$$X_a = 0.42 \frac{\text{g VSS}_a}{\text{g BOD}_L} \left((529 - 1.7) \frac{\text{mg BOD}_L}{\text{l}} \right) \frac{1}{1.3}$$
$$= 170 \text{ mg VSS}_a/\text{l}$$

$$X_i = 50 \text{ mg VSS}_i/\text{l} + 170 \frac{\text{mg VSS}_a}{\text{l}} (1 - 0.8)(0.15/\text{d})(2 \text{ d})$$
$$= 60 \text{ mg VSS}_i/\text{l};$$

$$X_v = X_a + X_i + S_p = 170 + 60 + \frac{71}{1.42} = 280 \text{ mg VSS/l}$$

Thus, the amount of active biomass is augmented by the hydrolysis of particulate COD, while the VSS also is augmented by the remaining biodegradable particulate COD.

4. The detailed computations for SMP are omitted, as they are exactly analogous to Example 3.1. The result is

UAP = 9.4 mg COD_p/l
BAP = 23.2 mg COD_p/l
SMP = 32.6 mg COD_p/l

5. The effluent concentrations of COD and BOD_L are affected by the changes in X_a, X_i and SMP, as well as by the residual particulate organic substrate S_p. All increase.

Soluble COD and BOD_L	$= S +$ SMP
	$= 1.7 + 32.7$
	$= 34.4$ mg COD/l
Total COD	$= S +$ SMP $+ 1.42 \cdot X_v$
	$= 1.7 + 32.7 + 1.42 \cdot 280$
	$= 432$ mg COD/l
Total BOD_L	$= S +$ SMP $+ 1.42 \cdot f_d \cdot X_a + S_p$
	$= 1.7 + 32.7 + 1.42 \cdot 0.8 \cdot 170 + 71$
	$= 299$ mg BOD_L/l

3.9 INHIBITION

The rates of substrate utilization and microbial growth can be slowed by the presence of inhibitory compounds. Examples of inhibitors are heavy metals, pesticides, antibiotics, aromatic hydrocarbons, and chlorinated solvents. Sometimes these materials are called toxicants, and their effects termed toxicity. Here, we use the terms inhibitor and inhibition, because they imply one of several different general phenomena affecting metabolic rates.

The range of possible inhibitors and their different effects on the microorganisms can make inhibition a confusing topic. In some cases, the inhibitor affects a single enzyme active in substrate utilization; in such a case, utilization of that substrate is

slowed. In other cases, the inhibitor affects some more general cell function, such as respiration; then, indirect effects, such as reduced biomass levels may slow utilization of a particular substrate. Finally, some reactions actually are increased by inhibition, as the cell tries to compensate for the negative impacts of the inhibitor.

Figure 3.6 shows the key places that inhibitors affect the primary flows of electrons and energy. Inhibition of a particular degradative enzyme may occur during the initial oxidation reactions of an electron-donor substrate. The immediate effect is a slowing of the degradation rate. In addition, the reduced electron flow can lead to a loss of biomass or slowing of other reactions requiring electrons (ICH_2) or energy (ATP). At the other end of the electron-transport chain, inhibition of the acceptor reaction prevents electron flow and energy generation, thereby leading to a loss of biomass. Interestingly, inhibition of the acceptor reaction can lead to a buildup of reduced electron carriers (ICH_2) and increased rates for reactions requiring reduced electron carriers as a cosubstrate (Wrenn and Rittmann, 1995). Decouplers reduce or eliminate energy generation, even though electrons flow from the donors to the acceptor. Decoupling inhibits cell growth and other energy-requiring reactions. In some cases, decoupling inhibition leads to an increase in acceptor utilization per unit biomass, as the cells attempt to compensate for a low energy yield by sending more electrons to the acceptor.

How an inhibitor affects growth and substrate-utilization kinetics can be expressed succinctly by using *effective kinetic parameters*. The kinetic expressions for substrate utilization and growth remain the same as before (i.e., Equations 3.6 and 3.9), but the effective kinetic parameters depend on the concentration of the inhibitor. Written out with effective parameters, Equations 3.6 and 3.9 are

$$r_{ut,\,eff} = -\frac{\hat{q}_{eff} S}{K_{eff} + S} X_a \qquad \textbf{[3.62]}$$

$$\mu_{eff} = Y_{eff}(-r_{ut,\,eff}) - b_{eff} \qquad \textbf{[3.63]}$$

* Corrected eq'n.

