in mg/L as P, K_P is the half-saturation coefficient for soluble phosphate, S_O is the dissolved oxygen concentration, and K_O is the half-saturation coefficient for dissolved oxygen. It should be noted that the expression for the effect of PHB concentration on biomass growth is written in terms of the amount of PHB available per unit of biomass COD because the PHB is not free in the medium, but is stored in the biomass. As a result, K_{PHB} has units of mg PHB COD/mg PAO COD. Because of biomass lysis, phosphate will continually be released to the medium. Consequently, S_P will never reach a zero concentration and phosphorus will always be available for growth. The values chosen for the half-saturation coefficients by Henze et al.[56] were 0.01 mg PHB COD/mg PAO COD, 0.20 mg P/L, and 0.20 mg O_2/L, for K_{PHB}, K_P, and K_O, respectively.

The stoichiometry of the aerobic growth reaction on a COD basis is the same as that in Eq. 3.33, except that PHB is the growth substrate. Consequently, the relationship between r_{XBP}, r_{XPHB}, and r_{SO} (with all in COD units) will be the same as the relationship between r_{XB}, r_{SS}, and r_{SO} in Eq. 3.34, or:

$$\frac{r_{XPHB}}{\left(-\frac{1}{Y_{PAO}}\right)} = \frac{r_{SO}}{(-1)\left[-\left(\frac{1-Y_{PAO}}{Y_{PAO}}\right)\right]} = \frac{r_{XBP}}{1} \tag{3.86}$$

where Y_{PAO} is the yield coefficient for PAOs growing on stored PHB. The value assumed for it in ASM No. 2 is 0.63 mg PAO COD/mg PHB COD.[56] It should be noted that the rates of PHB loss and oxygen consumption expressed by Eq. 3.86 are that associated only with PAO growth.

Storage of polyphosphate also occurs under aerobic conditions and the energy for it also comes from PHB utilization. The rate expression includes all of the parenthetical terms in Eq. 3.85. It has been observed, however, that storage of poly-P stops if its content in the PAOs becomes too high.[56] It is necessary to include a term that decreases the rate of Poly-P storage as the Poly-P concentration per unit of PAOs approaches a maximum value of K_{PMAX}. Considering these factors, the rate of Poly-P storage, r_{XPP}, can be expressed as:

$$r_{XPP} = \hat{q}_{PP}\left[\frac{X_{PHB}/X_{B,P}}{K_{PHB} + (X_{PHB}/X_{B,P})}\right]\left(\frac{S_P}{K_P + S_P}\right)\left(\frac{S_O}{K_O + S_O}\right) \cdot \left[\frac{K_{PMAX} - (X_{PP}/X_{B,P})}{K_{IPP} + K_{PMAX} - (X_{PP}/X_{B,P})}\right] X_{B,P} \tag{3.87}$$

where $\hat{q}_{PP}$ is the maximum specific rate of Poly-P storage, which has a typical value of 0.06 mg P/(mg PAO COD·h) at 20°C. K_{IPP} is the inhibition coefficient for Poly-P storage, with an assumed value of 0.02 mg P/mg PAO COD. All other terms were defined following Eq. 3.85. Soluble phosphate is removed from the medium in direct proportion to the amount incorporated into Poly-P. Furthermore, PHB is lost and oxygen is utilized proportionally as well. The relationship between the rates is determined from the stoichiometry as:

$$\frac{r_{XPHB}}{(-Y_{PHB})} = \frac{r_{SO}}{(-1)(-Y_{PHB})} = \frac{r_{SP}}{-1} = \frac{r_{XPP}}{1} \tag{3.88}$$

where Y_{PHB} is the PHB requirement for poly-P storage, which has a typical value of 0.20 mg PHB COD/mg P.[56] The rates of PHB loss and oxygen consumption in this

expression are those associated with only Poly-P storage. The total rates of each under aerobic conditions must be obtained by adding the expressions from Eqs. 3.86 and 3.88. Furthermore, Eq. 3.88 does not give the total rate of soluble phosphate loss since polyphosphate formation is not the only mechanism for removing soluble phosphate from the liquid. Rather, phosphorus is also a required nutrient for biomass synthesis. If $i_{P/XB}$ is the mass of phosphorus incorporated into cell material per unit of PAO COD formed, the total rate of removal of soluble phosphorus by the PAOs will be:

$$r_{SP} = -(i_{P/XB} \cdot r_{XBP}) - r_{XPP} \tag{3.89}$$

where r_{XBP} is given by Eq. 3.85. Cellular biomass contains about 2.5 percent phosphorus on a mass basis, so on a biomass COD basis, $i_{P/XB}$ has a value around 0.02 mg P/mg biomass COD. If heterotrophs and autotrophs are growing in the system, they will also consume soluble phosphate for incorporation into biomass with the same stoichiometry.

