2
Fundamentals of Biochemical Operations

Before we begin the systematic study of biochemical operations, it is necessary to develop a clear picture of what wastewater treatment engineers hope to accomplish through their use. Furthermore, if we are to develop the capability for their design, it is necessary to understand what is happening within them and to recognize the role of various types of microorganisms in those events.

2.1 OVERVIEW OF BIOCHEMICAL OPERATIONS

Biochemical operations only alter and destroy materials that microorganisms act upon, i.e., those that are subject to biodegradation or biotransformation. If soluble pollutants are resistant to microbial attack, they are discharged from a biochemical operation in the same concentration that they enter it, unless they are acted on by chemical or physical mechanisms such as sorption or volatilization (see Chapter 22). Insoluble pollutants entering a suspended growth biochemical operation become intermixed with the biomass and, for all practical purposes, are inseparable from it. Consequently, engineers consider this mixture of biomass and insoluble pollutants as an entity, calling it mixed liquor suspended solids (MLSS). If insoluble pollutants are biodegradable, their mass is reduced. On the other hand, if they are nonbiodegradable, their only means of escape from the system is through MLSS wastage and their mass discharge rate in the wasted MLSS must equal their mass input rate to the system. Attached growth processes usually have little impact on nonbiodegradable insoluble pollutants, although in some cases those pollutants are flocculated and settled along with the biomass discharged from the operation.

When wastewater treatment engineers design biochemical operations they use natural cycles to accomplish in a short time what nature would require a long time to accomplish, often with environmental damage. For example, if biodegradable organic matter were discharged to a stream, the bacteria in that stream would use it as a source of carbon and energy (electrons) for growth (see Chapter 1). In the process, they would incorporate part of the carbon into new cell material and the rest would be oxidized to carbon dioxide to provide the energy for that synthesis. The electrons removed during the oxidation would be transferred to oxygen in the stream, but if the supply of oxygen were insufficient, the dissolved oxygen (DO) concentration would be depleted, killing fish and causing other adverse effects. On the other hand, in a well designed biochemical operation, microbial growth is allowed to occur in

an environment where the appropriate amount of oxygen can be supplied, thereby destroying the organic matter and allowing the treated wastewater to be discharged without environmental harm.

The two major cycles employed in biochemical operations are the carbon and nitrogen cycles. Actually, most biochemical operations only use half of the carbon cycle, i.e., the oxidation of organic carbon, releasing carbon dioxide. While some biochemical operations use algae and plants to fix carbon dioxide and release oxygen, thereby using the other half of the carbon cycle, they are not as widely applied and will not be covered in this book. However, almost all of the nitrogen cycle is used, as illustrated in Figure 2.1. In domestic wastewaters, most nitrogen is in the form of ammonia (NH_3) and organic nitrogen, whereas industrial wastewaters sometimes contain nitrate (NO_3^-) nitrogen as well. Organic nitrogen is in the form of amino groups (NH_2^-), which are released as ammonia—in the process called ammonification—as the organic matter containing them undergoes biodegradation. The form in which bacteria incorporate nitrogen during growth is as ammonia. If an industrial wastewater has insufficient ammonia or organic nitrogen to meet the growth needs of the bacteria, but contains nitrate or nitrite (NO_2^-) nitrogen, they will be converted to ammonia through assimilative reduction for use in cell synthesis. On the other hand,

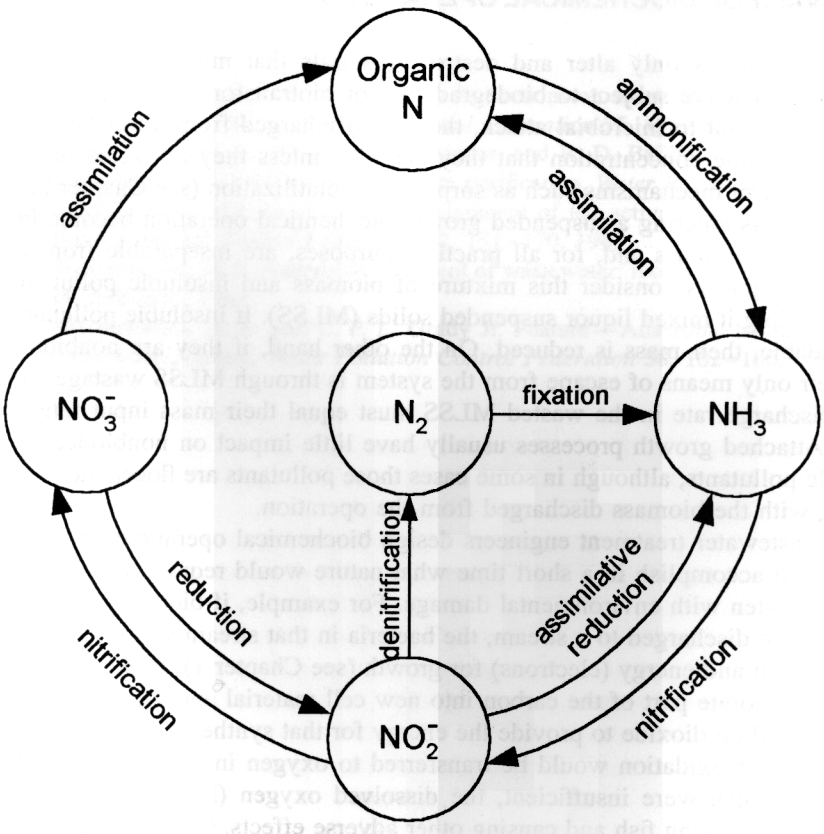

Figure 2.1 The nitrogen cycle.

if a wastewater contains ammonia-N in excess of that needed for cell synthesis, nitrification can occur, where the excess ammonia-N is oxidized to nitrate-N, going through the intermediate, nitrite. Discharge of nitrate to a receiving water is preferable to discharge of ammonia because nitrification in the receiving water can deplete the DO, just as degradation of organic matter can. In some cases, however, discharge of nitrate can have a deleterious effect on the receiving water, and thus some effluent standards limit its concentration. In that case, biochemical operations that use denitrification to convert nitrate and nitrite to nitrogen gas must be used to reduce the amount of nitrogen in the effluent. The only step in the nitrogen cycle not normally found in biochemical operations is nitrogen fixation, in which nitrogen gas is converted to a form that can be used by plants, animals, and microorganisms.

2.2 MAJOR TYPES OF MICROORGANISMS AND THEIR ROLES

Modern molecular biology has allowed scientists to investigate the relatedness among organisms by analysis of the nucleotide sequences within certain segments of their genes. Organization of this information into a phylogenetic tree has revealed that organisms fall into three primary groupings, or domains: Archaea, Bacteria, and Eucarya.[83] Members of the domains Archaea and Bacteria are microscopic and procaryotic,[a] i.e., they lack a nuclear membrane, whereas members of the domain Eucarya are eucaryotic, i.e., they have a nuclear membrane, and vary in size from microscopic (e.g., protozoa) to macroscopic (e.g., animals). The workhorses of biochemical operations belong to the domains Bacteria and Archaea, but protozoa and other microscopic Eucarya have a role as well. Thus it is important to have a clear picture of what various microorganisms do.

2.2.1 Bacteria

Bacteria can be classified in many ways; however, the most important from an engineering perspective is operational. Consequently, we will focus on it.

Like all organisms, members of the domain Bacteria derive energy and reducing power from oxidation reactions, which involve the removal of electrons. Thus, the nature of the electron donor is an important criterion for their classification. The two sources of electrons of most importance in biochemical operations are organic and inorganic compounds that are present in the wastewater or released during treatment. Bacteria that use organic compounds as their electron donor and their source of carbon for cell synthesis are called heterotrophic bacteria, or simply heterotrophs. Since the removal and stabilization of organic matter are the most important uses of biochemical operations, it follows that heterotrophic bacteria predominate in the systems. Bacteria that use inorganic compounds as their electron donor and carbon

[a]Recognition of the distinction between Bacteria and Archaea is relatively recent. Consequently, it is still common for members of both domains to be referred as bacteria, in reference to their procaryotic nature. In this book, the term bacteria (with an upper case "b") will be used to refer to procaryotes in general, without regard to their domain.

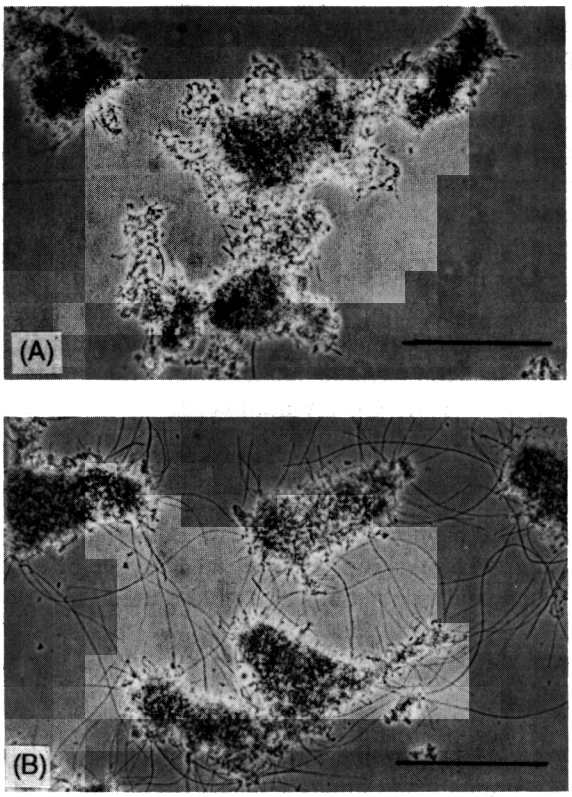

Figure 2.2 Photomicrographs of activated sludge floc: (a) Good settling biomass with optimal filaments; (b) poor settling biomass with excessive filaments. [Courtesy of M. G. Richard (Colorado State Univ.) and David Jenkins (Univ. California, Berkeley.)]

ganisms in anaerobic systems are the sulfate-reducing bacteria. It is generally desirable to design anaerobic operations to produce methane because it is a valuable product. If a wastewater contains high concentrations of sulfate, however, sulfate-reducing bacteria will compete for the electron donor, producing sulfide as a product. This not only reduces the amount of methane produced, but results in a product that is both dangerous and undesirable in most situations. Wastewater treatment engineers need to be aware of the growth characteristics of such nuisance organisms so that systems that discourage or prevent their growth can be designed.

Bacteria can also be classified according to their function in biochemical operations. Many act as primary degraders and attack the organic compounds present in the wastewater, beginning their degradation. If an organic compound is one normally found in nature (biogenic), the primary degraders usually will completely metabolize it in an aerobic environment, converting it to carbon dioxide, water, and new biomass. Such ultimate destruction is called mineralization and is the goal of most wastewater treatment systems. On the other hand, if an organic compound is synthetic and foreign to the biosphere (xenobiotic), it is possible that no single type of bacteria will be able to mineralize it. Instead, a microbial consortium may be

required, with secondary degraders living on the metabolic products excreted by the primary degraders. The more complex the organic compounds found in a wastewater, the more important secondary degraders will be. Secondary degraders are common in anaerobic environments, however, even when biogenic compounds are being degraded, because of the specialized needs of the bacteria involved. Other functions that are important in wastewater treatment systems are the production and elimination of nitrate-N through nitrification and denitrification, respectively. Consequently, it is not surprising that bacteria are classified according to those functions, as nitrifiers and denitrifiers. While the nitrifiers constitute a highly specialized group containing a limited number of species of aerobic, chemoautotrophic bacteria, the denitrifying bacteria constitute a diverse group of facultative heterotrophic bacteria containing many species. Finally, some species of bacteria have the ability to store and release phosphate in response to cyclical environmental conditions. Because they contain quantities of phosphate well in excess of other bacteria, these bacteria are often called phosphate accumulating organisms (PAOs).

As with the classification of pollutants in wastewaters, the classifications listed above are not exclusive, but overlap, with members of the domain Bacteria playing many roles. Nevertheless, these simple classification schemes are very helpful in describing the events occurring in biochemical operations and will be used throughout this book.