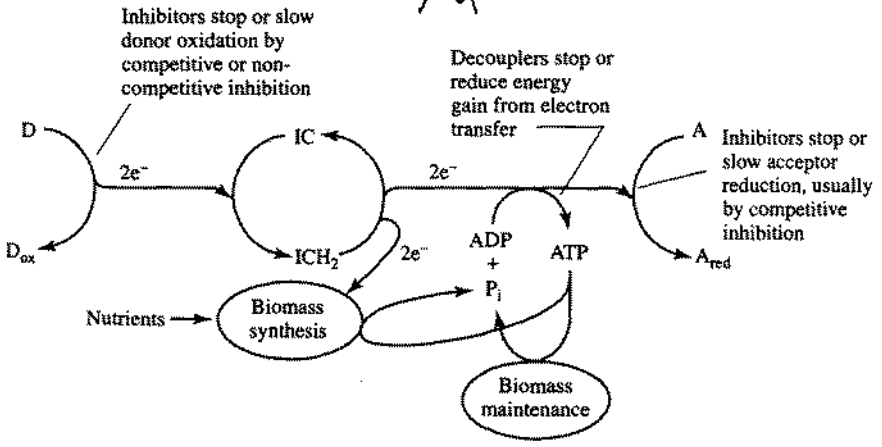

Figure 3.6 Illustration of how inhibitions can affect the primary flow of electrons and energy. D = electron donor, A = electron acceptor, and IC = intracellular electron carrier, such as NAD^+.
(After Rittmann and Sáez, 1993).

How $\hat{q}_{\text{eff}}$, K_{eff}, Y_{eff}, and b_{eff} are controlled by the inhibitor concentrations depends on the location and mode of the inhibition phenomenon. The most common types of inhibition and their effective parameters are reviewed here. Some aspects of inhibition were introduced in Chapter 1, and more details can be found in Rittmann and Sáez (1993).

A common type of inhibition for aromatic hydrocarbons and chlorinated solvents is *self-inhibition*, which also is called *Haldane* or *Andrews kinetics*. In this case, the enzyme-catalyzed degradation of the substrate is slowed by high concentrations of the substrate itself. It is not clear whether the self-inhibition occurs directly through action on the degradative enzyme or indirectly through hindering electrons or energy flow after the original donor reaction. In either situation, the effective parameters for self-inhibition are

$$\hat{q}_{\text{eff}} = \frac{\hat{q}}{1 + \dfrac{S}{K_{\text{IS}}}} \qquad \textbf{[3.64]}$$

$$K_{\text{eff}} = \frac{K}{1 + S/K_{\text{IS}}} \qquad \textbf{[3.65]}$$

where K_{IS} = an inhibition concentration of the self-inhibitory substrate ($M_s L^{-3}$). Y_{eff} and b_{eff} are not affected and remain Y and b, respectively.

Figure 3.7 contains two graphical presentations of the effect of substrate self-inhibition on reaction kinetics. The left figure illustrates how the reaction rate ($-r_{\text{ut}}$) varies with substrate concentration. At low substrate concentrations, the rate increases with an increase in S. However, a maximum rate is reached, and substrate concentrations beyond this become inhibitory, causing a decrease in reaction rate. When the Haldane reaction rate model is substituted for the Monod relation in the CSTR mass balances, the effect of θ_x on effluent substrate concentration is illustrated by the right graph in Figure 3.7. This graph indicates that, with θ_x greater than about 2 d, two values of S are possible for each value of θ_x. Which is the correct one? The answer depends on how the reactor arrives at the steady state.

First, we assume that the influent concentration is 45 mg/l, and that the reactor is being operated at a θ_x of 10 d, conditions indicated by the dotted lines in the figure. Under such conditions, the influent concentration is quite inhibitory to the microorganisms. If the reactor were initially filled with the untreated wastewater, seeded with microorganisms, and then operated at the θ_x of 10 d, the reactor would fail, because the specific growth rate of the organisms under these conditions is less than 0.1/d ($= 1/\theta_x$). However, if the wastewater were initially diluted so that the concentration in the reactor was about 33 mg/l, then the specific growth rate would be greater than 0.1/d, and the bacteria would reproduce faster than they are removed from the reactor. The reactor population would continue to increase, and the value of S in the reactor would continue to decrease until the microbial population size and substrate concentration reached their steady-state concentrations, about 1 mg/l for the substrate. The value of Figure 3.7, then, is not only to indicate what this steady-state concentration would be for a well-operating reactor, but also to indicate upper limits on substrate concentration beyond which reactor startup cannot proceed.

