chapter

3

MICROBIAL KINETICS

The previous chapters emphasized that microorganisms fuel their lives by performing oxidation/reduction reactions that generate the energy and reducing power needed to construct and maintain themselves. Because redox reactions are nearly always very slow unless catalyzed, microorganisms produce enzyme catalysts that increase the kinetics of their essential reactions to rates fast enough for them to exploit the chemical resources available in their environment. Engineers want to take advantage of these microbially catalyzed reactions, because the chemical resources of the microorganisms usually are the pollutants that the engineers must control. For example, the biochemical oxygen demand (BOD) is an organic electron donor for heterotrophic bacteria, NH_4^+ -N is an inorganic electron donor for nitrifying bacteria, NO_3^- -N is an electron acceptor for denitrifying bacteria, and PO_4^{3-} is a nutrient for all microorganisms.

In trying to employ microorganisms for pollution control, engineers must recognize two interrelated principles: First, metabolically active microorganisms catalyze the pollutant-removing reactions. The rate of pollutant removal depends on the concentration of the catalyst, or the active biomass. Second, the active biomass is grown and sustained through the utilization of its energy- and electron-generating primary substrates, which are its electron donor and electron acceptor. The rate of production of active biomass is proportional to the utilization rate of the primary substrates.

The connection between the active biomass (the catalyst) and the primary substrates is the most fundamental factor needed for understanding and exploiting microbial systems for pollution control. Because those connections must be made systematically and quantitatively for engineering design and operation, mass-balance modeling is an essential tool. That modeling is the foundation for the subject of this chapter.

3.1 BASIC RATE EXPRESSIONS

At a minimum, a model of a microbial process must have mass balances on the active biomass and the primary substrate that limits the growth rate of the biomass. In the vast majority of cases, the rate-limiting substrate is the electron donor. That convention is used here, and the term *substrate* now refers to the *primary electron*-

donor substrate. To complete the mass-balance equations, rate expressions for the growth of the biomass and utilization of the substrate must be supplied. Those two rate expressions are presented first.

The relationship most frequently used to represent bacterial growth kinetics is the so-called *Monod equation*, which was developed in the 1940s by the famous French microbiologist Jacques Monod. His original work related the specific growth rate of fast-growing bacteria to the concentration of a rate-limiting, electron-donor substrate,

$$\mu_{\text{syn}} = \left(\frac{1}{X_a} \frac{dX_a}{dt}\right)_{\text{syn}} = \hat{\mu} \frac{S}{K + S}$$
 [3.1]

in which

 μ_{syn} = specific growth rate due to synthesis (T⁻¹)

 X_a = concentration of active biomass ($M_x L^{-3}$)

t = time(T)

 $S = \text{concentration of the rate-limiting substrate } (M_s L^{-3})$

 $\hat{\mu}$ = maximum specific growth rate (T⁻¹)

 $K = \text{concentration giving one-half the maximum rate } (M_s L^{-3})$

This equation is a convenient mathematical representation for a smooth transition from a first-order relation (in S) at low concentration to a zero-order relation (in S) at high concentration. The Monod equation is sometimes called a saturation function, because the growth rate saturates at $\hat{\mu}$ for large S. Figure 3.1 shows how μ varies with S and that $\mu = \hat{\mu}/2$ when K = S. Although Equation 3.1 is largely empirical, it has widespread applicability for microbial systems.

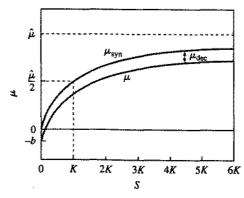


Figure 3.1 Schematic of how the synthesis and net specific growth rates depend on the substrate concentration. At S=20K, $\mu_{\text{syn}}=0.95\hat{\mu}$.

People who studied more slowly growing bacteria (environmental engineers are among the key examples of these people) discovered that active biomass has an energy demand for maintenance, which includes cell functions such as motility, repair and resynthesis, osmotic regulation, transport, and heat loss. Environmental engineers usually represent that flow of energy and electrons required to meet maintenance needs as *endogenous decay*. In other words, the cells oxidize themselves to meet maintenance-energy needs.

The rate of endogenous decay is

$$\mu_{\text{dec}} = \left(\frac{1}{X_a} \frac{dX_a}{dt}\right)_{\text{decay}} = -b$$
 [3.2]

in which

b = endogenous-decay coefficient (T⁻¹) μ_{dec} = specific growth rate due to decay (T⁻¹).

