substrate released is always less than the amount destroyed by lysis, as discussed in Section 2.4.1.

A conceptually similar, but less complex, model was developed by Dold et al.[26] for use in modeling wastewater treatment systems containing both aerobic and anoxic zones. Only one type of biomass is considered to be present: active, viable biomass. However, it is viewed as continually undergoing death and lysis, yielding particulate substrate and biomass debris. As in the model of Mason et al.,[73] particulate substrate is hydrolyzed to soluble substrate, and the soluble substrate is used by the viable biomass for growth, yielding new cell material. However, as above, because biomass yield values are always less than one, the amount of new biomass formed is always less than the amount destroyed by death and lysis, resulting in a net loss of biomass from the system (i.e., decay). A loss of viability results from the accumulation of biomass debris and particulate substrate.

The model of Dold et al.[26] is simpler than that of Mason et al.,[73] yet appears to be adequate for modeling many important wastewater treatment systems.[25] Furthermore, it can account for differences in decay observed as bacteria are cycled through aerobic, anoxic, and anaerobic conditions, whereas those differences cannot be accounted for by the traditional decay approach.[123] Finally, it has been adopted for use in a general model of single-sludge processes[54,55] that has been shown to adequately represent the dynamic performance of full-scale systems.[8] Thus, it will be used herein as an alternative to the traditional approach. The events in it are depicted in Figure 3.6.

The COD-based stoichiometry of the lysis:regrowth approach of Dold et al.[26] is:

$$\begin{aligned}&\text{Biomass COD} \rightarrow (1 - f'_D)\ \text{particulate substrate COD}\\ &\quad + f'_D\ \text{biomass debris COD}\end{aligned} \tag{3.60}$$

where f'_D is the fraction of active biomass contributing to biomass debris. No COD is lost during death and lysis. Rather active biomass COD is simply converted into an equivalent amount of COD due to biomass debris and particulate substrate. As a

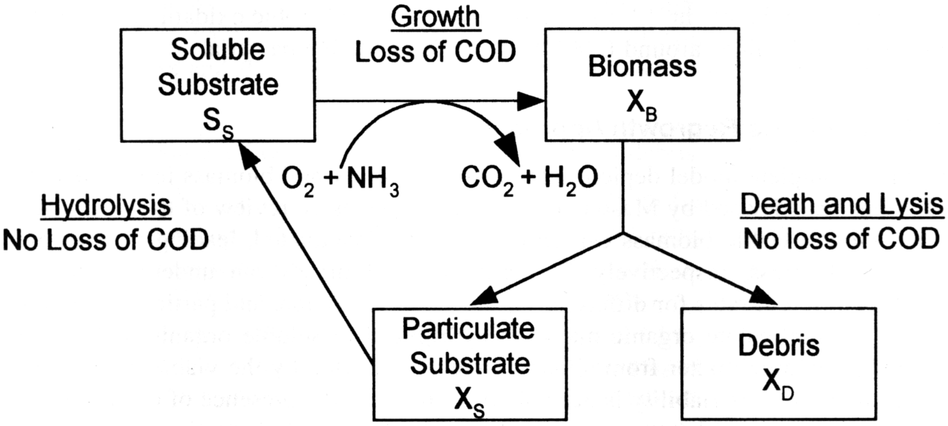

Figure 3.6 Schematic representation of the lysis:regrowth approach to modeling biomass decay and loss of viability.

consequence, no use of electron acceptor is directly associated with the loss of biomass, i.e., decay. Electron acceptor utilization occurs as soluble substrate, which arises from hydrolysis of particulate substrate, is used by active biomass for growth. As with the traditional approach, cell debris is assumed to be resistant to microbial attack within the time constraints of biochemical operations.