2.2.2 Archaea

Many Archaea are capable of growing in extreme environments, such as high temperatures (up to 90°C), high ionic strength, and highly reduced conditions. Consequently, members of this domain were first thought to be restricted to growth in such environments, although that proved to be incorrect. More recent studies have shown that Archaea are abundantly distributed in a wide variety of environments.[51] As our knowledge of the Archaea expands it is likely that wastewater treatment engineers will find more applications for them. Currently, however, their major use in biological wastewater treatment is in anaerobic operations, where they play the important role of producing methane. Methane-producing Archaea, commonly called methanogens, are obligate anaerobes that bring about the removal of organic matter from the liquid phase by producing an energy rich gas of low solubility. This allows capture of the energy in the pollutants in a useful form. Because methanogens are very limited in the substrates they can use, they grow in complex microbial communities with Bacteria, which carry out the initial attack on the pollutants and release the methanogens' substrates as fermentation products.

2.2.3 Eucarya

Although fungi can use soluble organic matter in competition with Bacteria, they seldom compete well in suspended growth cultures under normal conditions, and thus do not usually constitute a significant proportion of the microbial community.[22] On the other hand, when the supplies of oxygen and nitrogen are insufficient, or when the pH is low, fungi can proliferate, causing problems similar to those caused by filamentous bacteria. In contrast to suspended growth cultures, fungi commonly play an important role in attached growth cultures, making up a large part of the

biomass.[76] Under certain conditions, however, they can also become a nuisance in such systems by growing so heavily as to block interstices and impede flow.

Protozoa play an important role in suspended growth cultures by grazing on colloidal organic matter and dispersed bacteria, thereby reducing the turbidity remaining after the biofloc has been removed by sedimentation. Protozoa are also known to contribute to bioflocculation, but their contribution is thought to be less important than that of the floc-forming bacteria.[11] Although some protozoa can utilize soluble organic compounds for growth, it is doubtful that they can compete effectively with bacteria in that role and thus soluble substrate removal is generally considered to be due to bacterial action. Protozoa also play a significant role in attached growth bioreactors where the protozoan community is usually richer than it is in suspended growth cultures. Nevertheless, their role appears to be similar to that in suspended growth cultures.

Other Eucarya in suspended growth cultures are usually limited to rotifers and nematodes, but their presence depends very much on the way in which the culture is grown. Although these organisms feed upon protozoa and biofloc particles, their contribution to biochemical operations using suspended growth cultures is largely unknown because little change in process performance can be attributed to their presence. In contrast, because attached growth bioreactors provide a surface upon which higher organisms can graze, it is not uncommon for such reactors to have highly developed communities of macroinvertebrates in addition to rotifers and nematodes.[22] The nature of those communities depends largely on the physical characteristics of the bioreactor and in some cases the presence of the higher community has no deleterious effect on system performance. In other cases, however, the grazing community can disrupt development of the primary biofilm that is responsible for the removal of the pollutants, leading to a deterioration in system performance.

2.3 MICROBIAL ECOSYSTEMS IN BIOCHEMICAL OPERATIONS

An ecosystem is the sum of interacting elements (both biological and environmental) in a limited universe. Consequently, each biochemical operation will develop a unique ecosystem governed by the physical design of the facility, the chemical nature of the wastewater going to it, and the biochemical changes wrought by the resident organisms. The microbial community which develops in that ecosystem will be unique from the viewpoint of species diversity, being the result of physiological, genetic, and social adaptation. Thus, it is impossible to generalize about the numbers and types of species that will be present. Nevertheless, it would be instructive to consider the general nature of the community structures in biochemical operations and relate them to the environments in which the operations are performed. The objective of such an exercise is not the simple listing of the organisms present, but rather an understanding of the role that each important group plays in the operation.

Because the biochemical processes in aerobic and anoxic environments are based on respiration, whereas those in anaerobic environments are based on fermentation, there are large differences in the microbial communities involved. Thus, the biochemical environment provides a logical way for dividing this discussion.

2.3.1 Aerobic/Anoxic Operations

Suspended Growth Bioreactors. Activated sludge, aerated lagoons, and aerobic digesters have similar microbial ecosystems, although they differ somewhat in the relative importance of various groups. The microorganisms in those operations are all Bacteria and microscopic Eucarya, and generally may be divided into five major classes: (1) floc-forming organisms, (2) saprophytes, (3) nitrifying bacteria, (4) predators, and (5) nuisance organisms.[59] With the exception of nitrifying bacteria, these are not distinct physiological groups and, in fact, any particular organism may fit into more than one category at a time or may change categories as the selective pressures within the community change.

Floc-forming organisms play a very important role in suspended growth biochemical operations because without them the biomass cannot be separated from the treated wastewater nor can colloidal-sized organic pollutants be removed. Figure 2.2a shows typical, well-settling biomass. Originally it was thought that the bacterium *Zooglea ramigera* was primarily responsible for floc formation, but it has now been shown that a variety of bacteria are capable of flocculation,[58] although they constitute only a small percentage of the species found in a floc particle.[53] Classification of organisms into the floc-forming group is complicated by the fact that protozoa and fungi can also cause bacteria to flocculate.[11,58,59] Nevertheless, the predominant floc-forming organisms are generally considered to be bacteria,[11] with *Zooglea ramigera* playing an important role.[65] Flocculation is thought to be caused by aggregative growth and natural polyelectrolytes, although their origin is uncertain.

Saprophytes are organisms responsible for the degradation of organic matter. These are primarily heterotrophic bacteria and include most of those considered to be floc formers. Nonflocculent bacteria are also involved, but are entrapped within the floc particles. The saprophytes can be divided into primary and secondary degraders, as discussed previously, and the larger the number of substrates, the more diverse the community will be. The principal saprophytic genera are gram-negative and include *Achromobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus,* and *Pseudomonas*.[57]

Nitrification is the conversion of ammonia-N to nitrate-N and it may be performed by either heterotrophic or autotrophic bacteria.[52] In spite of the fact that over a hundred heterotrophic species have been cited as forming nitrite from ammonia,[78] significant amounts of nitrate are not thought to be generated heterotrophically in natural systems,[14] although studies suggest that this assumption should be investigated further.[55] Nevertheless, nitrification in wastewater treatment systems is generally considered to be due to autotrophic bacteria, primarily of the genera *Nitrosomonas* and *Nitrobacter*, which appear to grow in close physical association.[48] *Nitrosomonas* oxidizes ammonia-N to nitrite-N with hydroxylamine as an intermediate product, whereas *Nitrobacter* oxidizes nitrite-N to nitrate-N in a single step. The fact that nitrifying bacteria are autotrophic does not mean that they cannot incorporate exogenous organic compounds while obtaining their energy from inorganic oxidation, because they can.[32] The amount of such uptake will be small and will vary with the growth conditions, however, so that most equations depicting the stoichiometry of nitrification ignore it and use carbon dioxide as the sole carbon source. Nitrifying bacteria have several unique growth characteristics that are important to their impact on and survival in biochemical operations. The first is that

their maximal growth rate is smaller than that of heterotrophic bacteria. Consequently, if suspended growth bioreactors are operated in a way that requires the bacteria to grow rapidly, the nitrifying bacteria will be lost from the system and nitrification will stop even though organic substrate removal will continue. Second, the amount of biomass formed per unit of nitrogen oxidized is small. As a result, they may make a negligible contribution to the MLSS concentration even when they have a significant effect on process performance.

The main predators in suspended growth bioreactors are the protozoa, which feed on the bacteria. About 230 species have been reported to occur in activated sludge and they may constitute as much as 5% of the biomass in the system.[58] Ciliates are usually the dominant protozoa, both numerically and on a mass basis. Almost all are known to feed on bacteria and the most important are either attached to or crawl over the surface of biomass flocs. On occasion, both amoeba and flagellates may be seen in small numbers, but they are not thought to play a major role in well-settling, stable communities. As discussed earlier, it has been suggested that protozoa play a secondary role in the formation of biomass flocs and contribute to the absence of dispersed bacteria and colloidal organic material in stable communities.[11]

Nuisance organisms are those that interfere with proper operation of a biochemical reactor when present in sufficient numbers. In suspended growth bioreactors, most problems arise with respect to removal of the biomass from the treated wastewater, and are the result of filamentous bacteria and fungi. Although a very small number of filamentous bacteria is desirable to strengthen floc particles, too large a number is undesirable.[70] Even a small percentage by weight in the microbial community can make the effective specific gravity of the biomass flocs so low that the biomass becomes very difficult to remove by gravity settling. This leads to a situation known as bulking. A poor-settling biomass is shown in Figure 2.2b. For many years it was thought that the bacterium *Sphaerotilus natans* was the organism primarily responsible for bulking, but the conditions causing its growth were a puzzle because they appeared to be so contradictory. It was not until the pioneering work of Eikelboom[13] that it was realized that many types of filamentous organisms could be responsible for bulking, and that different organisms were favored by different growth conditions. Today, effective bulking control is based on identification of the causative organism and elimination of the condition favoring its growth.[31] Table 2.1 ranks the most abundant filamentous organisms found in bulking sludges in the United States and Table 2.2 lists the suggested causes for some. In Table 2.2, the term, "low F/M," refers to a low food to microorganism ratio; in other words, the system is being operated with a very low loading of organic matter into it. It should be noted that although *Nocardia* is a commonly found filamentous organism, it does not normally cause bulking because its filaments do not extend beyond the floc particle.[31]

The other major nuisance associated with suspended growth cultures is excessive foaming. This condition is caused primarily by bacteria of the genus *Nocardia* and the species *Microthrix parvicella*.[31] There is still controversy concerning the conditions responsible for excessive foaming in suspended growth cultures. Because *Nocardia* and *M. parvicella* have very hydrophobic cell surfaces, they migrate to air bubble surfaces, where they stay, thereby stabilizing the bubbles and causing foam.[31] Foaming also appears to be related to the concentration of hydrophobic organic compounds at the air–water interface, where they are metabolized by the *Nocardia* and *Nocardia*-like organisms that have collected there.[21]

Table 2.1 Filament Abundance in Bulking and Foaming Activated Sludge in the United States

Rank	Filamentous organism	Percentage of treatment plants with bulking or foaming where filament was observed	
		Dominant	Secondary
1	*Nocardia* spp	31	17
2	type 1701	29	24
3	type 021N	19	15
4	type 0041	16	47
5	*Thiothrix* spp	12	20
6	*Sphaerotilus natans*	12	19
7	*Microthrix parvicella*	10	3
8	type 0092	9	4
9	*Halsicomenobacter hydrossis*	9	45
10	type 0675	7	16
11	type 0803	6	9
12	*Nostocoida limicola* (Types I, II, and III)	6	18
13	type 1851	6	2
14	type 0961	4	6
15	type 0581	3	1
16	*Beggiatoa* spp	1	4
17	fungi	1	2
18	type 0914	1	1
—	all others	1	—

From Ref. 31.

Table 2.2 Conditions Associated with Dominant Filament Types

Suggested causative conditions	Filament types
Low DO	*H. hydrossis*, *M. parvicella*, *S. natans*, type 1701
Low F/M	*M. parvicella*, types 0041, 0092, 0675, 1851
Completely mixed bioreactors	*H. hydrossis*, *Nocardia* spp., *N. limicola*, *S. natans*, *Thiothrix* spp., types 021N, 1701, 1851
Septic wastewater/sulfide	*Beggiatoa*, *Thiothrix* spp., types 021N, 0914
Nutrient deficiency	*S. natans*, *Thiothrix* spp., type 021N; possibly *H. hydrossis*, types 0041, 0675
Low pH	fungi

From Ref. 31.

Although the ecosystems of activated sludge, aerated lagoons, and aerobic digestion are complex, they are not as complicated as those in suspended growth systems accomplishing biological nutrient removal. This is because biological nutrient removal systems also contain anoxic and anaerobic reactors, which provide opportunities for the growth of microorganisms that do not ordinarily grow in totally aerobic systems.