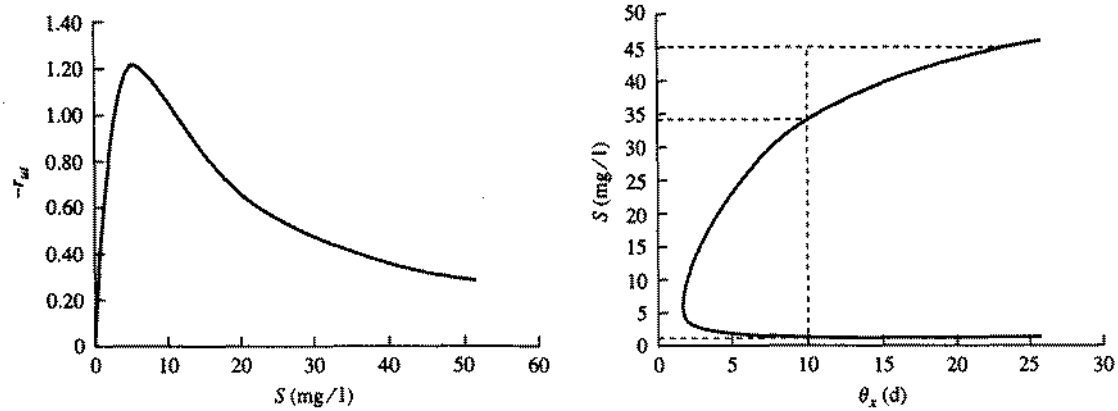

Figure 3.7 Haldane kinetics and the relationship between S and reaction rate (top) and between S and θ_x. The example is for orthochlorophenol with $K = 20$ mg/l, $K_{IS} = 1.5$ mg/l, $\hat{q} = 10$ mg/mg VSS_a-d, $Y = 0.6$ g VSS_a/g, $b = 0.15$/d, and $X_a = 1$ mg VSS/l.

The second type of inhibition is *competitive,* and a separate inhibitor is present at concentration I ($M_I L^{-3}$). The competitive inhibitor binds the catalytic site of the degradative enzyme, thereby excluding substrate binding in proportion to the degree to which the inhibitor is bound. The only parameter affected by I in competitive inhibition is K_{eff}:

$$K_{\text{eff}} = K\left(1 + \frac{I}{K_I}\right) \qquad \textbf{[3.66]}$$

where K_I = an inhibition concentration of the competitive inhibitor ($M_I L^{-3}$). A small value of K_I indicates a strong inhibitor. The rate reduction caused by a competitive inhibitor can be completely offset if S is large enough, because $\hat{q}_{\text{eff}}$ remains equal to $\hat{q}$. Competitive inhibitors usually are substrate analogues.

One example of competitive inhibition that is of interest in environmental engineering practice is that of cometabolism of trichloroethylene (TCE) by microorganisms that grow on substrates such as methane. Here, the first step in methane utilization is its oxidation to methanol by an enzyme called a methane monooxygenase (MMO), which replaces one hydrogen atom attached to the methane carbon with an -OH group. With TCE, MMO interacts to place an oxygen atom between the two carbon atoms to form an epoxide. The key factor is that methane and TCE compete for the same enzyme. The presence of TCE affects the rate at which methane is consumed and, in turn, the presence of methane affects the reaction rate of MMO with TCE. This is illustrated in Figure 3.8. The presence of 20 mg/l TCE reduces the rate of methane utilization considerably over that given by the Monod model, which does not involve competitive inhibition. Similarly, the rate of TCE utilization is greatly reduced as the methane concentration increases. The rate expression for TCE is similar to that for methane, except that the roles of the substrate and the inhibitor are reversed.

