3

Stoichiometry and Kinetics of Biochemical Operations

Stoichiometry is concerned with the relationships between the quantities of reactants and products in chemical reactions. Kinetics is concerned with the rates at which reactions take place. Because stoichiometry quantitatively relates a change in one reactant (product) to the change in another, when the reaction rate of one reactant (product) becomes known, stoichiometry may be used to determine the reaction rate of another in the reaction. In this chapter we will first examine these relationships on a generalized basis. Then we will apply them to the major biochemical events from Chapter 2 and examine the expressions that will be used to model the theoretical performance of biochemical operations in Parts II and IV.

3.1 STOICHIOMETRY AND GENERALIZED REACTION RATE

3.1.1 Alternative Bases for Stoichiometry

Stoichiometric equations are usually derived in molar units, but they are not the most convenient units for our purposes. This is because we must write mass balance equations for the various constituents being acted upon in a biochemical operation in order to model its performance. Thus, it would be more convenient if the stoichiometric equations for the reactions were written in mass units. Consequently, we need to know how to convert a molar-based stoichiometric equation into a mass-based one. Furthermore, we see in Chapter 2 that microorganisms gain their energy from oxidation/reduction reactions in which electrons are removed from the electron donor and passed ultimately to the terminal electron acceptor. This suggests that it would also be convenient to write electron balances. Unfortunately, as we saw earlier, we usually don't know the exact composition of the electron donor in a wastewater, making this difficult to do. However, we can experimentally determine the chemical oxygen demand (COD), which is a measure of available electrons, of the various constituents. Thus, we can accomplish the same thing by writing a mass balance on COD for each of the constituents that undergo a change in oxidation state. Consequently, we also need to know how to convert molar- or mass-based stoichiometric equations into COD-based equations.

The general formula for a stoichiometric equation can be written as:[62]

$$a_1A_1 + a_2A_2 + \cdots + a_kA_k \rightarrow a_{k+1}A_{k+1} + a_{k+2}A_{k+2} + \cdots + a_mA_m \tag{3.1}$$

where A_1 through A_k are the reactants and a_1 through a_k are their associated molar stoichiometric coefficients, and A_{k+1} through A_m are the products and a_{k+1} through a_m are their molar stoichiometric coefficients. Two characteristics allow recognition of a stoichiometric equation as molar-based. First, the charges are balanced. Second, the total number of moles of any given element in the reactants equals the number of moles of that element in the products.

When writing a mass-based stoichiometric equation it is common practice to normalize the stoichiometric coefficients relative to one of the reactants or products. Thus, each normalized mass-based stoichiometric coefficient represents the mass of the particular reactant used or product formed relative to the mass of the reference reactant used or product formed. If A_1 is the component that we want to use as the basis for our mass-based stoichiometric equation, its stoichiometric coefficient would be 1.0 and the new mass-based stoichiometric coefficient for every other component (referred to as a normalized stoichiometric coefficient, Ψ_i) would be calculated from:

$$\Psi_i = (a_i)(MW_i)/(a_1)(MW_1) \tag{3.2}$$

where a_i and MW_i are the molar stoichiometric coefficient and molecular weight, respectively, of component A_i, and a_1 and MW_1 have the same meanings for the reference component. Thus, the equation becomes:

$$A_1 + \Psi_2A_2 + \cdots + \Psi_kA_k \rightarrow \Psi_{k+1}A_{k+1} + \Psi_{k+2}A_{k+2} + \cdots + \Psi_mA_m \tag{3.3}$$

Two characteristics can be used to identify this type of stoichiometric equation: (1) the charges do not appear to be balanced, and (2) the total mass of reactants equals the total mass of products. In other words, the sum of the stoichiometric coefficients for the reactants equals the sum of the stoichiometric coefficients for the products. The latter characteristic makes a mass-based stoichiometric equation well-suited for use in mass balance equations for biochemical reactors.