The impact of having appropriately placed anoxic zones in a suspended growth system is to allow the proliferation of denitrifying bacteria. As discussed in Section 2.2.1, they are heterotrophic organisms that use nitrate-N and nitrite-N as electron acceptors in the absence of molecular oxygen. Denitrification can be accomplished by a large number of bacterial genera commonly found in wastewater treatment systems, including *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Proteus*, and *Pseudomonas*,[52] thereby making the establishment of a denitrifying culture relatively easy. However, there is uncertainty concerning the fraction of the heterotrophic bacteria in a biological nutrient removal system that can denitrify,[24] and it may well depend on the nature of the microorganisms entering the system in the wastewater,[25] as well as on the system configuration. Nevertheless, it is evident that the introduction of anoxic zones in suspended growth bioreactors will give a competitive advantage to denitrifying bacteria over heterotrophs that do not denitrify.

As described in Section 2.4.6, the placement of an anaerobic zone at the influent end of an otherwise aerobic suspended growth system establishes the conditions required for proliferation of phosphate accumulating organisms, thereby allowing development of a biomass that is rich in phosphorus. Although bacteria of the genus *Acinetobacter* were originally thought to be the major PAOs,[72] several other bacterial types have also been found to be capable of storing polyphosphate.[34,79] In fact, in one study, *Acinetobacter* was not the predominant PAO present; rather it was an unidentified gram-positive bacteria.[79] Identification of phosphate-accumulating bacteria in wastewater treatment systems is not an easy task because of the complicated growth environment required for the formation of polyphosphate granules. Nevertheless, through the development and application of new techniques, we can expect to learn more in the future about the microbial ecology of these important communities.

The previous discussion has indicated the various types of organisms that can be present in suspended growth bioreactor. However, it is very important to recognize that the types that are present in any given system will depend on the reactor configuration and the biochemical environment imposed. In later chapters we will see how these conditions, which are under engineering control, can be used to select the type of microbial community required to accomplish a specific objective.

Attached Growth Bioreactors. Attached growth bioreactors are those in which the microorganisms grow as a biofilm on a solid support. In a fluidized bed bioreactor (FBBR), the biofilm grows on small particles of sand or activated carbon that are maintained in a fluidized state by the force of water flowing upward. Packed bed bioreactors contain similar support particles, but the water being treated flows over them without displacing them. Thus, in both bioreactor types, the biofilm is surrounded by the fluid containing the substrate being removed. In a trickling filter (TF) or rotating biological contactor (RBC), on the other hand, the biofilm grows on a large surface over which the wastewater flows in a thin film (TF) or moves through

the wastewater (RBC). As a consequence, the fluid shear associated with the latter two is less than that associated with the first two. This has an impact on the type of microbial community involved.

Because FBBRs and packed beds are relatively new, few studies have been done to characterize the microbial communities involved. However, we would expect them to be very similar to those in suspended growth bioreactors, being comprised primarily of bacteria and protozoa. In contrast, TFs and RBCs contain more diverse microbial communities containing many other Eucarya, notably nematodes, rotifers, snails, sludge worms, and larvae of certain insects.[10] This more complex food chain allows more complete oxidation of organic matter, with the net result that less excess biomass is produced. This has the beneficial effect of decreasing the mass of solid material that must be disposed of.

The Bacteria form the base of the food chain by acting on the organic matter in the wastewater being treated. Soluble materials are taken up rapidly, while colloidal-sized particles become entrapped in the gelatinous layer built up by the bacteria to form the biofilm. There they undergo attack by extracellular enzymes, releasing small molecules that can be metabolized. The bacterial community is composed of primary and secondary saprophytes, much like suspended growth bioreactors, including members of the genera *Achromobacterium*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, *Sphaerotilus*, and *Zooglea*.[7] Unlike suspended growth cultures, however, the species distribution is likely to change with position in the reactor. Attached growth reactors can also contain nitrifying bacteria, such as members of the genera *Nitrosomonas* and *Nitrobacter*, which tend to be found in regions of the film where the organic substrate concentration is low.

Quite extensive communities of Eucarya are known to exist in trickling filters.[7,10,11,76] Over 90 species of fungi have been reported, and of these, more than 20 species are considered to be permanent members of the community. Their role is similar to that of the bacteria, i.e., saprophytic. Many protozoa have also been found, with large communities of Sarcodina, Mastigophora, and Ciliata being reported. Their roles are largely those of predators. During warm summer months algae can flourish on the upper surfaces of the biomass. Usually green algae and diatoms predominate. Finally, trickling filters also contain a large metazoan community, consisting of annelid worms, insect larvae, and snails. These feed on the microbial film and in some cases have been responsible for extensive film destruction.

Because of the diverse nature of the microbial community in attached growth bioreactors, the microbial interactions are extremely complex. Unfortunately, even less is known about the impact of these interactions on system performance than is known about them in suspended growth systems.

2.3.2 Anaerobic Operations

The microbial communities in anaerobic operations are primarily procaryotic, with members of both the Bacteria and the Archaea being involved. Although fungi and protozoa have been observed under some circumstances, the importance of eucaryotic organisms is questionable.[75] Thus, the emphasis here will be on the complex and important interactions between the Bacteria and the Archaea that are fundamental to the successful functioning of methanogenic communities. Because those interac-

tions occur in both suspended and attached growth systems, no distinctions will be made between the two.

General Nature of Anaerobic Operations. The multistep nature of anaerobic biochemical operations is depicted in Figure 2.3. Before insoluble organic materials can be consumed, they must be solubilized, just as was necessary in aerobic systems. Furthermore, large soluble organic molecules must be reduced in size to facilitate transport across the cell membrane. The reactions responsible for solubilization and size reduction are usually hydrolytic and are catalyzed by extracellular enzymes produced by bacteria. They are all grouped together as hydrolysis reactions (reaction 1) in Figure 2.3, but in reality many enzymes are involved, such as cellulases, amylases, and proteases. They are produced by the fermentative bacteria that are an important component of the second step, acidogenesis.

Acidogenesis is carried out by members of the domain Bacteria. Amino acids and sugars are degraded by fermentative reactions (reaction 2) in which organic compounds serve as both electron donors and acceptors. The principal products of reaction 2 are intermediary degradative products like propionic and butyric acids and the direct methane precursors, acetic acid and H_2. The H_2 production from fermentative reactions is small and originates from the dehydrogenation of pyruvate by mechanisms that are different from the production of the bulk of the H_2 produced.[18] In contrast, most of the H_2 produced comes from oxidation of volatile and long chain fatty acids to acetic acid (reactions 3 and 4) and arises from the transfer of electrons from reduced carriers directly to hydrogen ions, in a process called anaerobic oxidation.[18] Because of the thermodynamics of this reaction, it is inhibited by high partial pressures of H_2, whereas the production of H_2 from pyruvate is not.

The production of H_2 by anaerobic oxidation is very important to the proper functioning of anaerobic processes. First, H_2 is one of the primary substrates from which methane is formed. Second, if no H_2 were formed, acidogenesis would not result in the oxidized product acetic acid being the major soluble organic product. Rather, the only reactions that could occur would be fermentative, in which electrons released during the oxidation of one organic compound are passed to another organic compound that serves as the electron acceptor, yielding a mixture of oxidized and reduced organic products. Consequently, the energy level of the soluble organic matter would not be changed significantly because all of the electrons originally present would still be in solution in organic form. When H_2 is formed as the reduced product, however, it can escape from the liquid phase because it is a gas, thereby causing a reduction in the energy content of the liquid. In actuality, the H_2 does not escape. It is used as a substrate for methane production, but because methane is removed as a gas, the same thing is accomplished. Finally, if H_2 formation did not occur and reduced organic products were formed, they would accumulate in the liquid because they cannot be used as substrates for methane production. Only acetic acid, H_2, methanol, and methylamines can be used. As shown by reaction 5, some of the H_2 can be combined with carbon dioxide by H_2-oxidizing acetogens to form acetic acid,[86] but since the acetic acid can serve as a substrate for methanogens, the impact of this reaction is thought to be small.

The products of the acidogenic reactions, acetic acid and H_2, are used by methanogens, which are members of the domain Archaea, to produce methane gas. Two groups are involved: (1) aceticlastic methanogens, which split acetic acid into methane and carbon dioxide (reaction 6), and (2) H_2-oxidizing methanogens, which reduce

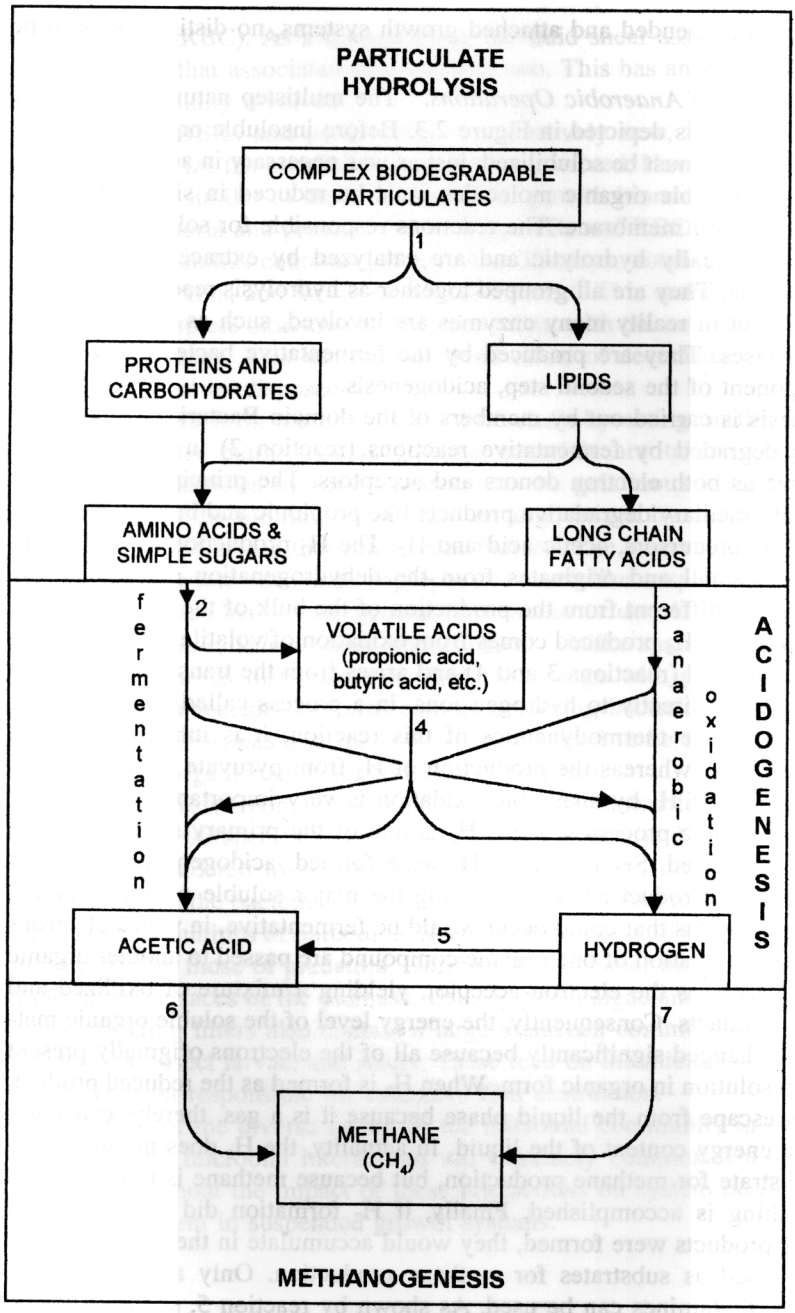

Figure 2.3 Multistep nature of anaerobic operations.

carbon dioxide (reaction 7). It is generally accepted that about two-thirds of the methane produced in anaerobic digestion of primary sludge is derived from acetic acid, with the remainder coming from H_2 and carbon dioxide.[18,86] With the exception of the electrons incorporated into the cell material formed, almost all of the energy removed from the liquid being treated is recovered in the methane. Chemical oxygen demand (COD),[67] a common measure of pollutant strength, is a measure of the electrons available in an organic compound, expressed in terms of the amount of oxygen required to accept them when the compound is completely oxidized to carbon dioxide and water. One mole of methane requires two moles of oxygen to oxidize it to carbon dioxide and water. Consequently, each 16 grams of methane produced and lost to the atmosphere corresponds to the removal of 64 grams of COD from the liquid.[42] At standard temperature and pressure, this corresponds to 0.34 m^3 of methane for each kg of COD stabilized.[43]

Microbial Groups and Their Interactions. The hydrolytic and fermentative bacteria comprise a rather diverse group of facultative and obligately anaerobic Bacteria. Although facultative bacteria were originally thought to be dominant, evidence now indicates that the opposite is true,[33] at least in sewage sludge digesters where the numbers of obligate anaerobes have been found to be over 100 times greater. This does not mean that facultative bacteria are unimportant, because their relative numbers can increase when the influent contains large numbers of them,[29] or when the bioreactor is subjected to shock loads of easily fermentable substrates.[38] Nevertheless, it does appear that most important hydrolytic and fermentative reactions are performed by strict anaerobes, such as *Bacteroides*, *Clostridia*, and *Bifidobacteria*,[66] although the nature of the substrate will determine the species present.