The nitrogen in the biomass is divided between biomass debris and particulate substrate, with the latter being called particulate biodegradable organic nitrogen. The nitrogen based stoichiometric equation depicting this is:

$$\text{Biomass N} \rightarrow \text{particulate biodegradable organic N} + \text{biomass debris N} \qquad (3.61)$$

Giving the same meanings to $i_{N/XB}$ and $i_{N/XD}$ as given above, Eq. 3.61 can be rewritten in terms of biomass COD and biomass debris COD:

$$i_{N/XB} \cdot \text{Biomass COD} \rightarrow \text{particulate biodegradable organic N} + i_{N/XD} \cdot \text{biomass debris COD} \qquad (3.62)$$

Thus, each unit of biomass COD lost to decay yields $(i_{N/XB} - i_{N/XD} \cdot f'_D)$ units of particulate biodegradable organic nitrogen. This differs from the traditional approach which leads directly to soluble ammonia nitrogen.

As in the traditional approach, the rate of loss of biomass COD to death and lysis is considered to be first order with respect to the active biomass concentration:

$$r_{XB} = -b_L \cdot X_B \qquad (3.63)$$

where b_L has units of hr^{-1}, just as b does. In a manner similar to the traditional approach, the rate of production of biomass debris COD is:

$$r_{XD} = b_L \cdot f'_D \cdot X_B \qquad (3.64)$$

And the rate of production of particulate substrate COD (X_S) is:

$$r_{XS} = (1 - f'_D) b_L \cdot X_B \qquad (3.65)$$

Note the similarity of this equation to Eq. 3.58, the equation for oxygen consumption in the traditional approach. This similarity arises from the retention in the particulate substrate of all electrons lost from active biomass, rather than their transfer to oxygen. Finally, the rate of production of particulate, biodegradable organic nitrogen (X_{NS}) is:

$$r_{XNS} = (i_{N/XB} - i_{N/XD} \cdot f'_D) b_L \cdot X_B \qquad (3.66)$$

It is important to realize that b_L is conceptually and numerically different from b and that f'_D is numerically different from f_D. This follows from the cycling of COD that occurs in the lysis:regrowth approach. Biomass COD is lost, releasing particulate substrate COD, which is hydrolyzed to soluble substrate COD, which is degraded by active biomass yielding new biomass, which is lost by death and lysis giving particulate substrate COD, etc. The net effect of the two approaches is the same because a given amount of biomass will be lost from a bioreactor regardless of how we conceptualize the actual events occurring. Since it is necessary for carbon to cycle around the system several times in the lysis:regrowth conceptualization to achieve the same loss of biomass that the traditional approach achieves in one pass,

aer

b_L must be numerically larger than b. Likewise, since the same amount of biomass debris is ultimately formed from the loss of a given amount of biomass by decay, f'_D must be numerically smaller than f_D. In fact, the values of the four parameters are related:[26]

$$f'_D \cdot b_L = f_D \cdot b \tag{3.67}$$

Furthermore,

$$f'_D = \left(\frac{1 - Y}{1 - Y \cdot f_D}\right) f_D \tag{3.68}$$

It was stated above that f_D has a value around 0.2. Given the Y values associated with the biomass for which f_D was estimated, Eq. 3.68 suggests that the value of f'_D is around 0.08.[25] The values of f_D and f'_D are not likely to vary greatly, and thus those values will be adopted herein. It should be noted, however, that the relationship between b_L and b also depends on Y:[25]

$$b_L = \frac{b}{[1 - Y(1 - f'_D)]} \tag{3.69}$$

Although it is common during parameter evaluation studies to measure both Y and b, neither f_D nor f'_D is commonly measured. Since Y can influence the relationship between b_L and b, it is recommended that Eq. 3.69 be used instead of Eq. 3.67 to convert measured b values to b_L values.[55]

An important assumption implicit in the lysis:regrowth approach is that within a given culture, cell lysis occurs all of the time with the same value of the rate coefficient b_L, regardless of the rate at which the bacteria are growing. The validity of this assumption has been confirmed by measuring the release of nucleic acids as direct evidence of cell lysis.[95]

For autotrophic growth, the relationship between b_L and b is different.[54,55] This is because autotrophic organisms do not use organic matter for growth. Thus, death and lysis will not lead to additional autotrophic biomass growth. (The amount of autotrophic biomass that will grow from the nitrogen released is negligible.) Rather, heterotrophic biomass will grow on the organic matter released. As a consequence, the lysis:regrowth and traditional approaches are the same for autotrophic biomass; the result is that the two parameter values are equal.