A similar approach can be used to write the stoichiometric equation in terms of compounds or components that change oxidation state by taking advantage of COD units.[62] In this case, the normalized stoichiometric coefficients are referred to as COD-based coefficients and are given the symbol Y. The COD-based coefficient, Y_i, for component A_i would be calculated from:

$$Y_i = (a_i)(MW_i)(COD_i)/(a_1)(MW_1)(COD_1) \tag{3.4}$$

$$Y_i = \Psi_i(COD_i)/(COD_1) \tag{3.5}$$

where COD_i and COD_1 are the COD per unit mass of component A_i and the reference component, respectively. They can be obtained by writing a balanced equation for the oxidation of the compound or component to carbon dioxide and water. Table 3.1 contains COD mass equivalents of several constituents that commonly change oxidation state in biochemical operations. Note that under oxidizing conditions, carbon dioxide has a COD of zero, since the carbon in it is already in the most oxidized state (+IV), as it is in bicarbonate and carbonate. Furthermore, oxygen is equivalent to negative COD since COD is oxygen demand, i.e., it represents loss of oxygen. Finally, it should be noted that any reactant or product containing only elements that do not change oxidation state during biochemical oxidation/reduction reactions will have a unit COD of zero, causing them to drop out of the COD-based stoichiometric equation.

Table 3.1 COD Mass Equivalents of Some Common Constituents

Constituent[a]	Change of oxidation state	COD equivalent[b]
Biomass, $C_5H_7O_2N$	C to +IV	1.42 g COD/g $C_5H_7O_2N$, 1.42 g COD/g VSS, 1.20 g COD/g TSS
Oxygen (as e^- acceptor)	O(0) to O(−II)	−1.00 g COD/gO_2[c]
Nitrate (as e^- acceptor)	N(+V) to N(0)	−0.646 g COD/g NO_3^-, −2.86 g COD/g N
Nitrate (as N source)	N(+V) to N(−III)	−1.03 g COD/g NO_3^-, −4.57 g COD/g N
Sulfate (as e^- acceptor)	S(+VI) to S(−II)	−0.667 g COD/g $SO_4^=$, −2.00 g COD/g S
Carbon dioxide (as e^- acceptor)	C(+IV) to C(−IV)	−1.45 g COD/g CO_2, −5.33 g COD/g C
CO_2, HCO_3^-, $H_2CO_3^=$	No change in an oxidizing environment	0.00
Organic matter in domestic wastewater, $C_{10}H_{19}O_3N$	C to +IV	1.99 g COD/g organic matter
Protein, $C_{16}H_{24}O_5N_4$	C to +IV	1.50 g COD/g protein
Carbohydrate, CH_2O	C to +IV	1.07 g COD/g carbohydrate
Grease, $C_8H_{16}O$	C to +IV	2.88 g COD/g grease
Acetate, CH_3COO^-	C to +IV	1.08 g COD/g acetate
Propionate, $C_2H_5COO^-$	C to +IV	1.53 g COD/g propionate
Benzoate, $C_6H_5COO^-$	C to +IV	1.98 g COD/g benzoate
Ethanol, C_2H_5OH	C to +IV	2.09 g COD/g ethanol
Lactate, $C_2H_4OHCOO^-$	C to +IV	1.08 g COD/g lactate
Pyruvate, CH_3COCOO^-	C to +IV	0.92 g COD/g pyruvate
Methanol, CH_3OH	C to +IV	1.50 g COD/g methanol
$NH_4^+ \rightarrow NO_3^-$	N(−III) to N(+V)	3.55 g COD/g NH_4^+, 4.57 g COD/g N
$NH_4^+ \rightarrow NO_2^-$	N(−III) to N(+III)	2.67 g COD/g NH_4^+, 3.43 g COD/g N
$NO_2^- \rightarrow NO_3^-$	N(+III) to N(+V)	0.36 g COD/g NO_2^-, 1.14 g COD/g N
$S \rightarrow SO_4^=$	S(0) to S(+VI)	1.50 g COD/g S
$H_2S \rightarrow SO_4^=$	S(−II) to S(+VI)	1.88 g COD/g H_2S, 2.00 g COD/g S
$S_2O_3^= \rightarrow SO_4^=$	S(+II) to S(+VI)	0.57 g COD/g $S_2O_3^=$, 1.00 g COD/g S
$SO_3^= \rightarrow SO_4^=$	S(+IV) to S(+VI)	0.20 g COD/g $SO_3^=$, 0.50 g COD/g S
H_2	H(0) to H(+I)	8.00 g COD/g H

[a] Listed in the same order as the reactants in Table 3.2.