As mentioned previously, the role of H_2 as an electron sink is central to the production of acetic acid as the major end product of acidogenesis. Reactions leading from long chain fatty acids, volatile acids, amino acids, and carbohydrates to acetic acid and H_2 are thermodynamically unfavorable under standard conditions, having positive standard free energies.[86] Thus, when the H_2 partial pressure is high, these reactions will not proceed and instead, fermentations occur, with the results discussed above. Under conditions in which the partial pressure of H_2 is 10^{-4} atmospheres or less, however, the reactions are favorable and can proceed, leading to end products (acetic acid and H_2) that can be converted to methane. This means that the bacteria that produce H_2 are obligately linked to the methanogens that use it. Only when the methanogens continually remove H_2 by forming methane will the H_2 partial pressure be kept low enough to allow production of acetic acid and H_2 as the end products of acidogenesis. Likewise, methanogens are obligately linked to the bacteria performing acidogenesis because the latter produce the substrates required by the former. Such a relationship between two microbial groups is called obligate syntrophy.

While the organisms responsible for the fermentative reactions are reasonably well characterized, less is known about the H_2-producing acetogenic bacteria. This is due in part to the fact that the enzyme system for H_2 production is under very strict control by H_2.[68] As a consequence, early studies which attempted to enumerate the H_2-forming bacteria underestimated them by allowing H_2 to accumulate during testing. However, because H_2 partial pressures are kept low in anaerobic biochemical operations,[71] H_2-forming bacteria play an important role, and thus they have been the subject of more intensive research in recent years. Several species have been

2.3.3 The Complexity of Microbial Communities: Reality Versus Perception

It is apparent from the preceding that the microbial communities in biochemical operations are very complex, involving many trophic levels and many genera and species within a trophic level. Unfortunately, most studies on community structure have been descriptive and the exact roles of many organisms have not even been defined, much less quantified. As a consequence, wastewater treatment engineers have tended to view the communities in biochemical operations as if they were monocultures consisting only of procaryotes of a single species. This is slowly changing, but the models used by engineers still reflect only the procaryotic portion of the community, and its divisions are usually limited to major groups, such as aerobic heterotrophs, floc-formers, denitrifiers, nitrifiers, PAOs, etc. In the chapters to follow, we will be exploring the performance of biochemical operations based on these divisions. While the resulting mathematical descriptions are adequate for establishing a fundamental understanding of system performance, and indeed, even for design, it is important to remember the complex nature of the microbial communities involved, and to temper acceptance of the models accordingly. As engineers and microbiologists continue to work together to understand these fascinating systems, we will eventually be able to consider community structure in a quantitative way, resulting in better system design and performance.

2.4 IMPORTANT PROCESSES IN BIOCHEMICAL OPERATIONS

Regardless of the nature and complexity of the microbial community involved, there are certain fundamental processes that occur universally in biochemical operations. The relative importance of these processes, and hence the outcome from a biochemical operation, depends on the physical configuration of the operation and the manner in which it is operated. Our ability to select and design the appropriate biochemical operation for a specific task depends on our recognition of the importance of the various processes in it and our capability for quantitatively expressing the rates of those processes. In this section we will introduce those processes in qualitative terms; in Chapter 3 we will describe them quantitatively.

2.4.1 Biomass Growth, Substrate Utilization, and Yield

When reduced to their barest essentials, biochemical operations are systems in which microorganisms are allowed to grow by using pollutants as their carbon and/or energy source, thereby removing the pollutants from the wastewater and converting them to new biomass and carbon dioxide, or other innocuous forms. Because of the role of enzymes in microbial metabolism, the carbon and/or energy source for microbial growth is often called the substrate, causing wastewater treatment engineers to commonly refer to the removal of pollutants during biomass growth as substrate utilization. If growth is balanced, which is the case for most (but not all) biochemical operations, biomass growth and substrate utilization are coupled, with the result that the removal of one unit of substrate results in the production of Y units of biomass,

where Y is called the true growth yield, or simply the yield.[b] Because of the coupling between biomass growth and substrate utilization, the rates of the two activities are proportional, with Y as the proportionality factor. Consequently, the selection of one as the primary event (or cause) and the other as the secondary event (or effect) is arbitrary. Both selections are equally correct and benchmark papers have been published using both substrate removal[36] and biomass growth[27] as the primary event. The point of view taken in this book is that biomass growth is the fundamental event, and the rate expressions presented in Chapter 3 are written in terms of it. It should be emphasized, however, that rate expressions for biomass growth and substrate utilization can be interconverted through use of the yield, Y.

Because of the central role that Y plays in the relationship between biomass growth and substrate utilization, it is an intrinsic characteristic. Consequently, a clear understanding of the factors that can influence its magnitude is important. The development of such an understanding requires consideration of the energetics of microbial growth, including energy conservation and energy requirements for synthesis.

Overview of Energetics. Microorganisms require four things for growth: (1) carbon, (2) inorganic nutrients, (3) energy, and (4) reducing power. As mentioned in Section 2.2.1, microorganisms derive energy and reducing power from oxidation reactions, which involve the removal of electrons from the substrate with their ultimate transfer to the terminal electron acceptor. Consequently, the energy available in a substrate depends on its oxidation state, which is indicative of the electrons available for removal as the substrate is oxidized. Highly reduced compounds contain more electrons, and have a higher standard free energy, than do highly oxidized compounds, regardless of whether they are organic or inorganic. As described in Chapter 1, most biochemical operations are used for the removal of soluble organic matter and the stabilization of insoluble organic matter. Consequently, in this discussion we will focus on carbon oxidation by heterotrophic bacteria. Since COD is a measure of available electrons, compounds with a high COD:C ratio are highly reduced, whereas those with a low COD:C rThe carbon in methane is in the most highly reduced state possible, with a COD:C ratio of 5.33 mg COD/mg C, whereas the carbon in carbon dioxide is in the most highly oxidized state with a COD:C ratio of zero. Thus, all organic compounds will have a COD:C ratio between these extremes.

As heterotrophic bacteria oxidize the carbon in organic compounds through their catabolic pathways, they convert them to metabolic intermediates of the central amphibolic pathways that are in a higher oxidation state than either the starting compound or the biomass itself. Those metabolic intermediates are used in the anabolic pathways for cell synthesis, but since they are in a higher oxidation state than the cell material being synthesized from them, electrons must be available in an appropriate form for reducing them. Those electrons arise from the original substrate during its catabolism and are transferred to the anabolic pathways through the use of carriers such as nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which alternate between the oxidized (NAD

[b]Throughout this book, the term "yield" will be considered to be synonymous with "true growth yield."

and NADP) and the reduced (NADH and NADPH) state. Thus NAD and NADP serve as electron acceptors for catabolic reactions, forming NADH and NADPH, which act as electron donors for biosynthetic reactions. The availability of NADH and NADPH is called reducing power.

Biosynthetic reactions also require energy in a form that can be used in coupled reactions to join the amphibolic intermediates into new compounds. That energy is provided primarily by adenosine triphosphate (ATP), and to a lesser degree by other nucleotides. ATP is generated by phosphorylation reactions from adenosine diphosphate (ADP) and when the ATP is used to provide energy in biosynthetic reactions, ADP is released for reuse. ATP can be formed from ADP by two types of phosphorylation reactions: substrate level and electron transport phosphorylation. During substrate level phosphorylation, ATP is formed directly by coupled reactions within a catabolic pathway. Only small amounts of ATP can be generated in this way. Much larger amounts can be generated during electron transport phosphorylation, which occurs as electrons removed during oxidation of the substrate (and carried in NADH) are passed through the electron transport (or terminal respiratory) chain, to the terminal electron acceptor, setting up a proton-motive force.[5] The magnitude of the proton motive force, and consequently, the amount of ATP that can be generated, depends on both the organism and the nature of the terminal electron acceptor.

An important concept to recognize about microbial energetics is that as a compound is degraded, all of the electrons originally in it must end up in the new cell material formed, in the terminal electron acceptor, or in the soluble organic metabolic intermediates excreted during growth. If a compound is mineralized, the amount of metabolic intermediates will be very small, so that essentially all electrons must end up either in the cell material formed or in the terminal acceptor. Because the yield is the amount of cell material formed per unit of substrate destroyed; because the amount of cell material formed depends on the amount of ATP generated; and because the amount of ATP generated depends on the electrons available in the substrate, the organism carrying out the degradation, and the growth environment, it follows that the yield also depends on the nature of the substrate, the organism involved, and the growth environment.

Effects of Growth Environment on ATP Generation. The electron transport chains found in most Bacteria and Eucarya share common features. They are highly organized and are localized within membranes. They contain flavoproteins and cytochromes which accept electrons from a donor like NADH and pass them in discrete steps to a terminal acceptor. All conserve some of the energy released by coupling the electron transfer to the generation of proton motive force, which drives a number of processes, such as the synthesis of ATP from ADP and inorganic phosphate, active transport, and flagellar movement. The electron transport chain in Eucarya is located in the mitochondria and is remarkably uniform from species to species. The electron transport chain in Bacteria is located in the cytoplasmic membrane and exhibits considerable variety among individual species in the identity of the individual components and in the presence or absence of sections of the chain. Nevertheless, the sequential organization of the components of the electron transport chain is determined by their standard oxidation–reduction potentials. Table 2.3 presents the potentials for the array of couples found in mitochondrial electron transport chains.[20] The couples in Bacteria are similar, but not necessarily identical. The transfer is in the direction of increasing redox potential until the final reaction with the terminal

Table 2.3 The Standard Oxidation–Reduction Potentials of a Number of Redox Couples of Interest in Biological Systems

Redox couple	E_0' (mV)
$H_2/2H^+ = 2e^-$	−420
Ferredoxin red./oxid.	−410
$NADPH/NADP^+$	−324
$NADH/NAD^+$	−320
Flavoproteins red./oxid.	−300 to 0
Cyt. b red./oxid.	+ 30
Ubiquinone red./oxid.	+100
Cyt. c red./oxid.	+254
Cyt. a_3 red./oxid.	+385
$O^{2-}/½O_2 + 2e^-$	+820

From Ref. 20.

acceptor is catalyzed by the appropriate enzyme. When the environment is aerobic, oxygen serves as the terminal acceptor and the enzyme is an oxidase.

ATP generation is associated with the transfer of electrons down the electron transport chain through electron transport phosphorylation, although it is not directly coupled to specific biochemical reactions that occur during that transfer.[3,5] Rather, the generation of ATP is driven by the proton motive force through chemiosmosis. The elements of the electron transport chain are spatially organized in the cytoplasmic membrane of Bacteria and the mitochondrial membrane of Eucarya in such a way that protons (hydrogen ions) are translocated across the membrane as the electrons move down the electron transport chain, i.e., toward more positive E_0' values. In Bacteria the transfer is from the cytoplasm (inside the cell) to the periplasmic space (outside the cell); in Eucarya, from inside the mitochondria to outside. The transfer of electrons across the membrane establishes a proton gradient which causes a diffusive counterflow of protons back across the membrane through proton channels established by a membrane-bound ATPase enzyme. This proton counterflow drives the synthesis of ATP from ADP and inorganic phosphate. The number of ATP synthesized per electron transferred to the terminal acceptor depends on the nature and spatial organization of the electron transport chain because they determine the number of protons that are translocated per electron transferred down the chain. In mitochondria, 3 ATP can be synthesized per pair of electrons transferred. However, in Bacteria the number will depend on the organization of the electron transport chain in the particular organism involved. This explains why the amount of ATP synthesized from the oxidation of a given substrate depends on the organism performing the oxidation.