3.8 SIMPLIFIED STOICHIOMETRY AND ITS USE

In Chapter 5, we use the concepts developed in Section 3.1.3 to construct mathematical models that incorporate the various events discussed in this chapter. There are many circumstances, however, in which the use of stoichiometric concepts would be very useful even without the development of rigorous equations. For example, examination of Eq. 3.13 expressing biomass growth and Eq. 3.52 expressing biomass decay by the traditional approach reveals that they could be combined into a single equation that incorporates both reactions. Since biomass is a product in Eq. 3.13 and a reactant in Eq. 3.52, the effect would be to reduce the net amount of biomass formed. Likewise, since nutrients are reactants in Eq. 3.13 and products in Eq. 3.52, the net amount of nutrients used would also be reduced. The electron acceptor, on the other hand, is a reactant in both equations, so the effect of combining them would be to increase the amount of electron acceptor required. Consideration of what is occurring when the equations are combined, in combination with the discussion of yield in Section 2.4.1, reveals that the net stoichiometric coefficient on biomass in a mass-based combined equation is the observed yield. In other words, it is the yield when maintenance energy needs and decay are taken into account. By making use of the fact that the observed yield is a function of the growth conditions imposed on the biomass, the combined equation may be used to show how the nutrient and electron acceptor requirements change as the growth conditions are changed.[110]

3.8.1 Determination of the Quantity of Terminal Electron Acceptor Needed

Although other stoichiometric equations can be used, the COD-based equation is the most useful for determining the quantity of terminal electron acceptor required for growth of heterotrophs. Writing the combined stoichiometric equation for heterotrophic biomass growth in COD units illustrates a very important point that will be used throughout this book. When ammonia serves as the nitrogen source, the sum of the oxygen (or oxygen equivalents of nitrate) used and the biomass (active plus

debris) formed (in COD units) must equal the COD removed from solution. This follows from the fact that COD is a measure of available electrons. In other words, all of the electrons available in a substrate being biodegraded are either removed and transferred to the terminal electron acceptor or they are incorporated into the biomass formed. As discussed in Section 3.2.1, when ammonia serves as the nitrogen source, no electrons are transferred to nitrogen during biomass synthesis. When nitrate serves as the nitrogen source, however, some of those electrons must be used to reduce nitrogen from the +V state to the −III state, and thus those electrons are incorporated into the biomass even though they will not be measured in the COD test. This is because nitrogen does not accept or give up electrons in the COD test. Consequently, if biomass is represented by $C_5H_7O_2N$, its COD must be multiplied by 1.4 for the balance to work, as suggested by Eq. 3.17. Thus, to generalize:

$$\text{COD removed} = O_2 \text{ equivalents of terminal electron acceptor used} + \alpha_N(\text{COD of biomass formed}) \tag{3.90}$$

where:

$\alpha_N = 1.0$ NH_4^+ as nitrogen source

$\alpha_N = 1.4$ NO_3^- as nitrogen source

Equation 3.90 is generally applicable and is much easier to use for determining the amount of terminal electron acceptor required than the writing of a molar or mass-based stoichiometric equation. Thus, it is widely employed and will be used frequently herein. Ammonia will be assumed to be the nitrogen source throughout this book, unless specifically stated otherwise. Thus, α_N will generally be set equal to 1.0.

