thesize new biomass, conservation of energy and Eq. 3.15 tell us that the remainder of the electrons originally available in the donor must end up in the new biomass formed. If we accept \( \text{C}_2\text{H}_6\text{O}_2\text{N} \) as being representative of biomass, we can see that carbon and nitrogen are the reduced elements that will house those electrons. Nitrogen in biomass is in the \(-\text{III}\) state, i.e., as amino nitrogen. If the nitrogen available for biomass synthesis is also in the \(-\text{III}\) state, as in ammonia, no electrons will be required to reduce it, and the electrons captured through synthesis will all be associated with the carbon. Consequently, the energy available in the carbon of the biomass is equal to the energy incorporated during synthesis, or \( f_e \) when expressed as a fraction of the electron donor. Thus, if we could measure the energy or electrons available in the biomass produced, we would have a measure of \( f_e \).

In Section 2.4.1 the yield was defined as the amount of biomass formed per unit of substrate used. However, it was also pointed out that when the electron donor is an organic compound, it is often convenient to express the yield as mass of biomass COD formed per mass of substrate COD destroyed. The COD test is a measure of electrons available from carbon. Since COD is oxygen demand, and oxygen has an equivalent weight of eight, there are eight grams of COD per electron equivalent, as can be seen by examining half-reaction 3 in Table 3.2. This allows interconversion of COD and electron equivalents. Consequently, the yield is also the number of electrons available from carbon in the new biomass per unit of electrons removed from the substrate, or the fraction of the electron donor captured through synthesis, \( f_e \). Thus, when ammonia nitrogen serves as the nitrogen source for heterotrophic biomass synthesis:

\[
f_e = Y_H \text{ (NH}_4\text{) as nitrogen source, organic electron donor) (3.16)}
\]

where \( Y_H \) is expressed on a COD basis and the subscript \( H \) indicates that the true growth yield is for heterotrophic biomass growth. The utility of Eq. 3.16 comes from the fact that the true growth yield, \( Y_H \), can be determined in COD units from data collected with full-, pilot-, or lab-scale bioreactors, thereby giving \( f_e \) for the system under study. The techniques for doing this will be discussed in Chapter 8.

As long as ammonia or amino nitrogen is available to the microorganisms, they will use it preferentially for biomass synthesis. If it is not available, the microorganisms will use nitrate-N. (If no nitrogen is available, cell synthesis cannot occur because an essential reactant is missing.) When nitrate is the nitrogen source, the nitrogen must be reduced from the \(+\text{V}\) state to the \(-\text{III}\) state before it can be assimilated. This requires some of the electrons available in the substrate and they are part of the energy required for synthesis, i.e., part of \( f_e \). However, the electrons required to reduce the nitrogen are not measured in the COD test because that test does not oxidize nitrogen, but leaves it in the \(-\text{III}\) state. In this case, the true growth yield expressed on a COD basis is not an accurate estimate of \( f_e \). Rather, \( Y_H \) will be smaller than \( f_e \). This artifact can be corrected, however, because we know the number of electrons required to reduce nitrate-N to the appropriate oxidation state. Assuming an empirical formula for biomass of \( \text{C}_2\text{H}_6\text{O}_2\text{N} \), it can be shown that:

\[
f_e = 1.40 Y_H \text{ (NO}_3^-\text{) as nitrogen source, organic electron donor) (3.17)}
\]

Thermodynamics suggests that the true growth yield obtained for growth with nitrate as the nitrogen source will be smaller than the true growth yield obtained when ammonia is available.\(^{52}\) For example, for carbohydrate as the electron and
carbon donor, the value of $Y_{H}$ would be about 20 percent smaller with nitrate as the nitrogen source.