3.4 SOLUBLE MICROBIAL PRODUCT FORMATION

As discussed in Section 2.4.3, soluble microbial products are thought to arise from two processes, one growth associated and the other non–growth-associated.[103]

Growth associated product formation results directly from biomass growth and substrate utilization. If soluble microbial product formation was occurring in appreciable amount, it would be necessary to modify the stoichiometric equation for microbial growth to account for it. Letting S_{MP} represent the concentration of soluble microbial products in COD units and Y_{MP} the product yield in units of product COD formed per unit of substrate COD used, Eq. 3.31 can be rewritten to account for soluble microbial product formation:

$$(1)S_S + [-(1 - Y_H - Y_{MP})]S_O \rightarrow Y_H X_{B,H} + Y_{MP} S_{MP} \qquad (3.70)$$

This shows that less electron acceptor is used when soluble products are formed because part of the COD of the substrate remains in the medium as those products. Rewriting this equation in the form of Eq. 3.9 with biomass as the reference constituent gives:

$$\left(-\frac{1}{Y_H}\right) S_S + (-1)\left[-\left(\frac{1 - Y_H - Y_{MP}}{Y_H}\right)\right] S_O + X_{B,H} + \frac{Y_{MP}}{Y_H} S_{MP} = 0 \qquad (3.71)$$

This tells us:

$$r_{SMP} = (Y_{MP}/Y_H) r_{XB} \qquad (3.72)$$

Combining Eq. 3.72 with Eq. 3.35 for r_{XB} gives:

$$r_{SMP} = (Y_{MP}/Y_H)\mu \cdot X_{B,H} \qquad (3.73)$$

The fact that r_{SMP} is proportional to μ shows that it is growth-associated.

Non–growth-associated product formation, also called biomass-associated product formation,[103] occurs as a result of cell lysis and decay. Rewriting Eq. 3.53 to incorporate soluble product formation into the COD-based stoichiometry of the traditional approach to decay gives:

$$\begin{aligned}&\text{Biomass COD} + [-(1 - f_D - f_{MP})]O_2 \text{ equivalents of electron}\\ &\quad \text{acceptor} \rightarrow f_D \text{ biomass debris COD} + f_{MP} \text{ soluble product COD}\end{aligned} \qquad (3.74)$$

where f_{MP} is the fraction of active biomass contributing to biomass-associated products. Using this with Eq. 3.56 gives the rate of production of biomass-associated product:

$$r_{SMP} = b \cdot f_{MP} \cdot X_{B,H} \qquad (3.75)$$

By analogy to biomass debris formation, a similar approach could be used to account for soluble microbial product formation in the lysis:regrowth approach, giving a parameter f'_{MP} which is smaller than f_{MP} in the same way that f'_D is smaller than f_D.

Combining Eqs. 3.73 and 3.75 suggests that the specific rate of soluble microbial product formation is linearly related to the specific growth rate. While such a relationship may be adequate for slowly growing cultures like those found in activated sludge systems, it is not adequate for more rapidly growing systems[49] and thus Eqs. 3.73 and 3.75 cannot be considered to be of general applicability to all systems. Although a relatively large body of research on soluble microbial product formation has been conducted,[49,103] it is still not sufficient to allow consensus on the rate expressions to be used. Thus, in spite of its known importance, soluble microbial product formation will not be incorporated into the models in Parts II and IV.

Insufficient information is available to provide typical values for f_{MP} and f'_{MP}, but, as indicated in Section 2.4.3, Y_{MP} values have been found to be less than 0.1.[42]

3.5 SOLUBILIZATION OF PARTICULATE AND HIGH MOLECULAR WEIGHT ORGANIC MATTER

The conversion of particulate and high molecular weight organic matter into forms small enough for bacteria to take up and degrade is an important step in biochemical operations for wastewater treatment because such materials are commonly present in wastewaters and also arise from lysis reactions as discussed previously. In spite of that, relatively few studies have focused on those reactions. Perhaps this is because many types of particulate materials are attacked by distinctly different mechanisms, even though they are collectively referred to as hydrolysis.