3.8.2 Determination of Quantity of Nutrient Needed

The amount of nitrogen required for heterotrophic biomass growth can also be calculated from the combined stoichiometric equation. Since the only use of nitrogen in the equation is for synthesis of biomass, the equation may be used to establish a relationship that is very useful for estimating nutrient requirements. If the ammonium ion requirement is expressed per unit of biomass COD formed, it is found to be 0.112 mg of NH_4^+ per mg of biomass COD formed. Or, expressed as the amount of nitrogen required, it is 0.087 mg of N per mg of biomass COD formed. Actually, this can be considered to be a generality that is independent of the source of the nitrogen, provided that $C_5H_7O_2N$ represents the composition of biomass. This suggests that once the observed yield has been determined, the amount of nitrogen required can be estimated easily, allowing adequate amounts to be provided if they are not naturally present in the wastewater. Likewise, each time a mg of biomass COD is destroyed, 0.087 mg of nitrogen will be released to the medium, and this fact must be considered in operations such as aerobic digestion which are designed to destroy biomass. As with determination of the electron acceptor requirement, the main purpose of traditional stoichiometric equations has been to provide simplified relationships such as these for engineering use. Thus, the conversion factor is generally used in lieu of writing a new balanced stoichiometric equation for each situation.

Table 3.3 Approximate Micronutrient Requirements for Bacterial Growth

Micronutrient	Approximate requirement,* μg/mg biomass COD formed
Potassium	10
Calcium	10
Magnesium	7
Sulfur	6
Sodium	3
Chloride	3
Iron	2
Zinc	0.2
Manganese	0.1
Copper	0.02
Molybdenum	0.004
Cobalt	<0.0004

* Estimates based on the judgment of the authors after considering information in Refs. 30, 99, and 127.

As mentioned in Section 3.2.1, the phosphorus requirement for normal microbial growth can be estimated as one-fifth of the nitrogen requirement on a mass basis. Consequently, about 0.017 mg of phosphorus will be required for each mg of heterotrophic or autotrophic biomass COD formed, and an equal amount will be released for each mg destroyed. If PAOs are in the system, the amount released by destruction of the biomass will be different and will depend on the amount of poly-P stored.

The provision of sufficient nutrients is essential if efficient wastewater treatment is to be achieved, because without them the microorganisms will not be able to perform their synthesis reactions. Although nitrogen and phosphorus are the nutrients needed in greatest quantity (macronutrients), many other elements are required by the microorganisms but are not normally included in the stoichiometric equation because of the complicating effect they would have. The need for them should not be ignored nor should their presence be taken for granted because severe problems can result if sufficient quantities are not available.[16,127] Table 3.3 lists the major micronutrients required for bacterial growth.[87] There is little agreement in the literature concerning their quantities in biomass. One reason is that different bacteria have different requirements. Another is that bacteria tend to adsorb cations, thereby making it difficult to determine exactly the quantity actually incorporated into biomass. The values listed in Table 3.3 are the authors' best estimates of the quantities required based on examination of several sources.[30,99,127]

3.9 EFFECTS OF TEMPERATURE

Temperature can exert an effect on biological reactions in two ways: by influencing the rates of enzymatically catalyzed reactions and by affecting the rate of diffusion

of substrate to the cells. The importance of both has not always been recognized and this has led to some confusion in the quantification of temperature effects. For example, temperature effects observed in the laboratory are often more pronounced than those observed in the field. This is due in part to the fact that full-scale reactors are apt to be diffusion controlled. Consequently, the temperature coefficients given below are provided simply to give an idea of the importance of temperature to various microbial processes. For system design, actual temperature effects should always be measured in prototype systems that simulate the anticipated mixing regime.

3.9.1 Methods of Expressing Temperature Effects

There are three techniques commonly used to quantify the effects of temperature on biochemical operations. The oldest is that of Arrhenius,[5] who first applied it in 1889 to quantify the effects of temperature on the enzymatic hydrolysis of sugar. It is:

$$k = A \cdot e^{-u/RT} \tag{3.91}$$

where k is the temperature dependent rate coefficient, A is a constant, u is the temperature coefficient, R is the gas constant, and T is the absolute temperature. The value of u may be obtained by plotting ln k versus 1/T and determining the slope. For normal SI units, the units of u are kJ/mole and a positive value means that k increases as the temperature is increased.

Although microorganisms have been found in extreme environments that can grow at temperatures approaching either the freezing point or the boiling point of water, most microorganisms exhibit a relatively narrow temperature range over which they can function. Within that range, most reaction rate coefficients increase as the temperature is increased, but then eventually decrease as the heat begins to inactivate cellular enzymes. The Arrhenius equation, as well as the others to be discussed below, are only applicable over the range where the coefficient increases with increasing temperature. Microorganisms are grouped into three categories depending on that temperature range. Of chief concern in biochemical operations are mesophilic organisms, which grow well over the range of 10–35°C. The two other groups, psychrophilic and thermophilic, have ranges on either side and find use under special conditions. Unless otherwise specified, all parameter values given in this book will be for mesophilic microorganisms.