There are often circumstances in which one needs to establish the stoichiometry of biomass growth and substrate utilization before experimentally determined values of $Y_{H}$ are available. Thus, it would be advantageous to have a theoretical basis for estimating $f$, or $Y_{H}$. This has led a number of workers to seek a thermodynamic approach for predicting yield values.\textsuperscript{51,52} However, as discussed in Section 2.4.1, this is a difficult task because of the large number of factors that influence the yield. The most successful approach to date is that of Heijnen et al.,\textsuperscript{51,52} which is based on the Gibbs energy dissipation per C-mole of biomass produced, the degree of reduction of the carbon donor, the nature of the nitrogen source, and the available Gibbs energy per electron between the electron donor and acceptor. Their technique is capable of predicting true growth yield values for both heterotrophic and autotrophic biomass growth for a variety of situations. The error is approximately 13% when tabular values of the best estimates of the Gibbs energy dissipation per C-mole of biomass produced are used, but increases to 19% when that dissipation is estimated with a correlation equation that relates it to the carbon chain length and the degree of reduction of the carbon source. Because one should fully understand the technique of Heijnen et al.\textsuperscript{52} before using it, and because the presentation required to establish that understanding is beyond the scope of this book, readers are referred to the original work if they desire to use such an approach.

### 3.2.2 Aerobic Growth of Heterotrophs with Ammonia as the Nitrogen Source

The best way to illustrate the use of half-reactions is by an example. We will develop the molar stoichiometric equation for aerobic growth of heterotrophs that was the starting point for Example 3.1.1.1.

**Example 3.2.2.1**

Write the stoichiometric equation for aerobic heterotrophic microbial growth on a carbohydrate using ammonia as the nitrogen source, under conditions such that the true growth yield ($Y_{H}$) is 0.71 mg of biomass COD formed per mg of carbohydrate COD removed.

To do this we must make use of Eqs. 3.14–3.16:

\[
R = R_d - f_e \cdot R_e - f_e \cdot R_e \\
f_e = Y_{H} = 0.71 \\
f_e = 1.00 - 0.71 = 0.29
\]

Therefore

\[
R = R_d - 0.29 \cdot R_e - 0.71 \cdot R_e
\]

The electron donor is carbohydrate and the acceptor is oxygen. Thus, from Table 3.2:

\[
R_d = 1/4 \text{CH}_2\text{O} + 1/4 \text{H}_2\text{O} = 1/4 \text{CO}_2 + \text{H}^+ + e^- \\
R_e = 1/2 \text{H}_2\text{O} = 1/4 \text{O}_2 + \text{H}^+ + e^-
\]
Since ammonia is the nitrogen source, \( R_e \) is:

\[
R_e = \frac{1}{20} \text{C}_6\text{H}_5\text{O}_2\text{N} + \frac{9}{20} \text{H}_2\text{O} = \frac{1}{5} \text{CO}_2 + \frac{1}{20} \text{HCO}_3^- \\
+ \frac{1}{20} \text{NH}_4^+ + \text{H}^+ + e^-
\]

Applying Eq. 3.14 gives:

\[
\begin{align*}
R_e &= 0.25 \text{CH}_2\text{O} + 0.25 \text{H}_2\text{O} = 0.25 \text{CO}_2 + \text{H}^+ + e^- \\
-0.29 R_e &= 0.0725 \text{O}_2 + 0.29 \text{H}^+ + 0.29 e^- = 0.145 \text{H}_2\text{O} \\
-0.71 R_e &= 0.142 \text{CO}_2 + 0.0355 \text{HCO}_3^- + 0.0355 \text{NH}_4^+ + 0.71 \text{H}^+ + 0.71 e^- \\
&= 0.0355 \text{C}_6\text{H}_5\text{O}_2\text{N} + 0.3195 \text{H}_2\text{O} \\
R &= 0.25 \text{CH}_2\text{O} + 0.0725 \text{O}_2 + 0.0355 \text{NH}_4^+ + 0.0355 \text{HCO}_3^- \\
&= 0.0355 \text{C}_6\text{H}_5\text{O}_2\text{N} + 0.108 \text{CO}_2 + 0.2145 \text{H}_2\text{O}
\end{align*}
\]