In the absence of molecular oxygen, other terminal acceptors may accept electrons from the electron transport chain, and the redox potentials for them, as well as for various donors, are given in Table 2.4.[20] In order for ATP to be generated by electron transport phosphorylation, the oxidation–reduction potential for the donor redox couple must be smaller (more negative) than the potential for the acceptor redox couple, there must be at least one site of proton translocation in the electron

Table 2.4 The Standard Oxidation–Reduction Potentials of Various Acceptor and Donor Redox Couples

Redox couple	E_0' (mV)
Acceptor	
$\frac{1}{2}O_2/H_2O$	+820
NO_3^-/NO_2^-	+433
NO_2^-/NO	+350
Fumarate/succinate	+ 33
SO_4^{2-}/SO_3^{2-}	− 60
CO_2/CH_4	−244
Donor	
$H_2/2H^+$	−420
$HCOOH/HCO_3^-$	−416
$NADH/NAD^+$	−320
Lactate/pyruvate	−197
Malate/oxaloacetate	−172
Succinate/fumarate	+ 33

From Ref. 20.

transport chain between the final acceptor and the point where the donor contributes its electrons, and the associated free energy change ($\Delta G^{0\prime}$) must exceed 44 kJ [$\Delta G^{0\prime} = -2F \cdot \Delta E_0'$, where F = 96.6 kJ/(V·mol)]. Nitrate and nitrite are important terminal electron acceptors in biochemical operations performing denitrification and the bacteria capable of using the nitrogen oxides as electron acceptors are biochemically and taxonomically diverse.[35] The enzyme nitrate reductase is responsible for the conversion of nitrate to nitrite. It is membrane bound and couples with the electron transport chain through a specific cytochrome b. The enzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase are involved in the reduction of nitrite to nitrogen gas and appear to be linked to the electron transport chain through specific c-type cytochromes.[20,35] It is possible that all of the reactions are coupled to the generation of proton motive force, but the number of ATPs synthesized per electron transported is less than the number associated with oxygen as the terminal acceptor because the available free energy change is less. Consequently, bacteria growing with nitrate as the terminal electron acceptor exhibit lower yields than bacteria growing under aerobic conditions.[46]

Under strictly anaerobic conditions, i.e., when neither oxygen nor the nitrogen oxides are present, many Bacteria generate their ATP through substrate level phosphorylation associated with fermentation reactions in which the oxidation of one organic substrate is coupled to the reduction of another. The second substrate is generally a product of the catabolic pathway leading from the oxidized substrate with the result that the fermentation pathway is internally balanced, with neither a net production nor a net requirement for reducing power. Several types of fermentation reactions are listed in Table 2.5. Because ATP generation occurs only by substrate level phosphorylation and a large part of the available electrons in the original substrate end up in the reduced organic products, bacteria receive relatively little energy in this mode of growth, and thus have low yield per unit of substrate processed. As

Table 2.5 Types of Fermentations of Various Microorganisms

Type of fermentation	Products	Organisms
Alcoholic	Ethanol, CO_2	Yeast
Lactic acid	Lactic acid	*Streptococcus, Lactobacillus*
Mixed acid	Lactic acid, acetic acid, Ethanol, CO_2, H_2	*Escherichia, Salmonella*
Butanediol	Butanediol, ethanol, lactic acid, acetic acid, CO_2, H_2	*Aerobacter, Serratia*
Butyric acid	Butyric acid, acetic acid, CO_2, H_2	*Clostridium butyricum*
Acetone-butanol	Acetone, butanol, ethanol	*Clostridium acetobutylicum*
Propionic acid	Propionic acid	*Propionibacterium*

discussed in Section 2.3.2, however, the production of H_2 allows more oxidized products like acetate to be produced. As a result, more ATP can be produced by bacteria when they generate H_2, allowing them to have a higher biomass yield per unit of substrate processed.

Methanogens are obligate anaerobes that have very restricted nutritional requirements, with the oxidation of acetate and hydrogen being their main sources of energy. Even though methane is produced from the reduction of carbon dioxide during the oxidation of H_2, methanogens lack the components of a standard electron transport chain, and thus carbon dioxide does not function as a terminal electron acceptor in a manner analogous to nitrate or oxygen.[20] Rather, reduction of carbon dioxide to methane involves a complex sequence of events requiring a number of unique coenzymes.[84] However, there is a sufficient free energy change during methane formation for the theoretical production of two molecules of ATP and it appears that a normal chemiosmotic mechanism is involved,[20] although it involves a sodium motive force as well as a proton motive force.[84] Regardless of the exact mechanisms involved, it is important to recognize that ATP generation in Archaea is different from that associated with both respiration and fermentation in Bacteria and Eucarya. Furthermore, like bacteria growing in anaerobic environments, methanogens have low yields.

Factors Influencing Energy for Synthesis. Energy for synthesis represents the energy required by microorganisms to synthesize new cell material. In the absence of any other energy requirements, the energy required for synthesis is the difference between the energy available in the original substrate and the energy associated with the cell material formed, or in the common units of the environmental engineer, the difference between the COD of the original substrate and the COD of the biomass formed. Consequently, the energy for synthesis and the yield are intimately linked. If the efficiency of ATP generation were the same for all bacteria, it would be possible to theoretically predict the energy for synthesis, and hence the yield, from thermodynamic considerations.[44] However, as we saw above, the amount of ATP generated per electron transferred differs from microorganism to microorganism, which means

that the efficiency of energy generation differs. This, coupled with the fact that the pathways of synthesis and degradation are not the same in all microorganisms, makes it difficult to use exactly the thermodynamic approaches for predicting yields that have been presented in the environmental engineering literature. Nevertheless, there are many instances in which it would be advantageous to have a theoretical prediction of the energy for synthesis or the yield prior to experimental work and a technique based on the Gibbs energy dissipation per unit of biomass produced appears to be best.[23] Regardless, thermodynamic concepts are most useful for understanding why different substrates and different terminal electron acceptors have different energies of synthesis and yields associated with them.

During biomass growth, energy is required to synthesize the monomers needed to make the macromolecules that form the structural and functional components of the cell. This suggests that more energy would be required for a culture to grow in a minimal medium containing only a single organic compound as the carbon and energy source than in a complex medium in which all required monomers were supplied. Actually, such a conclusion is false.[69] For example, the energy needed to synthesize all of the amino acids needed by a cell amounts to only about 10% of the total energy needed to synthesize new cell material. This is because macromolecules are too large to be transported into the cell and must be formed inside even when all of the needed monomers are provided in the medium. Consequently, although the complexity of the growth medium has some effect on the energy required for synthesis, it is not large.

Of more importance are the oxidation state and size of the carbon source.[23] The oxidation state of carbon in biomass is roughly the same as that of carbon in carbohydrate.[69] If the carbon source is more oxidized than that, reducing power must be expended to reduce it to the proper level. If the carbon source is more reduced, it will be oxidized to the proper level during normal biodegradation and no extra energy will be required. Therefore, as a general rule, a carbon source at an oxidation state higher than that of carbohydrate will require more energy to be converted into biomass than will one at a lower oxidation state. Pyruvic acid occupies a unique position in metabolism because it lies at the end of many catabolic pathways and the beginning of many anabolic and amphibolic ones. As such, it provides carbon atoms in a form that can be easily incorporated into other molecules. Indeed, three-carbon fragments play an important role in the synthesis of many compounds. If the carbon source contains more than three carbon atoms it will be broken down to size without the expenditure of large amounts of energy. If it contains less than three carbon atoms, however, energy must be expended to form three-carbon fragments for incorporation. Consequently, substrates containing few carbon atoms require more energy for synthesis than do large ones.

Carbon dioxide, which is used by autotrophic organisms as their chief carbon source, is an extreme example of the factors just discussed, being a single-carbon compound in which the carbon is in the highest oxidation state. Consequently, the energy for synthesis for autotrophic growth is much higher than for heterotrophic growth. As a result, the amount of biomass that can be formed per unit of available electrons in the energy source is quite low.

True Growth Yield. The true growth yield (Y) is defined as the amount of biomass formed per unit of substrate removed when all energy expenditure is for synthesis. In this context, the substrate is usually taken to be the electron donor,

although it can be defined differently. If the electron donor is an organic compound, it is common in environmental engineering practice to express Y in terms of the amount of soluble COD removed from the wastewater. This is because wastewaters contain undefined, heterogenous mixtures of organic compounds and the COD is an easily determined measurement of their quantity. In addition, the COD is fundamentally related to available electrons, having an electron equivalent of eight grams of oxygen. Thus, a Y value expressed per gram of COD removed can be converted to a Y value per available electron by multiplying by eight. If the electron donor is an inorganic compound, such as ammonia or nitrite nitrogen, it is common to express Y in terms of the mass of the element donating the electrons. Furthermore, regardless of the nature of the electron donor, it has been common practice to express the amount of biomass formed on a dry weight basis, i.e., mass of suspended solids (SS), or on the basis of the dry weight of ash-free organic matter, i.e., mass of volatile suspended solids (VSS). When grown on a soluble substrate, microorganisms have an ash content of about 15%, and thus the value of Y when expressed as VSS will be slightly less than the value of Y when expressed as suspended solids. As will be discussed later, there are advantages to expressing biomass concentrations on a COD basis rather than on a SS or VSS basis, and thus yields are sometimes expressed as the amount of biomass COD formed per unit of substrate COD removed from the medium. This convention will be used throughout this book. If we assume an empirical formula for the organic, i.e., ash-free, portion of biomass of $C_5H_7O_2N$, the COD of that organic portion can be calculated to be 1.42 g COD/g VSS.[30] Furthermore, if we assume the ash content of biomass to be 15%, the theoretical COD of biomass is 1.20 g COD/g SS. These values can be used to convert between the various ways of expressing the yield.

The nature of the substrate influences the yield. Hadjipetrou et al.[19] summarized data from one species, *Aerobacter aerogenes*, which was grown in unrestricted batch growth in minimal media on a number of substrates, and found Y to vary from 0.40 to 0.56 mg biomass COD formed per mg substrate COD removed. (The values were not reported on a COD basis, but were converted to it for this book.) Recognizing that the yield expressed on the basis of cell COD formed per unit of substrate COD removed is a measure of the amount of energy available in the substrate that was conserved through cell synthesis, it can be seen that 40 to 56% of the available energy was conserved while 44 to 60% was expended.

The species of organism will also affect Y, although the effect will not be as great as the effect of substrate. Payne[56] collected Y values for eight bacterial species growing aerobically on glucose in minimal media and found them to vary from 0.43 to 0.59 mg biomass COD formed per mg substrate COD removed. The data were from a number of different published reports and thus some of the variation may be due to differences in experimental conditions, rather than to species. Nevertheless, they clearly show that the microbial species has an impact. (As above, the values were not originally reported on a COD basis, but were converted to it for this book.)