If a rate coefficient is known at one temperature, it may be calculated at another through rearrangement of the Arrhenius equation:

$$\ln(k_1/k_2) = \frac{u(T_1 - T_2)}{(R \cdot T_1 \cdot T_2)} \tag{3.92}$$

Because the mesophilic temperature range is small when T is expressed in K, the term $(R \cdot T_1 \cdot T_2)$ does not vary appreciably and may be considered to be constant. Consequently, a more commonly used expression is:[89]

$$k_1 = k_2 \cdot e^{C(T_1 - T_2)} \tag{3.93}$$

where

$$C = \frac{u}{(R \cdot T_1 \cdot T_2)} \approx 0.0015\ u \tag{3.94}$$

for the normal mesophilic temperature range. Note that when Eq. 3.93 is used, the temperature may be expressed in °C because only the temperature difference enters into the equation. In that case the units of C are $°C^{-1}$. The value of C may be determined by plotting ln(k) versus T, giving a slope equal to C.

Finally, a third equation has found considerable use in the environmental engineering literature:[96]

$$k_1 = k_2 \cdot \theta^{(T_1 - T_2)} \tag{3.95}$$

Actually, Eqs. 3.93 and 3.95 are the same since:

$$C = \ln(\theta) \tag{3.96}$$

Thus, the coefficient θ may also be estimated by plotting ln(k) versus T, giving a slope equal to ln(θ). θ is dimensionless.

The temperature coefficients for the three equations may be interconverted by:

$$\ln(\theta) = C \approx 0.0015\ u \tag{3.97}$$

in which the temperature is expressed in °C or K.

3.9.2 Effects of Temperature on Kinetic Parameters

Biomass Growth and Substrate Utilization. It will be recalled from Eqs. 3.35 and 3.43 that biomass growth and substrate utilization are proportional to each other, with the yield being the proportionality coefficient. It will also be recalled from Figure 2.4 that temperature can influence the value of the yield. This suggests that temperature can influence growth and substrate utilization in quantitatively different ways. Nevertheless, because of the uncertainty associated with the impact of temperature on Y, most engineers assume it to be independent of temperature, thereby allowing the same temperature coefficient to be used for both growth and substrate utilization.

Two parameters are required to characterize biomass growth, $\hat{\mu}$ and K_S. The first is clearly a rate coefficient, and as such, its value increases with increasing temperature. The second describes how substrate concentration influences the specific growth rate, and thus the impact of temperature on it is less clear, with it increasing under some circumstances and decreasing under others. Consequently, there is no consensus about its relationship to temperature, and each situation must be experimentally determined.

Most studies of the impact of temperature have been done on the aerobic growth of heterotrophs. Two studies[17,85] have reviewed the literature, and have reported values of u for $\hat{\mu}$ ranging from 21.3 to 167.4 kJ/mole. The average value for the larger data base[17] (18 values) was 59.8 kJ/mole, which converts to C and θ values of 0.090 $°C^{-1}$ and 1.094, respectively. Very few studies reporting the effects of temperature on K_S were cited, and there was no consensus among them as to whether it increased or decreased with increasing temperature.

Very few studies have been done to quantify the effects of temperature on microbial growth under anoxic conditions. van Haandel et al.[123] recommend that a θ value of 1.20 ($C = 0.182\ °C,^{-1}$ u = 121 kJ/mole) be used for $\hat{q}$. This value is near the upper range for the aerobic values reported above, which suggests that it may be high. Until more data are available, it may be prudent to adopt a value more

Solubilization of Particulate and High Molecular Weight Organic Matter. As might be anticipated from the discussion in Section 3.5, relatively little work has been done on the effects of temperature on the hydrolysis of particulate substrate. However, because it is an enzymatic step, the hydrolysis coefficient, k_h, is likely to rise as the temperature is increased. From comparison of experimental data to simulation results from a complex system model, van Haandel et al.[123] concluded that a u value of 38.8 kJ/mole ($C = 0.058\ °C^{-1}$, $\theta = 1.060$) was appropriate for both aerobic and anoxic environments. No information was given for the effect of temperature on K_X, the half-saturation coefficient for hydrolysis.