This can be normalized to one mole of carbohydrate by dividing through by 0.25, giving Eq. 3.6, which was the starting point of Example 3.1.1.1:

\[
\begin{align*}
\text{CH}_2\text{O} + 0.29 \text{O}_2 + 0.142 \text{NH}_4^+ + 0.142 \text{HCO}_3^- &\rightarrow 0.142 \text{C}_6\text{H}_5\text{O}_2\text{N} \\
+ 0.432 \text{CO}_2 + 0.858 \text{H}_2\text{O}
\end{align*}
\]  

(3.6)

Equation 3.6 was converted to a COD-based stoichiometric equation in Example 3.1.1.1. If we rearrange that equation in the same form as Eq. 3.15, the result is:

\[
0.29 \text{O}_2 + 0.71 \text{C}_6\text{H}_5\text{O}_2\text{N} \text{ COD} = \text{CH}_2\text{O} \text{ COD}
\]  

(3.18)

We return to this equation to make three important points. First, note that the value of \( Y_H \) in Eq. 3.18 is 0.71 mg biomass COD formed/mg substrate COD used. This is the same as the \( Y_H \) value used to develop Eq. 3.6, as we would expect. Second, note that Eq. 3.18 expresses the same information as Eq. 3.15. In other words, since all of the electrons removed from the substrate must end up in either the electron acceptor or the biomass formed, we can state that the substrate COD removed must equal the biomass COD formed plus the oxygen used. Finally, since Eq. 3.18 expresses the same information as Eq. 3.15, we can see that the COD-based stoichiometric coefficient on oxygen is the same as \( f_e \). The balance portrayed by Eqs. 3.15 and 3.18 is a very important one that we will make extensive use of throughout this book.

### 3.2.3 Aerobic Growth of Heterotrophs with Nitrate as the Nitrogen Source

As previously discussed, consideration must be given to the form of nitrogen available for cell synthesis when writing the stoichiometric equation for cell growth. Ammonia will be used preferentially, and thus half-reaction 1 in Table 3.2 should be used when ammonia is available, even if nitrate is serving as the terminal electron acceptor. Only when nitrate is present as the sole nitrogen source should half-reaction 2 be used. In that case, when expressing the stoichiometric equation on a COD basis, it must be recognized that nitrogen changes oxidation state from +V to −III. As an example, consider the case of the aerobic growth of heterotrophs on carbohydrate with nitrate as the nitrogen source. In this case, the true growth yield is 0.57 mg
biomass COD/mg carbohydrate COD removed, reflecting the energy that must be
used to reduce the nitrogen. Applying Eq. 3.17 reveals that $f_\text{s}$ is 0.80, giving the
following molar stoichiometric equation:

$$\text{CH}_2\text{O} + 0.200 \text{ O}_2 + 0.114 \text{ NO}_3^- + 0.114 \text{ H}^+ \rightarrow 0.114 \text{ C}_2\text{H}_2\text{O}_2\text{N}$$

$$+ 0.429 \text{ CO}_2 + 0.657 \text{ H}_2\text{O}$$

(3.19)

After conversion to a mass basis by application of Eq. 3.2 this becomes:

$$\text{CH}_2\text{O} + 0.213 \text{ O}_2 + 0.236 \text{ NO}_3^- + 0.004 \text{ H}^+ \rightarrow 0.429 \text{ C}_2\text{H}_2\text{O}_2\text{N}$$

$$+ 0.629 \text{ CO}_2 + 0.395 \text{ H}_2\text{O}$$

(3.20)

Conversion of this equation to a mass of COD basis requires application of Eq. 3.5
using the unit CODs given in Table 3.1. Note that NO$_3^-$ has a unit COD of $-1.03$
mg COD/mg NO$_3^-$. This is equivalent to saying that each mg of nitrate that is reduced
to amino nitrogen in biomass accepts as many electrons as 1.03 mg of oxygen.
Application of Eq. 3.4 to Eq. 3.20 gives:

$$\text{CH}_2\text{O} \text{ COD} + (-0.200)\text{O}_2 + (-0.230)\text{O}_3 \text{ equivalents of NO}_3^- \rightarrow$$

$$0.570 \text{ C}_2\text{H}_2\text{O}_2\text{N} \text{ COD}$$

(3.21)

Equation 3.21 shows clearly that the COD (electron) balance would not be correct
if the change in oxidation state of the nitrogen was not considered. Failure to rec-
ognize this can lead to problems when COD balances are performed on operating
bioreactors.