The stoichiometry of hydrolysis is thought to be very simple, with organic material simply changing form. Consequently, most investigators have assumed that COD is conserved, i.e., that no energy is consumed. This is indicated in Figure 3.6. Because no energy is consumed, no electrons are removed and no terminal electron acceptor is used. Thus, the stoichiometric equation is simply:

$$\text{Particulate substrate COD} \rightarrow \text{soluble substrate COD} \tag{3.76}$$

This means that the rate of formation of soluble substrate COD is equal to the rate of loss of particulate substrate COD.

In the face of complex situations in which reactions are ill defined, it is common for engineers to choose the simplest possible reaction rate expression, and that is what a number of investigators have done, assuming that hydrolysis is first order with respect to the concentration of particulate substrate, X_S.[14,15,28,44,73] This approach, however, ignores the effect that the biomass concentration will have on the rate.

One group[55] performed an extensive literature survey before adopting a kinetic expression for the hydrolysis of particulate organic matter patterned after that of Dold et al.,[26] which is based on the work of Stenstrom:[117]

$$r_{XS} = -k_h \left[\frac{X_S/X_{B,H}}{K_X + (X_S/X_{B,H})} \right] X_{B,H} \tag{3.77}$$

In this expression k_h is the hydrolysis coefficient (hr^{-1}) and K_X is a half-saturation coefficient (mg particulate substrate COD/mg active biomass COD). An important characteristic of this expression is that even though the rate is first order with respect to the heterotrophic biomass concentration, it is controlled by the ratio of particulate substrate concentration to heterotrophic biomass concentration, rather than by the particulate substrate concentration alone. This is necessary because the reaction is thought to be surface mediated, depending on the presence of extracellular enzymes whose quantity will be proportional to the biomass concentration.[26]

Data on the values of k_h and K_X are very limited. Based primarily on the recommendations of Dold and Marais,[25] one group[54] adopted a value of 0.092 hr^{-1} for k_h and a value of 0.15 for K_X.

The rate of hydrolysis will also be influenced by the electron acceptor concentration,[25,54,55] even though no electron acceptor is used in the reaction. Under aerobic conditions, an interactive, dual nutrient limitation expression has been adopted in a manner similar to that in Eq. 3.46. Under anoxic conditions, an expression similar to that in Eq. 3.48 has been found to be appropriate, with nitrate stimulating anoxic hydrolysis and oxygen inhibiting it. In both expressions, the effect of the particulate substrate should be given by Eq. 3.77. Under anaerobic conditions of short duration, hydrolysis is assumed to stop. While this would not be true for long term anaerobic conditions, it is consistent with observations in biochemical operations that cycle bacteria between aerobic and anoxic conditions.[25] It would probably be satisfactory to use Eq. 3.77 alone to describe hydrolysis under fully anaerobic conditions, but with a k_h value much smaller than that used for aerobic conditions.

As seen in Eq. 3.61, biomass decay results in the formation of particulate biodegradable organic nitrogen. In addition, organic nitrogen will be associated with the particulate organic matter in the wastewater. All of this material will be converted into soluble, biodegradable organic nitrogen, S_{NS}, (i.e., the nitrogen associated with amino acids and other soluble, nitrogen containing organic substrates) as the particulate substrate is hydrolyzed. The rate of generation of S_{NS} (r_{SNS}) is numerically equivalent to the rate of loss of particulate organic nitrogen (r_{XNS}), which is proportional to the hydrolysis rate of particulate organic matter:[25]

$$r_{SNS} = -r_{XNS} = -(X_{NS}/X_S)r_{XS} \tag{3.78}$$

where X_{NS} is the concentration of particulate, biodegradable organic nitrogen.