Equation 3.2 says that the loss of active biomass is a first-order function. However, not all of the active biomass lost by decay is actually oxidized to generate energy for maintenance needs. Although most of the decayed biomass is oxidized, a small fraction accumulates as inert biomass. The rate of oxidation (or true respiration for energy generation) is

$$\left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{\text{resp}} = -f_d b \tag{3.3}$$

in which f_d = fraction of the active biomass that is biodegradable. The rate at which active biomass is converted to inert biomass is the difference between the overall decay rate and the oxidation decay rate,

$$-\frac{1}{X_a}\frac{dX_i}{dt} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{\text{inert}} = -(1 - f_d)b$$
 [3.4]

in which X_i = inert biomass concentration $(M_x L^{-3})$.

Overall, the net specific growth rate of active biomass (μ) is the sum of new growth (Equation 3.1) and decay (Equation 3.2):

$$\mu = \frac{1}{X_a} \frac{dX_a}{dt} = \mu_{\text{syn}} + \mu_{\text{dec}} = \hat{\mu} \frac{S}{K + S} - b$$
 [3.5]

Figure 3.1 also shows how μ varies with S and that μ can be negative for low enough S.

Because of our ultimate interest in substrate removal (e.g., BOD) and because biomass growth is "fueled" by substrate utilization, environmental engineers often prefer to regard the rate of substrate utilization as the basic rate, while cell growth is derived from substrate utilization. Then, the Monod equation takes the form

$$r_{\rm ut} = -\frac{\hat{q}S}{K+S}X_a \tag{3.6}$$

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in which

 r_{ut} = rate of substrate utilization $(M_sL^{-3}T^{-1})$ \hat{q} = maximum specific rate of substrate utilization $(M_sM_x^{-1}T^{-1})$

Substrate utilization and biomass growth are connected by

in which Y = true yield for cell synthesis $(M_x M_x^{-1})$. Yes, the true yield is exactly the same Y as you studied in Chapter 2 on *Stoichiometry*. It represents the fraction of electron-donor electrons converted to biomass electrons during synthesis of new biomass. The net rate of cell growth becomes

$$r_{\text{net}} = Y \frac{\hat{q}S}{K + S} X_a - bX_a$$
 [3.8]

in which r_{net} = the net rate of active-biomass growth $(M_x L^{-3} T^{-1})$. Of course,

$$\mu = r_{\text{net}}/X_a = Y \frac{\hat{q}S}{K+S} - b$$
 [3.9]

Some prefer to think of cell maintenance as being a shunting of substrate-derived electrons and energy directly for maintenance. This is expressed by

$$\mu = Y\left(\frac{\hat{q}S}{K+S} - m\right) \tag{3.10}$$

in which m = maintenance-utilization rate of substrate ($M_s M_x^{-1} T^{-1}$), which also was introduced in Chapter 2. When systems go to steady state, there is no difference in the two approaches to maintenance, and b = Ym. We use the endogenous-decay approach exclusively.

3.2 PARAMETER VALUES

The parameters describing biomass growth and substrate utilization cannot be taken as "random variables." They have specific units and ranges of values. In several cases, the values are constrained by the cell's stoichiometry and energetics, described in Chapter 2.

The true yield, Y, is proportional to f_s^o , presented under Stoichiometry. The thermodynamic method presented at the end of Chapter 2 is an excellent means to obtain a good first estimate of Y if experimental values are not available. Table 3.1 lists f_s^o and Y values for the most commonly encountered bacteria in environmental biotechnology. Table 3.1 illustrates that f_s^o ranges from its highest values (0.6 to 0.7 e⁻ eq cells/e⁻ eq donor) for aerobic heterotrophs to its lowest values (0.05 to 0.10 e⁻ eq cells/e⁻ eq donor) for autotrophs and acetate-oxidizing anaerobes. These values reflect the balancing of energy costs for synthesis with the energy gains from

Table 3.1 Typical f_s^0 , Y, \hat{q} , and $\hat{\mu}$ values for key bacterial types in environmental biotechnology