It is often convenient to express the COD equivalence of nitrate as a nitrogen
source on the basis of the nitrogen utilized for biomass synthesis, rather than on the
basis of nitrate. In that case, the conversion factor is $-4.57$ mg COD/mg N (or 4.57
mg O$_2$/mg N), as indicated in Table 3.1.

3.2.4 Growth of Heterotrophs with Nitrate as the
Terminal Electron Acceptor and Ammonia as the
Nitrogen Source

If nitrate were serving as the terminal electron acceptor under anoxic conditions, the
amount needed could be calculated from the stoichiometric equation obtained when
half-reaction No. 4 was used in place of No. 3 as $R_s$ in Eq. 3.14. Exactly the same
procedures would be followed for obtaining the molar and mass-based stoichiometric
equations. Consider the case when ammonia serves as the nitrogen source for cell
synthesis. Because it will allow illustration of an important point, we will assume
that the true growth yield from carbohydrate is 0.71 mg biomass COD/mg substrate
COD, which is the same as the $Y_s$ value used to develop Eq. 3.6 for growth under
aerobic conditions. Application of the appropriate techniques gives:

$$\text{CH}_2\text{O} + 0.232 \text{ NO}_3^- + 0.142 \text{ NH}_4^+ + 0.142 \text{ HCO}_3^- + 0.232 \text{ H}^+ \rightarrow$$

$$0.142 \text{ C}_2\text{H}_2\text{O}_2\text{N} + 0.432 \text{ CO}_2 + 0.116 \text{ N}_2 + 0.974 \text{ H}_2\text{O}$$

(3.22)
Converting this to a mass of carbohydrate basis by application of Eq. 3.2 gives:

\[
\begin{align*}
\text{CH}_2\text{O} & + 0.479 \text{NO}_3^- + 0.085 \text{NH}_4^+ + 0.289 \text{HCO}_3^- + 0.008 \text{H}^+ \\
0.535 \text{C}_5\text{H}_7\text{O}_2\text{N} & + 0.634 \text{CO}_2 + 0.108 \text{N}_2 + 0.584 \text{H}_2\text{O}
\end{align*}
\]

(3.23)

Because the true growth yield was assumed to be the same as in Example 3.1.1.1, the quantities of biomass formed in Eqs. 3.22 and 3.23 are the same as those in Eqs. 3.6 and 3.7, respectively.

Conversion of Eq. 3.23 to a COD basis requires inclusion of a conversion factor for the oxygen equivalence of nitrate nitrogen when it is being reduced to nitrogen gas, N\(_2\), which is the case when nitrate serves as the terminal electron acceptor. Examination of Table 3.1 reveals that the unit COD for the reduction of NO\(_3^-\) to N\(_2\) is \(-0.646\) mg COD/mg NO\(_3^-\). The sign is negative because the nitrate is accepting electrons. The source of this value may be seen from the half-reactions in Table 3.2 which reveal that 1/5 mole of nitrate is equivalent to 1/4 mole of oxygen. Conversion to a mass basis reveals that each gram of nitrate that is reduced to N\(_2\) can accept as many electrons as 0.646 grams of oxygen. Applying Eq. 3.4 with the appropriate conversion factors to Eq. 3.23 gives:

\[
\begin{align*}
\text{CH}_2\text{O} \text{ COD} & + (-0.29)\text{O}_2 \text{ equivalents of NO}_3^- \\
0.71 \text{ C}_5\text{H}_7\text{O}_2\text{N} \text{ COD}
\end{align*}
\]

(3.24)

Comparison of Eq. 3.24 to Eq. 3.8 reveals that they are the same. This follows from the fact that they are both expressed on a COD basis, that they both use ammonia as the nitrogen source for biomass synthesis, and that they both were derived for the same yield. Generally, however, the yield will be lower when nitrate serves as the terminal electron acceptor.\(^{51,52,79}\)

Often it is convenient to express the oxygen equivalence of nitrate as an electron acceptor on the basis of nitrogen rather than nitrate. In that case, the conversion factor is \(-2.86\) mg COD/mg N (or 2.86 mg O\(_2\)/mg N), as shown in Table 3.1.