[b] A negative sign implies that the constitutent is receiving electrons.

[c] By definition, oxygen demand is negative oxygen.

Example 3.1.1.1

Consider a typical molar-based stoichiometric equation for bacterial growth on carbohydrate (CH_2O) with ammonia as the nitrogen source:

$$CH_2O + 0.290\ O_2 + 0.142\ NH_4^+ + 0.142\ HCO_3^- \rightarrow$$
$$0.142\ C_5H_7O_2N + 0.432\ CO_2 + 0.858\ H_2O \quad (3.6)$$

where $C_5H_7O_2N$ is the empirical formula for cell mass. Note that the charges are balanced and that the number of moles of each element in the reactants equals the number in the products. The molar-based stoichiometric equation tells us that the biomass yield is 0.142 moles of biomass formed per mole of carbohydrate used and that 0.290 moles of oxygen are required per mole of carbohydrate used to synthesize that biomass.

Convert this equation to a mass based stoichiometric equation. To do this, we need the molecular weight of each reactant and product. These are CH_2O, 30; O_2, 32; NH_4^+, 18; HCO_3^-, 61; $C_5H_7O_2N$, 113; CO_2, 44; and H_2O, 18. Using these with the stoichiometric coefficients from Eq. 3.6 in Eq. 3.2 gives:

$$CH_2O + 0.309\ O_2 + 0.085\ NH_4^+ + 0.289\ HCO_3^- \rightarrow$$
$$0.535\ C_5H_7O_2N + 0.633\ CO_2 + 0.515\ H_2O \quad (3.7)$$

In this case, the charges are no longer balanced, but the sum of the stoichiometric coefficients for the reactants equals the sum for the products. The mass-based stoichiometric equation tells us that the biomass yield is 0.535 grams of biomass formed per gram of carbohydrate used and that 0.309 grams of oxygen are required per gram of carbohydrate used to synthesize that biomass.

Now convert the molar-based equation to a COD-based equation. To do this, use must be made of the unit CODs given in Table 3.1. In this case, the unit COD of ammonia is taken as zero because the nitrogen in cell material is in the same oxidation state as the nitrogen in ammonia, i.e., −III; it does not undergo a change of oxidation state. Carrying out the conversion represented by Eq. 3.4 yields:

$$CH_2O\ COD + (-0.29)O_2 \rightarrow 0.71\ C_5H_7O_2N\ COD \quad (3.8)$$

Note that only three constituents remain because they are the only ones that can be represented by COD in this case. Also note that like the mass-based equation, the sum of the stoichiometric coefficients for the reactants equals the sum of the stoichiometric coefficients for the products. Finally, note that the stoichiometric coefficient for oxygen carries a negative sign even though it is a reactant. That is because it is being expressed as COD. Thus, the COD-based stoichiometric equation tells us that the biomass yield is 0.71 grams of biomass COD formed per gram of carbohydrate COD used and that 0.29 grams of oxygen are required per gram of carbohydrate COD used to synthesize that biomass.

3.1.2 Generalized Reaction Rate

Stoichiometric equations can also be used to establish the relative reaction rates for reactants or products. Because the sum of the stoichiometric coefficients in a mass-based stoichiometric equation equals zero, its general form may be rewritten in the following way:[62]

$$(-1)A_1 + (-\Psi_2)A_2 + \cdots + (-\Psi_k)A_k + \Psi_{k+1}A_{k+1} + \cdots + \Psi_m A_m = 0 \quad (3.9)$$

where components 1 through k are reactants, components k+1 through m are products, and reactant A_1 is the basis for the normalized stoichiometric coefficients. Note that the normalized stoichiometric coefficients are given negative signs for reactants and positive signs for products. Since there is a relationship between the masses of the different reactants used or products formed, it follows that there is also a relationship between the rates at which they are used or formed. If we let r_i represent the rate of formation of component i (where i = 1 → k), it follows that:

$$\frac{r_1}{(-1)} = \frac{r_2}{(-\Psi_2)} = \frac{r_k}{(-\Psi_k)} = \frac{r_{k+1}}{(\Psi_{k+1})} = \frac{r_m}{(\Psi_m)} = r \quad (3.10)$$

where r is called the generalized reaction rate. As above, the sign on Ψ_i signifies whether the component is being removed or formed. Consequently, if the stoichiometry of a reaction has been determined in mass units, and the reaction rate has been determined for one component, then the reaction rates in mass units are known for all other components.