Organism Type	Electron Donor	Electron Acceptors	C-Source	f_s^0	Y	â	û
Aerobic, Heterotrophs	Carbohydrate BOD	02	BOD	0.7	0.49 gVSS/gBODL	27 gBOD _L /gVSS-d	13.2
	Other BOD	O_2	BOD	0.6	0.42 gVSS/gBODL	20 gBODL/gVSS-d	8.4
Denitrifiers	BOD	NO ₃	BOD	0.5	0.25 gVSS/gBOD _L	16 gBODL/gVSS-d	4
	H ₂	NO ₃	CO_2	0.2	0.81 gVSS/gH ₂	1.25 gH ₂ /gVSS-d	1
	S(s)	NO ₃	CO ₂	0.2	0.15 gVSS/gS	6.7 gS/gVSS-d	1
Nitrifying Autotrophs	NH [†]	O_2	CO_2	0.14	0.34 gVSS/gNH ₄ +N	2.7 gNH ₄ +N/gVSS-d	0.92
	NO2	O_2	CO ₂	0.10	0.08 gVSS/gNO ₂ -N	7.8 gNO ₂ -N/gVSS-d	0.62
Methanogens	acetate BOD	acetate	acetate	0.05	0.035 gVSS/gBODL	8.4 gBODL/gVSS-d	0.3
	H ₂	CO ₂	CO ₂	0.08	0.45 gVSS/gH ₂	1.1 gH ₂ /g VSS-d	0.5
Sulfide Oxidizing Autotrophs	H ₂ S	O_2	CO ₂	0.2	0.28 gVSS/gH ₂ S-S	5 gS/gVSS-d	1,4
Sulfate Reducers	H ₂	SO ₄ ²	CO ₂	0.05	0.28 gVSS/gH ₂	1.05 gH ₂ /gVSS-d	0.29
	acetate BOD	SO ₄ ²	acetate	0.08	0.057 gVSS/gBODL	8.7 gBODL/gVSS-d	0.5
Fermenters	sugar BOD	sugars	sugars	0.18	0.13 gVSS/gBODi	9.8 gBOD _L /gVSS-d	1.2

Y is computed assuming a cellular VSS_{σ} composition of C₅H₇O₂N, and NH $_4^+$ is the N source, except when NO $_3^-$ is the electron acceptor; then NO $_3^-$ is the N source. The typical units on Y are presented.

 $[\]hat{q}$ is computed using $\hat{q} = 1e^{-1} eq/gVSS_{a}d$.

 $[\]hat{\mu}$ has units of d^{-1} .

the donor-to-acceptor energy reaction. Chapter 2 showed that Y values are computed directly from f_s^o as a unit conversion. For example,

Aerobic heterotrophs:

$$Y = 0.6 \frac{e^- \text{ eq cells}}{e^- \text{ eq donor}} \cdot \frac{113 \text{ gVSS}}{20 \text{ e}^- \text{ eq cells}} \cdot \frac{1 \text{ e}^- \text{ eq donor}}{8 \text{ gBOD}_L}$$
$$= 0.42 \text{ gVSS/gBOD}_L$$

Denitrifying heterotrophs:

$$Y = 0.5 \frac{e^- \text{ eq cells}}{e^- \text{ eq donor}} \cdot \frac{113 \text{ gVSS}}{28 \text{ e}^- \text{ eq cells}} \cdot \frac{1 \text{ e}^- \text{ eq donor}}{8 \text{ gBOD}_L}$$
$$= 0.25 \text{ gVSS/gBOD}_L$$

H2-Oxidizing Sulfate Reducers:

$$Y = 0.05 \frac{e^- \text{ eq cells}}{e^- \text{ eq donor}} \cdot \frac{113 \text{ gVSS}}{20 \text{ e}^- \text{ eq cells}} \cdot \frac{2 \text{ e}^- \text{ eq donor}}{2 \text{ gH}_2}$$
$$= 0.28 \text{ gVSS/gH}_2$$

The wide range of Y values reflects a combination of changes in f_s^o values and different units for the donor.

The true yield also can be estimated experimentally from batch growth. A small inoculum is grown to exponential phase and harvested. The true yield is estimated from $Y = -\Delta X/\Delta S$, where ΔX and ΔS are the measured changes in biomass and substrate concentration from inoculation until the time of harvesting. The batch technique is adequate for rapidly growing cells, but can create errors when the cells grow slowly so that biomass decay cannot be neglected.

For the cells' usual primary substrates, the maximum specific rate of substrate utilization, \hat{q} , is controlled largely by electron flow to the electron acceptor. For 20 °C, the maximum flow to the energy reaction is about 1 e⁻ eq/gVSS-d. If this flow is termed \hat{q}_e , \hat{q} can be computed from

$$\hat{q} = \hat{q}_e / f_e^0 \tag{3.11}$$

Table 3.1 also lists typical \hat{q} values when $\hat{q}_e = 1 \text{ e}^-$ eq/gVSS-d. The range of \hat{q} values again reflects variations in f_e^o and different units for the donor. Table 3.1 also lists $\hat{\mu} = Y\hat{q}$ values. The table makes it clear that the fast growing cells are those that have a large f_s^o , which directly gives a large Y and indirectly gives a large \hat{q} . Thus, $\hat{\mu}$ is controlled mainly by the microorganism's stoichiometry and energetics.