The growth environment, including media complexity, type of terminal electron acceptor, pH, and temperature will all affect Y.[23] As explained above, biomass grown in complex media will have only slightly higher Y values than biomass grown in minimal media, whereas biomass grown with oxygen as the terminal electron acceptor will exhibit significantly higher yields than biomass grown with nitrate as the acceptor. The yield from fermentations will depend on the reduced end products and

the method of expressing the yield. If Y is expressed on the basis of the amount of the original substrate removed, ignoring the COD returned to the medium as reduced end products, the value will be very small, on the order of 0.03 to 0.04 mg biomass COD formed per mg substrate COD removed. However, when expressed on the basis of the COD actually utilized (accounting for the COD remaining as reduced end products), the Y value is not much different from that obtained with aerobic cultures.[1] On the other hand, when methane is produced, so that most of the reduced end product is lost from the system as a gas, then the COD removed from solution is actually much higher than the COD utilized by the microorganisms, making the yield per unit of COD removed about an order of magnitude lower than for aerobic growth. The pH of the medium has long been known to affect microbial growth, but the quantitative effects are unclear. The yield is likely, however, to have a maximum around pH 7 because that is optimal for so many physiological functions. Temperature also affects Y, as shown in Figure 2.4.[49] Although the significance of temperature is apparent, no generalizations can be made, and most engineers assume that Y is constant over the normal physiological temperature range. A final factor that

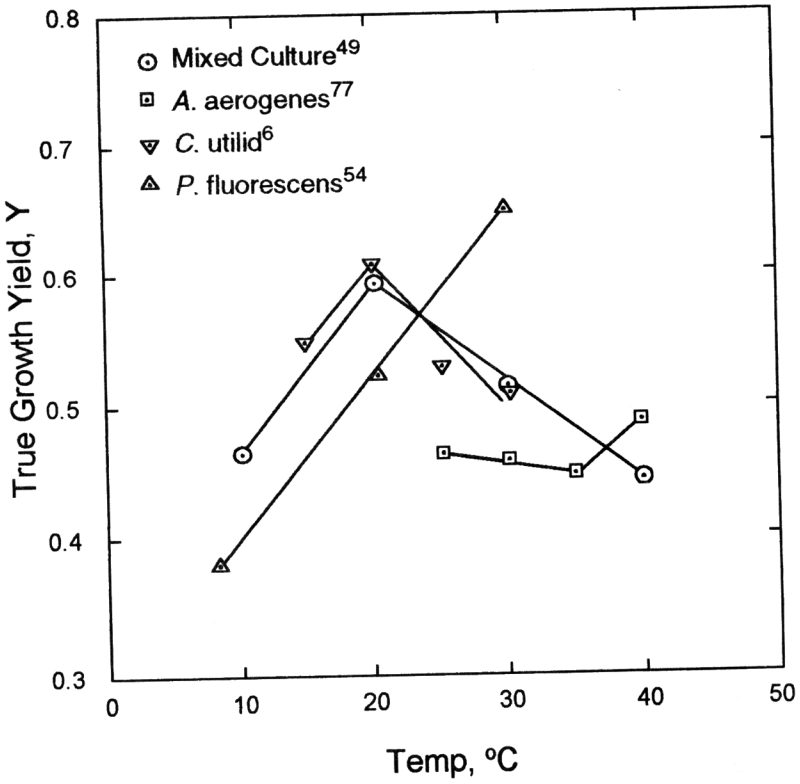

Figure 2.4 Effect of temperature on the true growth yield, Y. The units on Y are mg biomass formed per mg substrate COD removed. (From R. E. Muck and C. P. L. Grady, Jr., Temperature effects on microbial growth in CSTR's. *Journal of the Environmental Engineering Division, ASCE* **100**:1147–1163, 1974. Reprinted by permission of the American Society of Civil Engineers.).

may influence Y is the composition of the microbial community. When it is heterogeneous, the waste products from one species serve as growth factors for another, thereby converting a seemingly minimal medium into a complex one. Consequently, it might be anticipated that the yields from mixed microbial cultures would be slightly higher than those from pure cultures growing on the same medium. A comparison of the two revealed this to be the case.[28]

Constancy of Y in Biochemical Operations. Biochemical operations use mixed microbial communities to treat wastewaters containing mixtures of substrates. Thus, it is apparent that Y will depend on both the character of the wastewater and the particular community that develops on it. It is important that this variability be recognized by engineers designing biochemical operations, because then the estimated yield values will be interpreted in an appropriate way. As seen in Chapter 3, similar conclusions can be reached about the kinetic parameters associated with biochemical operations. This means that designers must utilize considerable judgement and allow for uncertainty. This situation does not prevent generalities from being made, however. For example, examination of a large number of yield values indicates that Y will generally lie within the range of 0.48–0.72 mg biomass COD formed per mg substrate COD utilized for aerobic heterotrophs degrading carbohydrates.[63] Under similar conditions, Y values for growth on a number of xenobiotic compounds, including substituted phenols, benzenes, and phthalate esters, lay within the range of 0.20–0.60 mg biomass COD formed per mg substrate COD removed.[16] One study[36] reported the range of yield values for autotrophs to be from 0.06 to 0.35 mg biomass COD per mg nitrogen oxidized, with values for *Nitrobacter* being lower than those for *Nitrosomonas*. Likewise, another study[85] reported the Y value for *Nitrobacter* to be 0.12 mg biomass COD per mg nitrogen oxidized and the value for *Nitrosomonas* to be 0.47. Although ranges such as these provide the engineer with an idea of the magnitudes to be expected, designs should only be based on estimates of Y obtained from laboratory and pilot-scale studies of the particular waste to be treated.

2.4.2 Maintenance, Endogenous Metabolism, Decay, Lysis, and Death

The yield values in the preceding section are those that result when all energy obtained by the biomass is being channeled into synthesis. Energy for synthesis is not the only energy requirement for microorganisms, however. They must also have energy for maintenance.[60]

Cellular processes, whether mechanical or chemical, require energy for their performance, and unless a supply is available these essential processes will cease and the cell will become disorganized and die. Mechanical processes include motility, osmotic regulation, molecular transport, maintenance of ionic gradients, and in the case of some Eucarya, cytoplasmic streaming. While it might be argued that motility can be dispensed with in some microorganisms, this argument would not hold for all because some require motility to find food. Osmotic regulation is quite important in all cells, even those protected by a rigid cell wall, and pump mechanisms, such as contractile vacuoles, exist in cells to counteract the normal tendency of osmotic pressure to pump water into them. Cell membranes are permeable to many small molecules, such as amino acids, and because of the high concentrations within the

cell these tend to diffuse into the medium. Active transport mechanisms operate to bring such molecules into the cell against the concentration gradient. Of a similar nature is the necessity for maintaining an ionic gradient across the cell membrane, which is closely linked to the proton motive force responsible for ATP synthesis. Maintenance of this gradient is thought to be a major consumer of maintenance energy.[73] Finally, cytoplasmic streaming and the movement of materials within Eucarya are often required for their proper functioning. They also require energy.

Chemical factors also contribute to maintenance energy needs. Microbial cells represent chemical organization and many of the components within them have higher free energies than the original compounds from which they were formed. In general, because of this organization, energy must be available to counteract the normal tendency toward disorder, i.e., to overcome entropy. The chemical processes contributing to the energy requirement for maintenance are those involved in resynthesis of structures such as the cell wall, flagella, the cell membrane, and the catabolic apparatus. For example, one study[39] suggested that energy for the resynthesis of proteins and nucleic acids was an important portion of the maintenance energy requirement for *Escherichia coli*.

A major point of controversy in the microbiological literature has concerned the impact on the maintenance energy requirement of the rate at which a culture is growing. Early investigations[60] suggested that the need for maintenance energy was independent of growth rate, but more recent research indicates the opposite.[73] Nevertheless, engineers generally consider maintenance energy needs to be independent of growth rate in biochemical operations for wastewater treatment and that is the approach that will be adopted in this book.

Given the existence of a need for maintenance energy, what energy sources can be used to supply it? The answer to that question depends on the growth conditions of the microorganisms. If an external (exogenous) energy supply is available, a portion of it will be used to meet the maintenance energy requirement and the remainder will be used for synthesis. As the rate of energy supply is decreased, less and less will be available for new growth and thus the net, or observed, yield will decline. When the point is reached at which the rate of energy supply just balances the rate at which energy must be used for maintenance, no net growth will occur because all available energy will be used to maintain the status quo. If the rate of energy supply is reduced still further, the difference between the supply rate and the maintenance energy requirement will be met by the degradation of energy sources available within the cell, i.e., by endogenous metabolism. This will cause a decline in the mass of the culture. Finally, if no exogenous energy source is available, all of the maintenance energy needs must be met by endogenous metabolism. When the point is reached at which all endogenous reserves have been exhausted, the cells deteriorate and die, or enter a resting state.

The nature of the materials serving as substrates for endogenous metabolism depends on both the species of the microorganism and the conditions under which the culture was grown. For example, when *E. coli* is grown rapidly in a glucose–mineral salts medium it stores glycogen.[39] If those cells are then placed in an environment devoid of exogenous substrate they will utilize the glycogen as an endogenous energy source. Amino acids and proteins show little net catabolism until the glycogen is gone. When grown in tryptone medium, on the other hand, *E. coli* accumulates little glycogen. As a result, endogenous metabolism utilizes nitrogenous

compounds immediately. Other organisms use still other compounds, including ribonucleic acid (RNA) and the lipid poly-β-hydroxybutyrate (PHB).

One question that has intrigued microbiologists concerns the route of energy flow when sufficient exogenous substrate is available to supply the maintenance energy requirements of the culture. Does endogenous metabolism continue under those circumstances so that part of the energy released from degradation of the substrate is used to resupply the energy reserves being degraded by endogenous metabolism? Or, alternatively, does endogenous metabolism cease so that the energy released from degradation of the exogenous substrate goes directly for maintenance functions? The evidence is still not conclusive. Actually, although such questions are of fundamental scientific significance, they have little bearing on the macroscopic energy balances used by engineers to mathematically model biochemical operations. In fact, as we see in Section 3.3.2, some models avoid the entire issue by introducing the concept of cell lysis and regrowth.

The amount of biomass actually formed per unit of substrate used in a biochemical operation, referred to as the observed yield (Y_{obs}), is always less than Y. One reason for this is the need for maintenance energy. The more energy that must be expended for maintenance purposes, the less available for synthesis and the smaller the quantity of biomass formed per unit of substrate degraded. Other factors also contribute to the difference, however. For example, consider the effect of predation. In a complex microbial community such as that found in the activated sludge process, protozoa and other Eucarya prey on the bacteria, reducing the net amount of biomass formed. To illustrate the effect of predation, assume that the value of Y for bacteria growing on glucose is 0.60 mg bacterial biomass COD formed per mg of glucose COD used. Thus, if 100 mg/L of glucose COD were used, 60 mg/L of bacterial biomass COD would result. Now assume that the value of Y for protozoa feeding on bacteria is 0.70 mg protozoan biomass COD formed per mg of bacterial biomass COD used. If the protozoa consumed all of the bacteria resulting from the glucose, the result would be 42 mg/L of protozoan biomass. As a consequence, if we observed only the net amount of biomass formed, without distinction as to what it was, we would conclude that 42 mg/L of biomass COD resulted from the destruction of 100 mg/L of glucose COD. Therefore, we would conclude that the observed yield was 0.42, which is less than the true growth yield for bacteria growing on glucose. Macroscopically, it is impossible to distinguish between the various factors acting to make the observed yield less than the true growth yield. Consequently, environmental engineers lump them together under the term "microbial decay," which is the most common way they have modeled their effect in biochemical operations.[36]

Another process leading to a loss of biomass in biochemical operations is cell lysis.[41] The growth of bacteria requires coordination of the biosynthesis and degradation of cell wall material to allow the cell to expand and divide. The enzymes responsible for hydrolysis of the cell wall are called autolysins and their activity is normally under tight regulation to allow them to act in concert with biosynthetic enzymes during cell division. Loss of that regulation, however, will lead to rupture of the cell wall (lysis) and death of the organism. When the cell wall is ruptured, the cytoplasm and other internal constituents are released to the medium where they become substrates for other organisms growing in the culture. In addition, the cell wall and cell membranes, as well as other structural units, begin to be acted upon

by hydrolytic enzymes in the medium, solubilizing them and making them available as substrates as well. Only the most complex units remain as cell debris, which is solubilized so slowly that it appears to be refractory in most biochemical operations.[45,47] The arguments for how lysis results in the loss of biomass are similar to those associated with predation, illustrated above. The yield exhibited by bacteria growing on the soluble products released by lysis is of the same magnitude as the yield associated with growth on other biogenic substrates. Consequently, if 100 mg/L of biomass is lysed, only 50–60 mg/L of new biomass will result from regrowth on the lysis products. Thus, the net effect of lysis and regrowth is a reduction in biomass within the system. In general, starvation itself does not initiate lysis, although the events that trigger it are not yet clear. Nevertheless, engineers seeking to model the decline in observed yield associated with situations in which the microbial community is growing slowly have focused on cell lysis as the primary mechanism.[12,26]

The final event impacting on the amount of active biomass in a biochemical operation is death. Traditionally, a dead cell has been defined as one that has lost the ability to divide on an agar plate[62] and studies based on this definition have shown that a large proportion of the microorganisms in slowly growing cultures are nonviable, or dead.[61,62,74] In addition, as summarized by Weddle and Jenkins,[80] a large number of studies using indirect evidence involving comparisons of substrate removal rates and enzyme activities have concluded that large portions of the MLSS in wastewater treatment systems are inactive. However, a later study,[40,41] using more sophisticated techniques for identifying dead bacteria, has suggested that a very low fraction of the cells present at low growth rates are actually dead. Instead, many are simply nonculturable by standard techniques, although they are still alive. Furthermore, the more recent work[40] suggests that dead cells do not remain intact for long, but rather lyse, leading to substrates and biomass debris, as discussed above. The presence of biomass debris acts to make the mass of viable microorganisms less than the mass of suspended solids in the system. Even though the predecessor of this book used a model[15] that explicitly considered cell death, it now appears that direct consideration of the phenomenon is not warranted.[40,41] Rather, the fact that only a portion of the MLSS in a biological wastewater treatment system is actually viable biomass can be attributed to the accumulation of biomass debris rather than to the presence of dead cells.