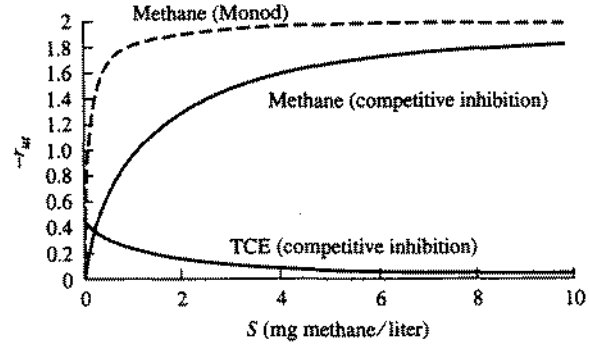

Figure 3.8 Reaction rates for methane and TCE as governed by competitive inhibition kinetics. Shown for comparison is the rate of methane oxidation in the absence of inhibition. $X_a = 1$ mg VSS_a/l, $\hat{q}$ (methane) = 2 mg/mg VSS_a-d, K (methane) = 0.1 mg/l, $\hat{q}$ (TCE) = 0.5 mg/mg VSS_a-d, K_I (TCE) = 2 mg/l, and I (TCE) = 20 mg/l.

A third type of inhibition is *noncompetitive inhibition* by a separate inhibitor. A noncompetitive inhibitor binds with the degradative enzyme (or perhaps with a coenzyme) at a site different from the reaction site, altering the enzyme conformation in such a manner that substrate utilization is slowed. The only parameter affected is $\hat{q}_{eff}$:

$$\hat{q}_{eff} = \frac{\hat{q}}{1 + I/K_I} \qquad \textbf{[3.67]}$$

In the presence of a noncompetitive inhibitor, high S cannot overcome the inhibitory effects, since the maximum utilization rate is lowered for all S. This phenomenon is sometimes called allosteric inhibition, and allosteric inhibitors need not have any structural similarity to the substrate.

Figure 3.9 illustrates the different effects that competitive and noncompetitive inhibitors have on reaction rates, in comparison with the Monod model. The values of I, K, and K_I are assumed to be the same, 1 mg/l, making the value $(1 + I/K_I) = 2$. With a competitive inhibitor, the impact is primarily on K, and the inhibitor causes the effective K to increase (from 1 to 2 as indicated by the horizontal line in the middle of the graph). If the substrate concentration (S) is high enough, the reaction rate eventually approaches $\hat{q}$. With the noncompetitive inhibitor, the effect is on $\hat{q}$, causing the apparent value to decrease as the inhibitor concentration increases (from 2 to 1 as indicated by the upper and middle horizontal lines in the illustration). The value of K, the substrate concentration at which the rate is one-half of the maximum value, in effect, remains unchanged (shown as 1 in the figure by the vertical line). By noting which coefficient in the Monod reaction (K or $\hat{q}$) appears to change when an

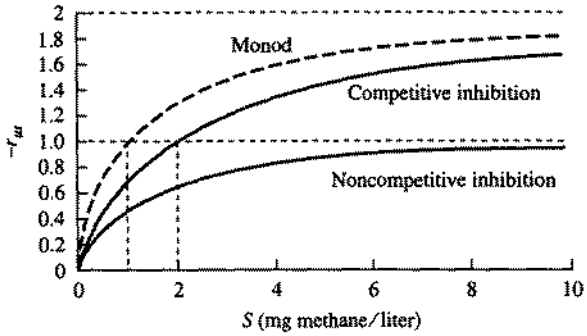

Figure 3.9 Effect of noncompetitive versus competitive inhibition on reaction kinetics. $K = 1$ mg/l, $K_I = 1$ mg/l, $X_a = 1$ mg VSS_a/l, $\hat{q} = 2$ mg/mg VSS_a-d, and $I = 1$ mg/l.

inhibitor is added, one can determine whether the inhibitor is acting in a competitive or a noncompetitive manner.

In some cases, competitive and noncompetitive impacts occur together. This situation is termed *uncompetitive inhibition*. Both effective parameters, $\hat{q}_{\text{eff}}$ and K_{eff}, vary as they do for the individual cases:

$$\hat{q}_{\text{eff}} = \frac{\hat{q}}{1 + I/K_I} \qquad \textbf{[3.68]}$$

$$K_{\text{eff}} = K(1 + I/K_I) \qquad \textbf{[3.69]}$$

Mixed inhibition is a more general form of uncompetitive inhibition in which the K_I values in Equations 3.68 and 3.69 can have different values.