3.6 AMMONIFICATION AND AMMONIA UTILIZATION

Ammonification is the conversion of soluble organic nitrogen into ammonia-N that occurs as bacteria consume soluble organic matter containing nitrogen. Actually, the true rate of ammonification is difficult to measure because ammonia-N is being consumed by the bacteria as they grow, and the only measurable event is the net accumulation or loss of ammonia in the medium. If the amount of nitrogen available in the organic substrate is just sufficient to meet the biosynthetic needs of the new biomass, there will be no net change in the ammonia-N concentration in the medium. On the other hand, if that amount exceeds the need, the ammonia concentration in the medium will increase, whereas if that amount is less than the need, the ammonia concentration will decrease. It should be recognized, however, that whether organic nitrogen is incorporated directly into new biomass depends on its form. The nitrogen in simple compounds like amino acids may be incorporated directly as the amino acids are used for protein synthesis, while nitrogen in complex synthetic organic chemicals may be released to the medium as ammonia.

In an effort to make this complex situation mathematically tractable, most modelers assume that all nitrogen goes through the medium before being used. Thus, ammonification is assumed to release all organic nitrogen to the medium as ammonia, and nitrogen utilizing reactions are assumed to obtain their ammonia from the medium. Whether ammonia accumulates or is removed depends on the relative rates of its production and utilization.

Because ammonification occurs as heterotrophic biomass destroys nitrogen containing soluble organic matter, it is likely that its rate is proportional to the rate of soluble substrate removal. Relatively little work has been done to investigate the rate of ammonification in complex substrates in which only a part of the soluble organic matter contains nitrogen, and it is uncertain whether a direct proportionality can be assumed between soluble substrate removal and ammonification. Consequently, ammonification has been represented as a reaction that is first order with respect to both the heterotrophic biomass concentration and the concentration of soluble, biodegradable organic nitrogen:[25,54,55]

$$r_{SNS} = -k_a \cdot S_{NS} \cdot X_{B,H} \tag{3.79}$$

where k_a is the ammonification rate coefficient (L/(mg biomass COD · hr)). Very little information is available about its value. As discussed above, the assumed stoichiometry of ammonification is such that all nitrogen removed from nitrogen containing soluble organic matter is released as ammonia, although some may ultimately be used for biomass synthesis. Thus, the rate of production of ammonia nitrogen (S_{NH}) through ammonification is:

$$r_{SNH} = -r_{SNS} \tag{3.80}$$

Ammonia is removed from solution by two reactions. First, it is used in the synthesis of new biomass as seen in Eq. 3.6 and others presented in Section 3.2. Second, it is used as a substrate by autotrophic biomass. The rate expression for the second use is the same as any other substrate, as discussed in Section 3.2.6. The rate expression for the first use can be determined from the generalized rate expression and the stoichiometry of growth. Since $i_{N/XB}$ is the mass of nitrogen per unit of biomass COD, the rate of ammonia removal through biomass growth is simply:

$$r_{SNH} = -i_{N/XB} \cdot \mu \cdot X_B \tag{3.81}$$

Equation 3.81 is true for both heterotrophic and autotrophic growth, and thus the general symbol for biomass, X_B, has been used in it.

3.7 PHOSPHORUS UPTAKE AND RELEASE

Biological phosphorus removal is a complex process that is dependent on the growth of specialized phosphate accumulating organisms (PAOs), which store phosphorus as polyphosphate (poly-P), as discussed in Section 2.4.6. Because biological phosphorus removal is still the subject of active experimental investigation, there is little consensus concerning all of the rate expressions describing it. Furthermore, the microbial events involved are subject to complex control through the concentrations of several constituents. As a consequence, a discussion beyond the scope of this chapter would be needed to fully define the kinetics and stoichiometry of biological phosphorus removal. There are, however, two events that should be considered briefly because they are distinctly different from the events discussed above. These are the uptake and release of phosphorus by the PAOs.