Equations 3.9 and 3.10 also hold true for COD-based stoichiometric equations. The normalized stoichiometric coefficients (Ψ_i) are simply replaced with appropriate COD-based coefficients (Y_i).

Example 3.1.2.1

Biomass is growing in a bioreactor at a rate of 1.0 g/(L·h) and the growth conforms to the stoichiometry expressed by Eq. 3.7. At what rate are carbohydrate and oxygen being used in the bioreactor to support that growth?

Rewriting Eq. 3.7 in the form of Eq. 3.9 gives:

$$-CH_2O - 0.309\ O_2 - 0.085\ NH_4^+ - 0.289\ HCO_3^- + 0.535\ C_5H_7O_2N + 0.633\ CO_2 + 0.515\ H_2O = 0$$

Use of Eq. 3.10 allows determination of the generalized reaction rate:

$$r = \frac{r_{C_5H_7O_2N}}{0.535} = \frac{1.0}{0.535} = 1.87\ \text{g CH}_2\text{O/(L·h)}$$

Note that the generalized reaction rate is expressed in terms of the constituent that serves as the basis for normalization of the stoichiometric equation. The rates of carbohydrate and oxygen utilization can now also be determined from Eq. 3.10:

$$r_{CH_2O} = (-1.0)(1.87) = -1.87\ \text{g CH}_2\text{O/(L·h)}$$

$$r_{O_2} = (-0.309)(1.87) = -0.58\ \text{g O}_2\text{/(L·h)}$$

3.1.3 Multiple Reactions—The Matrix Approach

In Chapter 2 we learn that there are many important events occurring in biochemical operations. Consequently, multiple reactions will take place simultaneously, and all must be considered when mass balance equations are written for biochemical operations. Extension of the concepts above to multiple reactions simplifies the presentation of those mass balances and allows the fates of all reactants to be easily visualized.[54,62]

Consider a situation in which i components (where i = 1 → m) participate in j reactions (where j = 1 → n), in which case $\Psi_{i,j}$ represents the normalized mass-based stoichiometric coefficient for component i in reaction j. This situation gives a group of mass-based stoichiometric equations:

$$\begin{array}{lllllll}
(-1)A_1 & + \cdots + & (-\Psi_{k,1})A_k & + & (+\Psi_{k+1,1})A_{k+1} & + \cdots + & (+\Psi_{m,1})A_m = 0\ r_1 \\
(+\Psi_{1,2})A_1 & + \cdots + & (-1)A_k & + & (+\Psi_{k+1,2})A_{k+1} & + \cdots + & (+\Psi_{m,2})A_m = 0\ r_2 \\
\vdots & & \vdots & & \vdots & & \vdots \\
(-\Psi_{1,n})A_1 & + \cdots + & (+\Psi_{k,n})A_k & + & (+\Psi_{k+1,n})A_{k+1} & + \cdots + & (-1)A_m = 0\ r_n
\end{array} \tag{3.11}$$

Note that A_1 does not necessarily represent the component chosen as the basis for the normalized stoichiometric coefficients. Rather, a different component may be selected for each reaction so that each resulting normalized stoichiometric coefficient has appropriate physical meaning. Nevertheless, because the equations are mass-based, the sum of the normalized stoichiometric coefficients in each equation must equal zero, as indicated in Eq. 3.11. This allows a continuity check to be made for each reaction. Furthermore, also note that any component A_i may be a reactant in one reaction and a product in another. This means that the overall rate of formation of that component will be the net rate obtained by considering the sum of the rates for all reactions in which it participates:

$$r_i = \sum_{j=1}^{n} \Psi_{i,j} \cdot r_j \tag{3.12}$$

If the net rate of formation is negative, the component is being consumed and if it is positive the component is being produced. The same approach can be used for COD-based stoichiometric equations by replacing $\Psi_{i,j}$ with $Y_{i,j}$. This approach will be applied in Part II when models are developed for biochemical reactors, and will be particularly useful when complex systems with several components and reactions are considered.