Temperature affects \hat{q} . For temperatures up to the microorganism's optimal temperature, the substrate-utilization rate roughly doubles for each 10 °C increase in temperature. This phenomenon can be approximated by

$$\hat{q}_T = \hat{q}_{20}(1.07)^{T-20}$$
 [3.12]

where T is in °C and \hat{q}_{20} is the \hat{q} value for 20 °C. If \hat{q}_{20} is not known, the relationship can be generalized to

$$\hat{q}_T = \hat{q}_{TR} (1.07)^{(T-T^R)}$$
 [3.13]

in which T^R is any reference temperature (°C) for which \hat{q}_{TR} is known.

The endogenous decay rate (b) depends on species type and temperature. The b values tend to correlate positively with $\hat{\mu}$ values. For example, aerobic heterotrophs have b values of 0.1 to 0.3/d at 20 °C, while the slower-growing species have b < 0.05/d. The temperature effect on b can be expressed by a $(1.07)^{T-T^R}$ relationship parallel to Equation 3.13. Endogenous decay coefficients normally encompass several loss phenomena, including lysis, predation, excretion of soluble materials, and death.

McCarty (1975) found that the biodegradable fraction (f_d) is quite reproducible and has a value near 0.8 for a wide range of microorganisms.

The Monod half-maximum-rate concentration (K) is the most variable and least predictable parameter. Its value can be affected by the substrate's affinity for transport or metabolic enzymes. In addition, mass-transport resistances, usually ignored for suspended growth, often are "lumped" into the Monod kinetics by an increase in K. When mass transport is not included in K and simple electron-donor substrates are considered, K values tend to be low, less than 1 mg/l and often as low as the μ g/l range. For more difficult to degrade compounds and when mass-transport resistance is de facto included, K values range from a few mg/l to 100s of mg/l for electron donors. Terminal electron acceptors for respiration often have very low K values, well below 1 mg/l. On the other hand, direct use of O_2 as a cosubstrate in oxygenation reactions may have a higher K value, around 1 mg/l. The information provided here for K should be taken as general guidance only. More exact values require analyzing experimental results.

3.3 BASIC MASS BALANCES

Writing mass balances requires specifying a control volume. All the mass balances, their solutions, and important trends can be obtained by considering one of the simplest systems: a steady-state chemostat. Figure 3.2 illustrates the key features of a chemostat: a completely mixed reactor having uniform and steady concentrations of active cells (X_a) , substrate (S), inert biomass (X_i) , and any other constituents we wish to consider. Substrate (S) includes soluble compounds or suspended materials that are hydrolyzed to soluble compounds within the biological reactor. The chemostat's volume is V, and it receives a constant feed flow rate Q having substrate concentration S^0 . For now, we preclude inputs of active or inert biomass. The effluent has flow rate Q and concentrations X_a , X_i , and S.

We must provide mass balances on the active biomass and the rate-limiting substrate (assumed to be the electron donor), because they are the active catalysts and the material responsible for the accumulation of the catalysts, respectively. The steady-

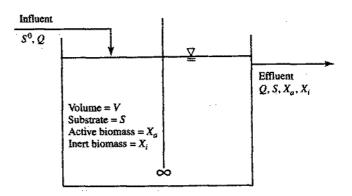


Figure 3.2 Schematic of a chemostat.

state mass balances are:

Active Biomass:
$$0 = \mu X_a V - Q X_a$$
 [3.14]

Substrate:
$$0 = r_{ut}V + Q(S^0 - S)$$
 [3.15]

Equations 3.6 and 3.9 provide the functions for μ and $r_{\mu t}$, respectively. Substituting them gives

$$0 = Y \frac{\hat{q}S}{K + S} X_a V - b X_a V - Q X_a$$
 [3.16]

for biomass and

$$0 = \frac{\hat{q}S}{K+S} X_a V + Q(S^0 - S)$$
 [3.17]

for substrate.