It will be recalled from Section 2.4.6 that two conceptual models exist for biological phosphorus removal, the Comeau/Wentzel model and the Mino model, illustrated in Figures 2.5 and 2.6, respectively. The major difference between them is that the Mino model incorporates glycogen formation and utilization, whereas the Comeau/Wentzel model does not. In developing a mathematical model for biological phosphorus removal, one group decided that the introduction of a detailed mechanistically based mathematical model for the process was premature.[56] Rather, they preferred to recommend the simplest mathematical model that allows adequate prediction of biological phosphorus removal. Consequently, they basically followed the Comeau/Wentzel model. Furthermore, they assumed that PAOs cannot use nitrate as a terminal electron acceptor and that they can only grow on the PHB stored in the cell. Although these assumptions are severe restrictions, we have chosen to use activated sludge model (ASM) No. 2 as the basis for the rate expressions presented herein because it represents a consensus among several investigators in the field.[56] It should be recognized, however, that more complete expressions are likely to be developed as more research is done.

Under anaerobic conditions, PAOs do not grow, but store acetic acid as PHB through the cleavage of Poly-P with the associated release of soluble phosphate. (In the following equations, all phosphate concentrations are expressed as phosphorus, P, and all organic materials are expressed as COD). The rate of removal of acetic acid, r_{SA}, can be modeled with an interactive, dual limiting nutrient expression:

$$r_{SA} = -\hat{q}_A \left(\frac{S_A}{K_A + S_A}\right)\left[\frac{X_{PP}/X_{B,P}}{K_{PP} + (X_{PP}/X_{B,P})}\right] X_{B,P} \tag{3.82}$$

where $\hat{q}_A$ is the maximum specific rate of acetic acid uptake, hr^{-1}, S_A is the acetic acid concentration in COD units, K_A is a half-saturation coefficient for acetic acid, X_{PP} is the poly-P concentration in the biomass, expressed as a liquid phase P concentration, K_{PP} is a half-saturation coefficient for poly-P in the same units, and $X_{B,P}$ is the concentration of PAO biomass in COD units. The stoichiometry of the reaction is such that each mg/L of acetic acid COD removed from the medium forms a mg/L of PHB COD in the biomass. Thus, the rate expression for PHB (X_{PHB}) formation, r_{XPHB}, is:

$$r_{XPHB} = -r_{SA} \tag{3.83}$$

Furthermore, Y_P units of soluble phosphate, S_P, are released for each unit of acetic acid stored as COD, increasing S_P by an amount equal to the decrease in stored Poly-P concentration. Thus:

$$r_{SP} = -r_{XPP} = Y_P \cdot r_{XPHB} \tag{3.84}$$

The value of Y_P selected by Henze et al.[56] was 0.40 mg P/mg COD, reflecting the average stoichiometry for the process. The values of K_{SA} and K_{PP} were chosen to be small to make the parenthetical terms in Eq. 3.82 serve as switching functions that change rapidly from one to zero, thereby turning the reaction on and off. The value chosen for K_{SA} was 4.0 mg COD/L whereas the value chosen for K_{PP} was 0.01 mg P/mg PAO COD.

Under aerobic conditions, the PAOs grow by using the stored PHB as a carbon and energy source. This is assumed to be their only substrate for growth, even though they are capable of growth on soluble substrates. Because little soluble substrate is likely to be present in the aerobic portion of a biological phosphorus removal process, Henze et al.[56] ignored it to simplify the model. Furthermore, because the process can only occur under aerobic conditions, a switching function for oxygen was included to make the rate go to zero when oxygen is absent. Considering all of these factors, the rate of PAO growth can be described by:

$$r_{XBP} = \hat{\mu}_P \left[\frac{X_{PHB}/X_{B,P}}{K_{PHB} + (X_{PHB}/X_{B,P})}\right]\left(\frac{S_P}{K_P + S_P}\right)\left(\frac{S_O}{K_O + S_O}\right) X_{B,P} \tag{3.85}$$

where $\hat{\mu}_P$ is the maximum specific growth rate coefficient for PAOs, X_{PHB} is the stored PHB concentration in mg/L as COD, S_P is the soluble phosphate concentration 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:

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

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 → n) involving i components (where i = 1 → 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.