3.2 BIOMASS GROWTH AND SUBSTRATE UTILIZATION

3.2.1 Generalized Equation for Biomass Growth

It will be recalled from Section 2.4.1 that biomass growth and substrate utilization are coupled. Furthermore, we see in Section 2.4.2 that environmental engineers account for maintenance energy needs through the decay reaction. This means that as long as the production of soluble microbial products is negligible, the only use of substrate is for biomass growth. Consequently, when a stoichiometric equation for biomass growth is written with the substrate as the basis, the stoichiometric coefficient for the biomass term will be the biomass true growth yield. With this in mind, the generalized equation for microbial growth can be written as:

$$\text{Carbon source} + \text{energy source} + \text{electron acceptor} + \text{nutrients} \rightarrow \text{biomass} + CO_2 + \text{reduced acceptor} + \text{end products} \qquad (3.13)$$

For modeling purposes, it would be desirable to be able to write a quantitative equation in the same form for any situation, no matter what the carbon source, energy source, or electron acceptor. Using the concept of half-reactions, McCarty[77] has devised a technique whereby this may be done.

Half-Reaction Approach. In the absence of significant soluble microbial product formation, all nonphotosynthetic microbial growth reactions consist of two components, one for synthesis and one for energy. The carbon in the synthesis component ends up in biomass, whereas any carbon associated with the energy component becomes carbon dioxide. Such reactions are also oxidation–reduction reactions and thus involve the transfer of electrons from a donor to an acceptor. For heterotrophic growth the electron donor is an organic substrate, whereas for autotrophic growth the electron donor is inorganic. To allow consideration of all of these factors, McCarty[77] has written three types of half-reactions: one for cell material (R_c), one for the electron donor (R_d), and one for the electron acceptor (R_a). These are presented in Table 3.2 for a variety of substances. Reactions 1 and 2 represent R_c for the formation of biomass. Both are based on the empirical formula $C_5H_7O_2N$, but one uses ammonia nitrogen as the nitrogen source whereas the other uses nitrate. Reactions 3–6 are half-reactions R_a for the electron acceptors oxygen, nitrate, sulfate, and carbon dioxide, respectively. Reactions 7–17 are half-reactions R_d for organic electron donors. The first of these represents the general composition of domestic wastewater, while the next three are for wastes composed primarily of proteins, carbohydrates, and lipids, respectively. Reactions 11–17 are for specific organic compounds of interest in some biochemical operations. The last nine reactions represent possible autotrophic electron donors. Reactions 19–21 are for nitrification. To facilitate their combination, the half-reactions are all written on an electron equivalent basis, with the electrons on the right side.

The overall stoichiometric equation (R) is the sum of the half-reactions:

$$R = R_d - f_e \cdot R_a - f_s \cdot R_c \qquad (3.14)$$

The minus terms mean that half-reactions R_a and R_c must be inverted before use. This is done by switching the left and right sides. The term f_e represents the fraction of the electron donor that is coupled with the electron acceptor, i.e., the portion used for energy, hence the subscript e, and f_s represents the fraction captured through synthesis. As such, they quantify the endpoint of the reaction. Furthermore, in order for Eq. 3.14 to balance:

$$f_e + f_s = 1.0 \qquad (3.15)$$

This equation is equivalent to stating that all electrons originally in the electron donor end up either in the biomass synthesized (f_s) or in the electron acceptor (f_e). This is an important fundamental concept that we will return to later.

Empirical Formulas for Use in Stoichiometric Equations. As can be seen by examining Table 3.2, it was necessary to assume empirical formulas for biomass and alternative organic electron donors in order to write the half-reactions.