It should be noted from the preceding that the COD conversion factor for nitrate as a nitrogen source is different from the COD conversion factor for nitrate as a terminal electron acceptor because the final oxidation state of nitrogen is different in the two cases. This becomes especially important when nitrate serves as both the nitrogen source and the terminal electron acceptor. The safest way to handle this situation is to keep the two uses of nitrate separate in writing the stoichiometric equation, and to apply the appropriate conversion factor for each when converting the equation to a COD basis.

### 3.2.5 Aerobic Growth of Autotrophs with Ammonia as the Electron Donor

Nitrifying bacteria are autotrophic microorganisms that obtain their energy from the oxidation of reduced nitrogen. As discussed previously, *Nitrosomonas* oxidizes ammonia-N to nitrite-N and *Nitrobacter* oxidizes nitrite-N to nitrate-N. The molar stoichiometric equations for their growth can be obtained by the half-reaction technique discussed previously, which requires knowledge of \(f_e\). For autotrophic biomass growth, yield is often expressed as the mass of biomass COD formed per mass of inorganic element oxidized; for example, mg of biomass COD per mg of ammonia-
N removed for *Nitrosomonas*. To convert this yield value to an electron equivalent basis for determining \( f_e \), it is necessary to know that *Nitrosomonas* oxidizes ammonia-N (−III) to nitrite-N (+III), for a six electron change. Thus, the equivalent weight for nitrogen in this case is \( 14/6 = 2.33 \) grams/equivalent, which means that:

\[
f_e = 0.291 \ Y_{Nitrosomonas} \ (NH_4^+ \ as \ nitrogen \ source \ and \ electron \ donor)
\]

(3.25)

For *Nitrobacter*, nitrite-N (+III) serves as the electron donor and is oxidized to nitrate-N (+V), for a two electron change. Ammonia-N, however, serves as the nitrogen source. Consequently:

\[
f_e = 0.875 \ Y_{Nitrobacter} \ (NH_4^+ \ as \ nitrogen \ source, \ NO_2^- \ as \ electron \ donor)
\]

(3.26)

for this organism, where \( Y_{Nitrobacter} \) has units of mg biomass COD formed/mg nitrite-N removed. Often nitrifying bacteria are considered together as a group and nitrification is treated as a single reaction converting ammonia-N to nitrate-N. In that case, nitrogen undergoes an eight electron change so that:

\[
f_e = 0.219 \ Y_A \ (NH_4^+ \ as \ nitrogen \ source \ and \ electron \ donor)
\]

(3.27)

where \( Y_A \) represents the true growth yield for autotrophic nitrifying biomass and has units of mg biomass COD formed/mg ammonia-N removed.

Application of the half-reaction technique using typical yield values and Eq. 3.2 provides the mass-based stoichiometric equations for nitrification. For *Nitrosomonas*, when \( NH_4^+ \) is the basis, the equation is:

\[
NH_4^+ + 2.457 O_2 + 6.716 HCO_3^- \rightarrow 0.114 C_6H_5O_2N + 2.509 NO_2^- + 1.036 H_2O + 6.513 H_2CO_3
\]

(3.28)

When \( NO_2^- \) is the basis, the equation for *Nitrobacter* is:

\[
NO_2^- + 0.001 NH_4^+ + 0.014 H_2CO_3 + 0.003 HCO_3^- + 0.339 O_2 \rightarrow 0.006 C_6H_5O_2N + 0.003 H_2O + 1.348 NO_3^- \]

(3.29)

Furthermore, combining the two reactions reveals that the overall stoichiometry is:

\[
NH_4^+ + 3.300 O_2 + 6.708 HCO_3^- \rightarrow 0.129 C_6H_5O_2N + 3.373 NO_3^- + 1.041 H_2O + 6.463 H_2CO_3
\]