Other Important Microbial Processes. Insufficient data are available to allow quantification of the effects of temperature on other processes, such as phosphorus release, but it is likely that appropriate temperature coefficients will be developed for them in the future.

3.10 KEY POINTS

1. Stoichiometric equations may be written on a mass basis rather than a molar basis. When this is done the total mass of reactants equals the total mass of products. When a stoichiometric equation is written on a mass of COD basis, only constituents containing elements that change oxidation state are included. The COD of the reactants must equal the COD of the products.
2. When nitrate serves as the terminal electron acceptor, nitrogen changes oxidation state from +V to 0. Consequently, the oxygen equivalence of nitrate is −2.86 mg COD/mg N (2.86 mg O_2/mg N). When nitrate serves as the nitrogen source for biomass growth, the nitrogen is reduced to the amino level; i.e., from the +V to the −III state. In that case the oxygen equivalence is −4.57 mg COD/mg N (4.57 mg O_2/mg N).
3. If the general form of the mass-based stoichiometric equation is written as:

$$(-1)A_1 + (-\Psi_2)A_2 + \cdots + (-\Psi_k)A_k + \Psi_{k+1}A_{k+1} + \cdots + (\Psi_m)A_m = 0$$

then, r, the generalized reaction rate is given by:

$$r = \frac{r_1}{(-1)} = \frac{r_2}{(-\Psi_2)} = \frac{r_k}{(-\Psi_k)} = \frac{r_{k+1}}{(\Psi_{k+1})} = \frac{r_m}{(\Psi_m)}$$

Furthermore, if there are j reactions (where $j = 1 \rightarrow n$) involving i components (where $i = 1 \rightarrow m$), the overall reaction rate for component i will be given by:

$$r_i = \sum_{j=1}^{n} \Psi_{i,j} \cdot r_j$$

If r_i is negative, component i is being consumed, whereas if it is positive, the component is being produced.

4. Knowledge of the yield is required before the stoichiometric equation for microbial growth can be written. If McCarty's half-reaction approach is used to write the stoichiometric equation, f_S, the fraction of the electron donor captured through synthesis, is directly related to the yield.
5. When the electron donor is an organic compound, ammonia serves as the nitrogen source, and the yield is expressed as biomass COD formed per unit of substrate COD used, f_S and Y are equal. For other circumstances, f_S may be either greater than or smaller than Y.
6. Bacteria divide by binary fission. Thus, their rate of growth is first order with respect to the concentration of active biomass present:

$$r_{XB} = \mu X_B$$

The rate coefficient, μ, is called the specific growth rate coefficient. It is influenced by the substrate concentration. If the substrate is noninhibitory, the most commonly used expression is that of Monod:

$$\mu = \hat{\mu} \frac{S_S}{K_S + S_S}$$

If the substrate is inhibitory to its own biodegradation, the Andrews equation is commonly used:

$$\mu = \hat{\mu} \frac{S_S}{K_S + S_S + S_S^2/K_I}$$

7. When the substrate concentration is large relative to K_S, the Monod equation may be simplified to an expression that is zero order with respect to the substrate concentration. When the substrate concentration is small relative to K_S, the specific growth rate coefficient is approximately first order with respect to the substrate concentration.
8. Complementary nutrients are those that meet different needs by growing microorganisms whereas substitutable nutrients are those that meet the same need. The effects of limitation by two complementary nutrients may be depicted by interactive and noninteractive models. The interactive approach is more appropriate for modeling wastewater treatment systems.
9. Biochemical operations can be designed most easily when the nutrient the system is being designed to control acts as the growth limiting nutrient for the biomass in the system.
10. The kinetic parameters in the Monod and Andrews equations depend strongly on the species of microorganism and the substrate upon which the microorganisms are growing. Since wastewater treatment operations use mixed cultures, and wastewaters contain many compounds, the parameters used to describe such operations should be characterized by ranges rather than by single values.
11. Nitrifying bacteria have lower maximum specific growth rate coefficients than heterotrophic bacteria and are more sensitive to pH and to low dissolved oxygen concentrations.