Various empirical formulas have been proposed to represent the organic composition of microbial cells. One of the oldest and most widely accepted in the field

Table 3.2 Oxidation Half-Reactions[a]

Reaction number	Half-reactions
Reactions for bacterial cell synthesis (R_c)	
Ammonia as nitrogen source:	
1.	$\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$
Nitrate as nitrogen source:	
2.	$\frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O = \frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^-$
Reactions for electron acceptors (R_a)	
Oxygen:	
3.	$\frac{1}{2}H_2O = \frac{1}{4}O_2 + H^+ + e^-$
Nitrate:	
4.	$\frac{1}{10}N_2 + \frac{3}{5}H_2O = \frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^-$
Sulfate:	
5.	$\frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O = \frac{1}{8}SO_4^{=} + \frac{19}{16}H^+ + e^-$
Carbon dioxide (methanogenesis):	
6.	$\frac{1}{8}CH_4 + \frac{1}{4}H_2O = \frac{1}{8}CO_2 + H^+ + e^-$
Reactions for electron donors (R_d)	
Organic donors (heterotrophic reactions):	
Domestic wastewater:	
7.	$\frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O = \frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^-$
Protein (amino acids, proteins, nitrogenous organics):	
8.	$\frac{1}{66}C_{16}H_{24}O_5N_4 + \frac{27}{66}H_2O = \frac{8}{33}CO_2 + \frac{2}{33}NH_4^+ + \frac{31}{33}H^+ + e^-$
Carbohydrate (cellulose, starch, sugars):	
9.	$\frac{1}{4}CH_2O + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^-$
Grease (fats and oils):	
10.	$\frac{1}{46}C_8H_{16}O + \frac{15}{46}H_2O = \frac{4}{23}CO_2 + H^+ + e^-$
Acetate:	
11.	$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$
Propionate:	
12.	$\frac{1}{14}CH_3CH_2COO^- + \frac{5}{14}H_2O = \frac{1}{7}CO_2 + \frac{1}{14}HCO_3^- + H^+ + e^-$
Benzoate:	
13.	$\frac{1}{30}C_6H_5COO^- + \frac{13}{30}H_2O = \frac{1}{5}CO_2 + \frac{1}{30}HCO_3^- + H^+ + e^-$

Table 3.2 Continued

Reaction number	Half-reactions
Ethanol:	
14. $\frac{1}{12} CH_3CH_2OH + \frac{1}{4} H_2O$	$= \frac{1}{6} CO_2 + H^+ + e^-$
Lactate:	
15. $\frac{1}{12} CH_3CHOHCOO^- + \frac{1}{3} H_2O$	$= \frac{1}{6} CO_2 + \frac{1}{12} HCO_3^- + H^+ + e^-$
Pyruvate:	
16. $\frac{1}{10} CH_3COCOO^- + \frac{2}{5} H_2O$	$= \frac{1}{5} CO_2 + \frac{1}{10} HCO_3^- + H^+ + e^-$
Methanol:	
17. $\frac{1}{6} CH_3OH + \frac{1}{6} H_2O$	$= \frac{1}{6} CO_2 + H^+ + e^-$
Inorganic donors (autotrophic reactions):	
18. Fe^{++}	$= Fe^{+3} + e^-$
19. $\frac{1}{8} NH_4^+ + \frac{3}{8} H_2O$	$= \frac{1}{8} NO_3^- + \frac{5}{4} H^+ + e^-$
20. $\frac{1}{6} NH_4^+ + \frac{1}{3} H_2O$	$= \frac{1}{6} NO_2^- + \frac{4}{3} H^+ + e^-$
21. $\frac{1}{2} NO_2^- + \frac{1}{2} H_2O$	$= \frac{1}{2} NO_3^- + H^+ + e^-$
22. $\frac{1}{6} S + \frac{2}{3} H_2O$	$= \frac{1}{6} SO_4^= + \frac{4}{3} H^+ + e^-$
23. $\frac{1}{16} H_2S + \frac{1}{16} HS^- + \frac{1}{2} H_2O$	$= \frac{1}{8} SO_4^= + \frac{19}{16} H^+ + e^-$
24. $\frac{1}{8} S_2O_3^= + \frac{5}{8} H_2O$	$= \frac{1}{4} SO_4^= + \frac{5}{4} H^+ + e^-$
25. $\frac{1}{2} SO_3^= + \frac{1}{2} H_2O$	$= \frac{1}{2} SO_4^= + H^+ + e^-$
26. $\frac{1}{2} H_2$	$= H^+ + e^-$

