are used as substrates by other organisms. The transfer of carbon and energy in the activated sludge process is between many groups of organisms in a "web-like" pattern.*

hydrogen to produce methane, the hydrogen pressure in the anaerobic digester decreases. This decrease in hydrogen pressure enables acetogenic bacteria to produce acetate (CH_3COOH). Acetoclasitc methane-forming bacteria use acetate to produce methane and carbon dioxide. The hydrogenotrophic methane-forming bacteria also combine the carbon dioxide produced by the acetogenic bacteria with hydrogen to form methane.

However, when the hydrogenotrophic methane-forming bacteria are inhibited, the hydrogen pressure increases in the anaerobic digester. The increase in hydrogen pressure inhibits acetogenic bacteria. This results in a decrease in acetate production and consequently a decrease in methane production by acetoclastic methane-forming bacteria.

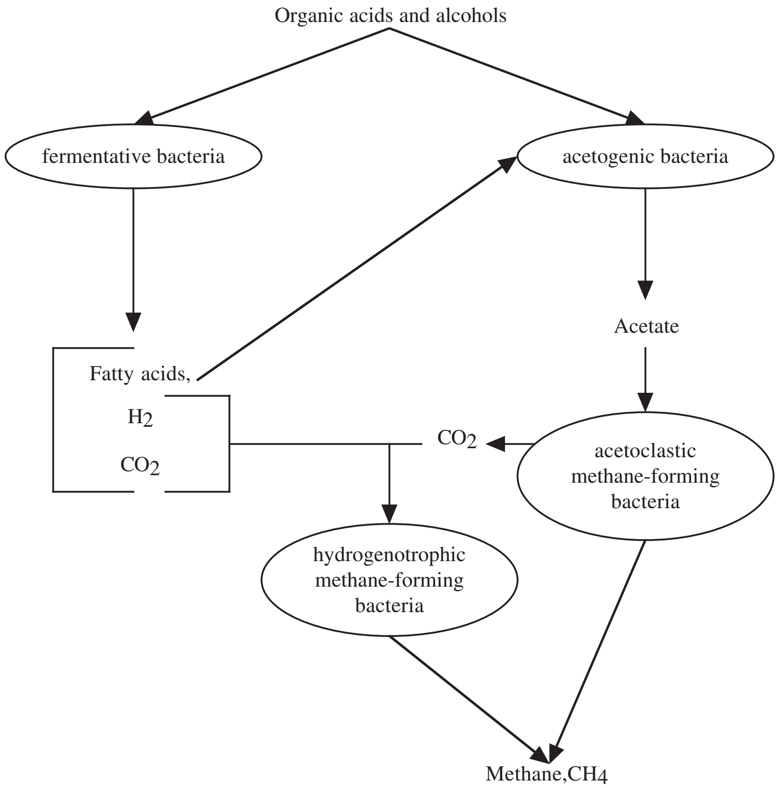

FIGURE 2.3 *Symbiotic relationships in an anaerobic digester.*

ACTIVATED SLUDGE PROCESS, SIGNIFICANT ABIOTIC AND BIOTIC FACTORS

Significant abiotic factors in the activated sludge process include alkalinity, ionized ammonia (NH_4^+), dissolved oxygen, hydraulic retention time (HRT), nutrients, pH, quantity and types of substrates, return activated sludge (RAS) rate, temperature, toxic wastes, and turbulence. Significant biotic factors include denitrifying bacteria, filamentous organisms, floc-forming bacteria, mean cell residence time (MCRT) or sludge age, mixed liquor volatile suspended solids (MLVSS) concentration, nitrifying bacteria, and relative abundance and dominant groups of protozoa.

ANAEROBIC DIGESTER, SIGNIFICANT ABIOTIC AND BIOTIC FACTORS

Significant abiotic factors in the anaerobic digester include ionized ammonia (NH_4^+), alkalinity, carbon dioxide, hydrogen, nitrate (NO_3^-), nutrients, pH, quantity and types of substrates, sulfate (SO_4^{2-}), temperature, toxic wastes, and volatile acids. Significant biotic factors include acetogenic bacteria, fermentative (acid-forming) bacteria, hydrolytic bacteria, methane-forming bacteria, solids retention time (SRT), sulfur-reducing bacteria, and volatile suspended solids (VSS).

Within each biological treatment unit, different groups of organisms transfer carbon and energy from one trophic (food) level to the next trophic level (Figures 2.1 and 2.2). In the activated sludge process, carbon and energy enter the process as nonliving substrates or BOD. In the soluble form, BOD is absorbed by a variety of organisms, mostly bacteria. Some of the absorbed BOD is transformed into new bacterial cells (sludge) or living BOD. Each organism in the food chain or food web represents BOD, because living organisms are consumed (predator–prey relationships); for example, bacteria are consumed by protozoa and metazoa, and dead organisms are decomposed by living organisms.

As carbon and energy move up the food chain or food web, the quantity (weight) or biomass of each group of organism in the higher trophic level decreases (Figure 2.4). With each move to a higher trophic level, more carbon and energy are lost in waste products and heat, thus leaving less carbon and energy for the synthesis of cellular material (biomass). However, the transfer of carbon and energy from one group of organisms to another is not as simple as a food chain, because several groups of organisms often feed off the same substrates or lower trophic level. The transfer or movement of carbon and energy here is referred to as a food web. The food web better illustrates the activated sludge process than the food chain, because organisms here work mostly side-by-side (Figure 2.2). For example, nitrifying bacteria oxidize nBOD, while organotrophic bacteria oxidize cBOD, and biological phosphorus removal occurs, while organotrophic bacteria oxidize cBOD.

The food chain better illustrates the anaerobic digester than the food web, because bacteria in the digester work in step-by-step fashion to produce methane

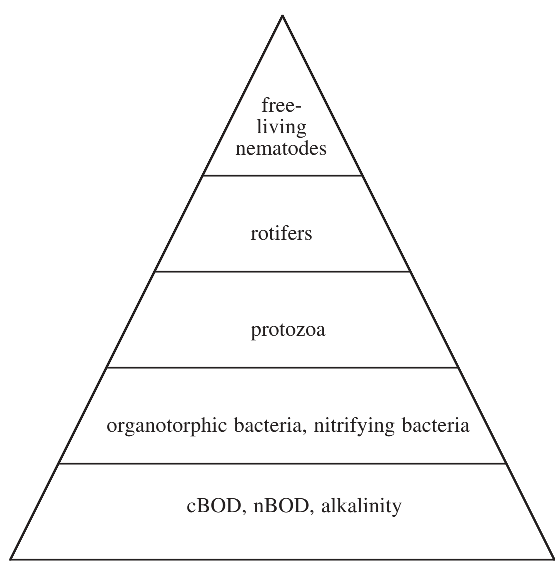

FIGURE 2.4 *Activated sludge food pyramid. As carbon and energy move up the food pryramid from one tropic level to the next, a smaller quantity of organisms (biomass) is produced in the higher trophic level, due to the loss of some carbon and energy as carbon dioxide and waste products. The loss occurs as a result of the biochemical reactions involved in the degradation of the substrate (biomass) from the lower trophic level.*