(3.30)

From these it can be seen that a large amount of alkalinity (HCO_3^-) is used during the oxidation of ammonia to nitrate: 6.708 mg HCO_3^-/mg NH_4^+ removed, which is equivalent to 8.62 mg HCO_3^-/mg NH_3-N removed. The vast majority of that alkalinity utilization is associated with neutralization of the hydrogen ions released during the oxidation of ammonia-N. Only a small part of the alkalinity is incorporated into the cell material. If the wastewater contains insufficient alkalinity and if pH control is not practiced, the pH will drop below the normal physiological range, retarding the activity of both the autotrophs and the heterotrophs, hurting system performance. The equations also tell us that considerable oxygen is required for nitrification: 3.30 mg O_2/mg NH_4^+ removed, which is equivalent to 4.33 mg O_2/mg of NH_3-N actually oxidized to nitrate-N. Of that amount 3.22 mg O_2 will be used by *Nitrosomonas* and 1.11 will be used by *Nitrobacter*. The oxygen requirement of the nitrifying bacteria can have a significant impact on the total amount of oxygen required by a biochem-
tical operation. Finally, it can be seen that relatively little biomass will be formed, reflecting the low yields associated with autotrophic growth. For every mg of NH₄⁺ removed, only 0.129 mg of biomass will be formed, which is equivalent to 0.166 mg biomass/mg NH₄⁺-N removed. Most of that, 0.146 mg biomass/mg NH₄⁺-N removed, will be due to the growth of *Nitrosomonas*, and only 0.020 mg biomass/mg NH₄⁺-N removed will be due to *Nitrobrobacter*. Overall, the growth of nitrifying bacteria will have little impact on the quantity of biomass in a biochemical operation, but will have a large impact on the oxygen and alkalinity requirements.

### 3.2.6 Kinetics of Biomass Growth

Equation 3.8 was the COD-based stoichiometric equation for aerobic growth of heterotrophic biomass with ammonia as the nitrogen source. Recognizing that the stoichiometric coefficient on biomass is the same as the true growth yield, \( Y_H \), and that both substrate (\( S_S \)) and active heterotrophic biomass (\( X_{B,H} \)) are measured in COD units, it may be rewritten in terms of the true growth yield as:

\[
(1) S_S + [-(1 - Y_H)] S_O \rightarrow Y_H X_{B,H} \tag{3.31}
\]

where \( S_O \) is oxygen, which is expressed in COD units, and thus carries a negative sign as indicated in Table 3.1. Putting this in the form of Eq. 3.9, while retaining COD units, gives:

\[
(-1) S_S + (-1)[- (1 - Y_H)] S_O + Y_H X_{B,H} = 0 \tag{3.32}
\]

This equation is based on substrate as the reference constituent. Alternatively, it could be rewritten with active heterotrophic biomass as the reference constituent, and that is the convention used herein:

\[
\left( - \frac{1}{Y_H} \right) S_S + (-1) \left[ - \left( \frac{1 - Y_H}{Y_H} \right) \right] S_O + X_{B,H} = 0 \tag{3.33}
\]

Application of Eq. 3.27 gives:

\[
\frac{r_{SS}}{- \frac{1}{Y_H}} = \frac{r_{SO}}{(-1) \left[ - \left( \frac{1 - Y_H}{Y_H} \right) \right]} = \frac{r_{XB}}{1} = r \tag{3.34}
\]

where \([r] = \text{mg COD/(L⋅hr)}\). Thus, once \( r_{XB} \) has been defined, the other rates are also known.

Similar equations can be written for the growth of heterotrophs with nitrate as the terminal electron acceptor and for the aerobic growth of autotrophs. The derivation of such equations is left as an exercise for the reader.

Bacteria divide by binary fission. Consequently, the reaction rate for bacterial growth can be expressed as first order with respect to the active biomass concentration:

\[
r_{XB} = \mu \cdot X_B \tag{3.35}
\]

\(^*\)S represents soluble constituents and X represents particulate constituents, with the subscript denoting the particular constituent involved.