[a]Adapted from McCarty.[77]

of wastewater treatment is the one introduced in Section 2.4.1 and used in Example 3.1.1.1, $C_5H_7O_2N$.[61] Other formulas consisting of the same elements have been used, but they all result in about the same COD per unit of biomass.[75] Another formula has been proposed that includes phosphorus, $C_{60}H_{87}O_{23}N_{12}P$.[76] While awareness of the need for phosphorus by biomass is essential, it is not necessary to include phosphorus in the empirical formula because the mass required is generally about one-fifth of the mass of nitrogen required. This allows the phosphorus requirement to be calculated even when the simpler empirical formula is used.

All empirical formulas for biomass seek to represent in a simple way material composed of a highly complex and integrated mixture of organic molecules. Furthermore, because the relative quantities of those molecules change as the growth conditions of the culture change,[57] it would be purely fortuitous if a single chemical formula for biomass applied to all cases. An estimate of the constancy of the overall elemental composition can be obtained by measuring the COD and heat of combustion of biomass grown under various conditions, because constancy of those parameters would imply that the ratios of the elements C, H, O, and N were relatively constant. Investigations of that sort have indicated that the elemental composition is indeed a function of the growth conditions.[41] Thus, while an empirical formula can be written for biomass, its applicability to all situations is doubtful and one should view with caution equations said to depict "the biochemical reaction" exactly. Nevertheless, the concepts stated in Eq. 3.13 are still valid and many important relationships can be demonstrated through their use. Consequently, for illustrative purposes, the formula $C_5H_7O_2N$ will be used to represent biomass throughout this book. As discussed in Section 2.4.1, it has a COD of 1.42 mg COD/mg VSS, or 1.20 mg COD/mg SS.

In a laboratory or research situation, the exact composition of the electron donor is usually known. For example, if glucose were the energy source, its empirical formula $C_6H_{12}O_6$ would be used in the stoichiometric equation. Furthermore, if a synthetic medium contained several organic electron donors, the half-reaction for each could be written separately and then they could be combined to get R_d for the mixture by multiplying each half-reaction by the fractional contribution (on an electron equivalent basis) of its electron donor in the medium and adding them together.

An actual wastewater presents a more difficult situation because the chemical composition of the electron donor is seldom known. One approach would be to analyze the waste for its carbon, hydrogen, oxygen and nitrogen contents, and construct an empirical formula from the results. A half-reaction could then be written for that particular formula.[77] For example, as shown in Table 3.2, the empirical formula for the organic matter in domestic wastewater has been estimated to be $C_{10}H_{19}O_3N$. Alternatively, if the COD, organic carbon, organic nitrogen, and volatile solids content of a wastewater are known, they can be used to generate the half-reaction.[77] Finally, if a wastewater contains predominately carbohydrate, protein, and lipid, knowledge of their relative concentrations can be used to write the equation for microbial growth because each can be represented by a generalized empirical formula: CH_2O, $C_{16}H_{24}O_5N_4$, and $C_8H_{16}O$, respectively. As with other mixtures, the half-reaction for each is multiplied by the fraction of the component in the wastewater and the three are added to get R_d.

The nature of the electron acceptor depends on the environment in which the biomass is growing. If the environment is aerobic, the acceptor will be oxygen. If it is anaerobic, the acceptor will depend on the particular reaction taking place. For example, if lactic acid fermentation is occurring, pyruvic acid is the acceptor, whereas carbon dioxide is the acceptor for methanogenesis. Finally, nitrate can serve as the electron acceptor under anoxic conditions. Half-reactions have been written for all of these, as shown in Table 3.2.

Determination of f_s. Once the electron donor and the electron acceptor have been identified, either f_e or f_s must be determined before the balanced stoichiometric equation can be written. Generally, f_s is easier to estimate because it can be related to the true growth yield expressed on a COD basis. If f_e is the fraction of the electron donor transferred to the electron acceptor to provide the energy with which to syn-