In summary, as a result of several mechanisms, biochemical reactors exhibit two important characteristics: (1) the observed yield is less than the true growth yield and (2) active, viable bacteria make up only a fraction of the "biomass." One simplified conceptualization of the events leading to these characteristics is that bacteria are continually undergoing death and lysis, releasing organic matter to the environment in which they are growing. Part of that organic matter is degraded very, very slowly, making it appear to be resistant to biodegradation and causing it to accumulate as biomass debris. As a consequence, only a portion of the "biomass" is actually viable cells. The remainder of the released organic matter is used by the bacteria as a food source, resulting in new biomass synthesis. However, because the true growth yield is always less than one, the amount of new biomass produced is less than the amount destroyed by lysis, thereby making the observed yield for the overall process less than the true growth yield on the original substrate alone.

2.4.3 Soluble Microbial Product Formation

Much of the soluble organic matter in the effluent from a biological reactor is of microbial origin and is produced by the microorganisms as they degrade the organic substrate in the influent to the bioreactor. The major evidence for this phenomenon has come from experiments in which single soluble substrates of known composition were fed to microbial cultures and the resulting organic compounds in the effluent were examined for the presence of the influent substrate.[64] The bulk of the effluent organic matter was not the original substrate and was of higher molecular weight, suggesting that it was of microbial origin. These soluble microbial products are thought to arise from two processes, one growth-associated and the other non–growth-associated. Growth-associated product formation results directly from biomass growth and substrate utilization. As such, it is coupled to those events through another yield factor, the microbial product yield, Y_{MP}, and the biodegradation of one unit of substrate results in the production of Y_{MP} units of products. Values of Y_{MP} for a variety of organic compounds have been found to be less than 0.1.[16] Non–growth-associated product formation is related to decay and lysis and results in biomass-associated products. They are thought to arise from the release of soluble cellular constituents through lysis and the solubilization of particulate cellular components. Although little is known about the characteristics of these two types of soluble microbial products, they are thought to be biodegradable, although some at a very low rate. Compared to other aspects of biochemical operations, little research has been done on the production and fate of soluble microbial products and few researchers have attempted to model the contribution of such products to the organic matter discharged from wastewater treatment systems.[64,50] Nevertheless, an awareness of their existence is necessary for an accurate understanding of the response of biochemical operations.

2.4.4 Solubilization of Particulate and High Molecular Weight Organic Matter

Bacteria can only take up and degrade soluble organic matter of low molecular weight. All other organic material must be attacked by extracellular enzymes that release low molecular weight compounds that can be transported across cellular membranes. Many organic polymers, particularly those of microbial origin, such as cell wall components, proteins, and nucleic acids, are composed of a few repeating subunits connected by bonds that can be broken by hydrolysis. Consequently, the microbial process of breaking particulate and high molecular weight soluble organic compounds into their subunits is commonly referred to as hydrolysis, even though some of the reactions involved may be more complicated.

Hydrolysis reactions play two important roles in biochemical reactors for wastewater treatment. First, they are responsible for the solubilization of cellular components released as a result of cell lysis, preventing their buildup in the system. Because cell lysis occurs in all microbial systems, hydrolysis reactions are even important in bioreactors receiving only soluble substrate. Second, many biochemical operations receive particulate organic material, in which case hydrolysis is essential to bring about the desired biodegradation. In spite of its central position in the functioning of biochemical operations, relatively few studies have sought to under-

stand the kinetics and mechanisms of hydrolysis.[8,12,17] Nevertheless, it has important impacts on the outcome of biochemical operations and must be considered for a complete understanding of their functioning.

2.4.5 Ammonification

Ammonification is the name given to the release of ammonia nitrogen as amino acids and other nitrogen containing organic compounds undergo biodegradation. It occurs as a normal result of the biodegradation process, during which amino groups are liberated and excreted from the cell as ammonia. The rate of ammonification will depend on the rate of nitrogen containing substrate utilization and the carbon to nitrogen ratio of that substrate. Ammonification is very important in wastewater treatment processes for nitrogen control because organic nitrogen is not subject to oxidation by nitrifying bacteria. They can only oxidize nitrogen to nitrate after it has been converted to ammonia and released to the medium.

2.4.6 Phosphorus Uptake and Release

If a suspended growth bioreactor system is configured as two zones in series with the first zone anaerobic and the second aerobic, PAOs, which possess a special metabolic capability not commonly found in other bacteria, will proliferate and store large quantities of inorganic phosphate as polyphosphate, thereby allowing phosphorus removal from the wastewater via biomass wastage. Although PAOs are often present in significant numbers in totally aerobic suspended growth cultures, they only develop the ability to store large quantities of phosphate when they are subjected to alternating anaerobic and aerobic conditions by being recycled between the two zones.[37] This follows from their unique capability to store carbon at the expense of phosphate under anaerobic conditions and to store phosphate at the expense of carbon under aerobic conditions. Two scenarios have been postulated to explain the functioning of PAOs. One was developed independently by Comeau et al.[9] and Wentzel et al.,[82] whereas the other was developed by Arun et al.[2] The former is referred to as the Comeau–Wentzel model whereas the latter is called the Mino model.[81] The difference between the two models is the result of the metabolic diversity among PAOs, and since it is not yet known which model is the more generally applicable, both will be presented.

Comeau–Wentzel Model. We will first consider the events occurring in the anaerobic zone. Because of fermentations that occur in sewers, much of the soluble organic matter in domestic wastewater is in the form of acetate and other short chain fatty acids. Furthermore, when the wastewater enters an anaerobic bioreactor, additional quantities of fatty acids are formed by fermentative reactions performed by facultative heterotrophs. As indicated in Figure 2.5A, acetate is transported across the cell membrane by passive diffusion (as undissociated acetic acid), but once inside, it is activated to acetyl-CoA by coupled ATP hydrolysis, yielding ADP. Although not shown in the diagram, ATP is also used to maintain the proton motive force that has been lost by transport of the proton associated with the undissociated acetic acid. The cell responds to the decreasing ATP/ADP ratio by stimulating ATP resynthesis from stored polyphosphate (Poly-P_n). A portion of the acetyl-CoA is metabolized through the TCA cycle to provide the reducing power ($NADH + H^+$) required for

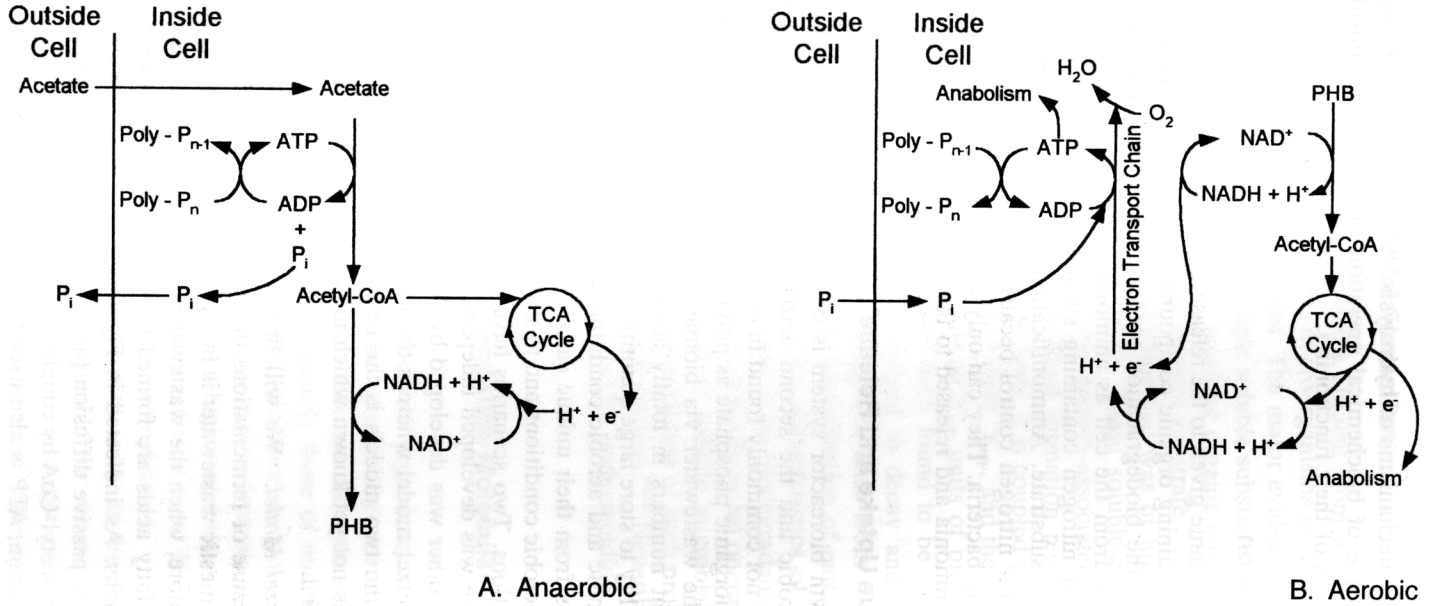

Figure 2.5 Schematic diagram depicting the Comeau–Wentzel model for the uptake and release of inorganic phosphate by PAOs: A. Anaerobic conditions; B. aerobic conditions. (Adapted from Wentzel et al.[81])

the synthesis of PHB. The remainder of the acetyl-CoA is converted into PHB, with about 90% of the acetate carbon being conserved in that storage polymer. Without the presence of the polyphosphate to provide energy for ATP resynthesis, acetate would build up in the cell, acetate transport would stop, and no PHB formation would occur. The hydrolysis of the polyphosphate to form ATP increases the intracellular concentration of inorganic phosphate, P_i, which is released to the bulk solution, along with cations (not shown) to maintain charge balance.

When the wastewater and the associated biomass enter the aerobic zone, the wastewater is low in soluble organic matter, but the PAOs contain large PHB reserves. Furthermore, the wastewater is rich in inorganic phosphate, while the PAOs have low polyphosphate levels. Because they have oxygen as an electron acceptor in the aerobic zone, the PAOs perform normal aerobic metabolism for growth by using the stored PHB as their carbon and energy source, generating ATP through electron transport phosphorylation, as illustrated in Figure 2.5B. Furthermore, as the ATP–ADP ratio increases, polyphosphate synthesis is stimulated, thereby removing phosphate and associated cations (not shown) from solution and regenerating the stored polyphosphate in the cells. Because of the large amount of energy provided by the aerobic metabolism of the stored PHB, the PAOs are able to take up all of the phosphate released in the anaerobic zone plus the phosphate originally present in the wastewater.

The continual cycling between the anaerobic and aerobic zones gives the PAOs a competitive advantage over ordinary heterotrophic bacteria, because without the capability to make and use polyphosphate, the ordinary heterotrophs are not able to take up organic matter in the anaerobic zone. It should be noted that while most systems that remove phosphate through the use of PAOs employ aerobic zones for the regeneration of the stored polyphosphate, some PAOs can use nitrate and nitrite as alternative electron acceptors,[37] allowing anoxic conditions to be used as well.