The last inhibition type considered is *decoupling*. Decoupling inhibitors, such as aromatic hydrocarbons, often act by making the cytoplasmic membrane permeable for protons. Then, the proton-motive force across the membrane is reduced, and ATP is not synthesized in parallel with respiratory electron transport. Sometimes, decouplers are called protonophores. A decrease in Y_{eff} and/or an increase in b_{eff} can model the effects of decoupling inhibition:

$$Y_{\text{eff}} = \frac{Y}{1 + I/K_I} \qquad \textbf{[3.70]}$$

$$b_{\text{eff}} = b(1 + I/K_I) \qquad \textbf{[3.71]}$$

The other parameters are not necessarily changed. An interesting aspect of decoupling is that the rate of electron flow to the primary acceptor and per unit active biomass can increase. This is shown mathematically for steady state:

$$r_{A,\,\text{eff}} = \frac{\hat{q}_{\text{eff}} S}{K_{\text{eff}} + S}\left[1 - Y_{\text{eff}}\left(1 - \frac{f_d b_{\text{eff}} \theta_x}{1 + b_{\text{eff}} \theta_x}\right)\right] \qquad \textbf{[3.72]}$$

in which $r_{A,\,\text{eff}}$ = specific rate of electron flow to the acceptor ($M_s M_x^{-1} T^{-1}$), and

all units of mass are proportional to electron equivalents (e.g., COD). Equation 3.72 is valid for all types of inhibition and shows how self-, competitive, and noncompetitive inhibitions normally slow $r_{A,\,\text{eff}}$. However, $r_{A,\,\text{eff}}$ increases when decoupling increases b_{eff} and/or decreases Y_{eff}.

Sometimes products of a reaction act as inhibitors. The classic example of product inhibition is in alcohol fermentation, where ethanol is produced as an end product from sugar fermentation. In wine manufacture, fermentation of sugar can occur until the ethanol concentration reaches 10 to 13 percent, at which point the alcohol becomes toxic to the yeast, and fermentation stops. This is why the alcohol content of wines normally is in the range of 10 to 13 percent.

3.10 OTHER ALTERNATE RATE EXPRESSIONS

The Monod model for microbial growth and substrate utilization, as represented by Equations 3.1 and 3.6, respectively, is the most widely used model for kinetic analysis and reactor design. However, other models sometimes are used for special circumstances. The previous section reviewed models for inhibition. This section reviews alternate models when inhibition is not the issue.

One popular alternative is the Contois model, which is represented by

$$r_{\text{ut}} = -\frac{\hat{q}S}{BX_a + S}X_a \qquad \textbf{[3.73]}$$

in which B = a constant $[M_s M_x^{-1}]$. The Contois equation shows a dependence of the specific reaction rate on the concentration of active organisms present. A high organism concentration slows the reaction rate, which approaches a first-order reaction that depends on S, but not X_a:

$$-r_{\text{ut}} = \frac{\hat{q}}{B}S \qquad X_a \to \infty \qquad \textbf{[3.74]}$$

The Contois equation is useful for describing the rate of hydrolysis of suspended particulate organic matter, such as are present in primary and waste activated sludges (Henze et al., 1995). It has been noted that hydrolysis rates of biodegradable sludge particles tend to follow first-order kinetics, as is shown by comparing Equations 3.57 and 3.74. The hydrolysis rate is relatively independent of microorganism concentration, even at fairly small organism concentrations. This independence may occur because extracellular enzymes, not the bacteria, carry out the hydrolysis reactions. For hydrolysis, typical values for the ratio $\hat{q}/B$ are on the order of 1 to 3 d^{-1}.

Two other alternate equations are the Moser and Tessier equations, shown by Equations 3.75 and 3.76, respectively.

$$r_{\text{ut}} = -\frac{\hat{q}S}{K + S^{-\gamma}}X_a \qquad \textbf{[3.75]}$$

$$r_{\text{ut}} = -\hat{q}\left(1 - \tilde{e}^{S/K}\right)X_a \qquad \textbf{[3.76]}$$

* Corrected