Mino Model. The Mino model, illustrated in Figure 2.6, is very similar to the Comeau–Wentzel model, the major difference being the role of glycogen, a carbohydrate storage polymer. In this case, in the anaerobic zone the reducing power required for synthesis of PHB from acetyl-CoA comes from the metabolism of glucose released from the glycogen. Glucose is oxidized to pyruvate through the Entner–Doudoroff (ED) or Embden–Meyerhof–Parnas (EMP) pathway, depending in the type of PAO, thereby providing some of the ATP required to convert acetate to acetyl-CoA and some of the reducing power needed for PHB synthesis. Pyruvate, in turn, is oxidatively decarboxylated to acetyl-CoA and carbon dioxide, with the electrons released also being used in the synthesis of PHB. Thus, all of the acetate taken up is stored as PHB, as is part of the carbon from the glycogen. In the aerobic zone, PHB is broken down as in the Comeau–Wentzel model to provide for biomass synthesis as well as for phosphate uptake and storage as polyphosphate. In addition, however, PHB is also used to replenish the stored glycogen.

2.4.7 Overview

A diagram depicting the overall sum of the events occurring in an aerobic bioreactor receiving a soluble substrate is shown in Figure 2.7. Bacteria consume the substrate (S_{S1}) and grow, leading to more bacteria, with the relationship between substrate consumption and biomass growth being given by the true growth yield, Y. There

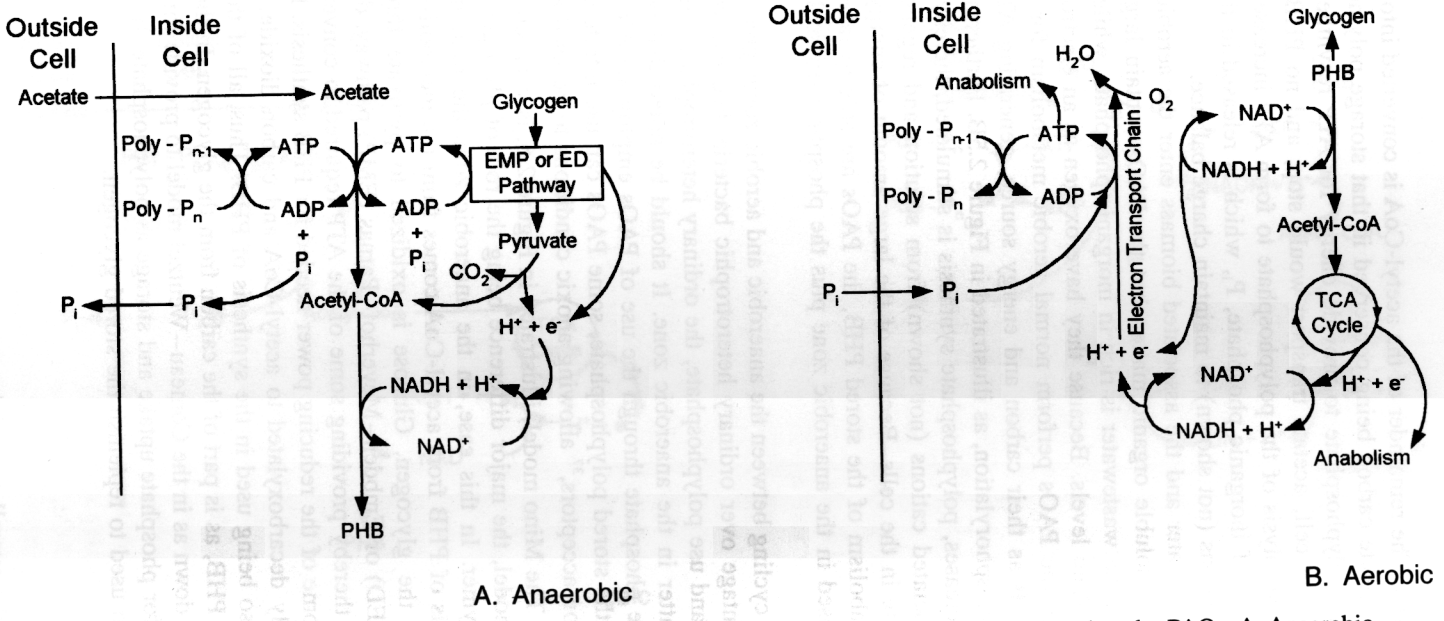

Figure 2.6 Schematic diagram depicting the Mino model for the uptake and release of inorganic phosphate by PAOs: A. Anaerobic conditions; B. aerobic conditions. (Adapted from Wentzel et al.[81])

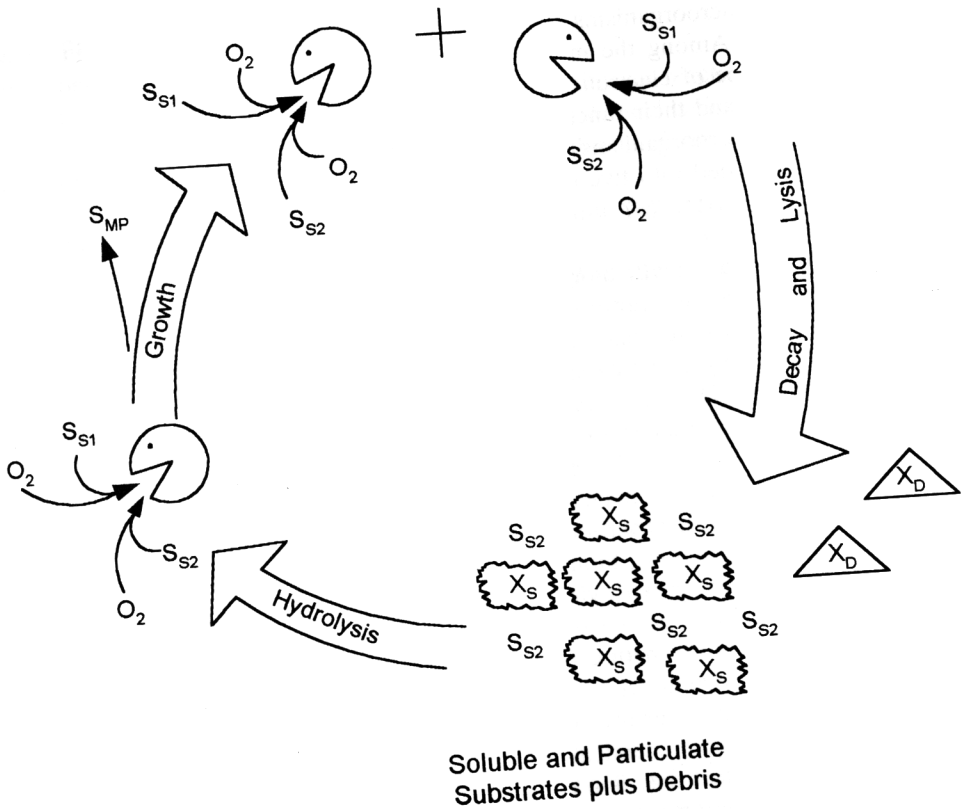

Figure 2.7 Overview of fundamental events occurring in an aerobic bioreactor receiving a soluble substrate (S_{S1}). (Adapted from Mason et al.[40])

will also be soluble microbial product (S_{MP}) formation associated with that substrate consumption and growth. Concurrently with growth, the biomass will be undergoing decay and lysis, releasing soluble (S_{S2}) and particulate (X_S) substrate to the medium. Cell debris (X_D), which is degraded so slowly that it appears to be nonbiodegradable, and biomass associated products (S_D) are also released. The particulate cell fragments (X_S) undergo hydrolysis, freeing more substrate (S_{S2}) that can be used by the cells. Part of the microbial products may undergo biodegradation, but others may be degraded so slowly that they appear inert. As might be imagined by the previous discussion in this section, more complicated conceptualizations could be depicted. However, this one contains the essential elements required to model biological processes and it will be used in later chapters for that purpose.

2.5 KEY POINTS

1. Biochemical operations use the carbon and nitrogen cycles to remove organic and nitrogenous pollutants from wastewaters.

2. The microorganisms in biochemical operations can be classified in several ways. Among the most important are: the type of electron donor used, the type of electron acceptor employed, their physical growth characteristics, and their function.
3. The microorganisms in aerobic/anoxic suspended growth bioreactors may be divided into five overlapping groups: (1) floc-forming organisms, (2) saprophytes, (3) nitrifying bacteria, (4) predators, and (5) nuisance organisms.
4. Attached growth bioreactors have more diverse microbial communities encompassing more trophic levels than suspended growth bioreactors.
5. Methanogenic anaerobic cultures are highly interdependent ecosystems with many complex interactions between Bacteria and Archaea. Acetic acid and H_2 play a central role in those interactions, being products of Bacteria and substrates for Archaea.
6. There are two major groups of methanogens: (1) those that oxidize H_2 and (2) those that cleave acetic acid. Both are essential to the proper functioning of anaerobic cultures receiving complex substrates.
7. In most situations, biomass growth and substrate utilization are coupled, with the true growth yield, Y, serving as the coupling factor. The yield is the amount of biomass formed per unit of substrate removed. Its value depends on the nature of the substrate, the organism involved, and the growth environment.
8. Heterotrophic bacteria obtain their energy from the oxidation of organic carbon. Hence, chemical oxygen demand (COD), which is a measure of available electrons, is a convenient way in which to express the concentration of organic matter in wastewaters. When an organic compound is mineralized, all of the electrons available in it must end up either in the biomass formed or in the terminal electron acceptor. Consequently, COD is also a convenient technique for expressing the concentration of biomass.
9. Yield values for heterotrophic biomass cover a very broad range, but seldom exceed 0.75 mg biomass COD formed per mg substrate COD removed because of the energy required for synthesis.
10. As a result of maintenance energy needs and decay, death, and lysis, biochemical reactors exhibit two characteristics: (1) the observed yield is less than the true growth yield, and (2) active, viable bacteria make up only a fraction of the "biomass."
11. Soluble microbial product formation is associated with substrate utilization and with biomass decay and lysis. As a consequence, much of the soluble organic matter leaving a biochemical operation is of microbial origin.
12. Hydrolysis reactions are important for the biodegradation of particulate substrates and cellular components released by biomass death and lysis.
13. Ammonification is the release of ammonia nitrogen as nitrogen containing organic compounds undergo biodegradation.
14. PAOs will only store large amounts of phosphorus as polyphosphate granules when they are cycled between substrate-rich anaerobic and substrate-poor aerobic environments.

2.6 STUDY QUESTIONS

1. Draw a sketch of the nitrogen cycle, labeling all reactions. Then explain the following terms and their importance in biochemical operations: ammonification, assimilation, nitrification, denitrification, and assimilative reduction.
2. Define or explain the following terms and their use in classifying the microorganisms in biochemical operations: electron donor, electron acceptor, heterotroph, autotroph, nitrifier, denitrifier, methanogen, obligate aerobe, obligate anaerobe, facultative anaerobe, biofloc, primary degrader, and secondary degrader.
3. Describe the roles of and give examples of microorganisms in each of the following groups commonly found in aerobic/anoxic suspended growth bioreactors: floc-forming organisms, saprophytes, nitrifying bacteria, predators, and nuisance organisms.
4. Draw a sketch depicting the multistep nature of methanogenic anaerobic cultures and use it to describe the roles of the major groups of microorganisms involved.
5. Why is the maintenance of a low partial pressure of H_2 necessary to the proper functioning of a methanogenic anaerobic culture? What is the role of methanogens in the maintenance of the required conditions?
6. There are two major groups of methanogens. Describe them, list their growth characteristics, and contrast their roles in anaerobic cultures.
7. Why does the value of the true growth yield, Y, depend on the nature of the substrate, the microorganism involved, and the growth environment?
8. Why is it convenient to express the concentrations of organic substrates and biomass in COD units?
9. Give a "typical" yield value for heterotrophic biomass growing on carbohydrates and then explain why there is considerable variability associated with Y in biochemical operations.
10. Explain why the observed yield in a biochemical reactor is less than the true growth yield. While so doing, explain what is meant by the term "decay."
11. Why does cell lysis in a biochemical operation make the observed yield less than the true growth yield and the viability less than 100%?
12. What is the difference between growth-associated and nongrowth-associated product formation?
13. Why are hydrolysis reactions important to the performance of all biochemical operations, even those receiving only soluble substrate?
14. Describe the scenarios that have been postulated to explain the functioning of phosphate accumulating bacteria.

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