*Equations 3.16 and 3.17 can be solved to yield the steady-state values of S and X_a . The strategy is to solve Equation 3.16, the active-biomass balance, first for S.

$$S = K \frac{1 + b\left(\frac{V}{Q}\right)}{Y\hat{q}\left(\frac{V}{Q}\right) - \left(1 + b\left(\frac{V}{Q}\right)\right)}$$
 [3.18]

Once we know S, we solve the substrate balance, Equation 3.17. To do this, we first rearrange Equation 3.16 to obtain $[\hat{q}SX_aV/(K+S)]$, which is substituted into Equation 3.17 to yield

$$X_a = Y(S^0 - S) \frac{1}{1 + b\left(\frac{V}{Q}\right)}$$
 [3.19]

This two-step strategy is a generally useful one and can be used for more complicated systems or when the growth and substrate-utilization relationships differ from those in Equations 3.6 and 3.9.

The ratio V/Q is not the most convenient form. It can be substituted immediately by either of two alternate forms:

hydraulic detention time
$$(T) = \theta = V/Q$$
 [3.20]

or

dilution rate
$$(T^{-1}) = D = Q/V$$
 [3.21]

Engineers generally are more comfortable with using θ to simplify Equations 3.18 and 3.19, while microbiologists usually use D.

A much more general and powerful substitution is the solids retention time (SRT), which also is commonly called the mean cell residence time (MCRT) or the sludge age. These three terms normally refer to the same quantity, which is denoted θ_x , has units of time, and always is defined as

$$\theta_x = \frac{\text{active biomass in the system}}{\text{production rate of active biomass}} = \mu^{-1}$$
 [3.22]

Equation 3.22 illustrates the two critical features of θ_x . First, it is the reciprocal of the net specific growth rate. Thus, θ_x is a fundamental descriptor of the physiological status of the system, because it gives us direct information about the specific growth rate of the microorganisms. Second, the word definition in Equation 3.22 must be converted to quantitative parameters to apply θ_x to any system.

For our chemostat, any biomass produced in the system must exit in the effluent: thus, the denominator is QX_a . The total active biomass (the numerator) is VX_a . Thus,

$$\theta_x = \frac{VX_a}{OX_a} = \theta = \frac{1}{D}$$
 [3.23]

which emphasizes that $\theta_x = \theta$ in our chemostat, as long as the chemostat is at steady state.

Rewriting Equations 3.18 and 3.19 in the (most useful) θ_x form gives

$$S = K \frac{1 + b\theta_x}{Y\hat{q}\theta_x - (1 + b\theta_x)}$$
 [3.24]

$$X_a = Y\left(\frac{S^0 - S}{1 + b\theta_x}\right)$$
 [3.25]

Figure 3.3 illustrates how S and X_a are controlled by θ_x . Although the chemostat is a simple system, several key trends shown in Figure 3.3 occur for all suspended-growth processes.

1. When θ_x is very small, $S = S^0$, and $X_a = 0$. This situation, termed washout, has no substrate removal and, therefore, no accumulation of active biomass. The θ_x value at which washout begins is called θ_x^{\min} , which forms the boundary between having steady-state biomass and washout. θ_x^{\min} is computed by letting $S = S^0$ in Equation 3.24 and solving for θ_x :

$$\theta_x^{\min} = \frac{K + S^0}{S^0(Y\hat{q} - b) - bK}$$
 [3.26]

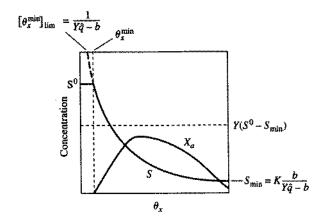


Figure 3.3 Sketch of how S and X_a vary with θ_x and what are the limiting values for θ_x , S, and X_a .

 θ_r^{min} increases with increasing S^0 , but asymptotically reaches a limiting value

$$\left[\theta_x^{\min}\right]_{\lim} = \frac{1}{Y\hat{a} - b}$$
 [3.27]

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which defines an absolute minimum θ_x (or maximum μ) boundary for having steady-state biomass. $[\theta_x^{\min}]_{\lim}$ is a fundamental delimiter of a biological process: washout at low θ_x .

- 2. For all $\theta_x > \theta_x^{\min}$, S declines monotonically with increasing θ_x . Equation 3.24 is used to compute S.
- 3. For very large θ_x , S approaches another key limiting value: S_{\min} , the minimum substrate concentration capable of supporting steady-state biomass. S_{\min} can be computed by letting θ_x approach infinity in Equation 3.24:

$$S_{\min} = K \frac{b}{Y\hat{q} - b}$$
 [3.28]

If $S < S_{\min}$, the cells net specific growth rate is negative (recall Equation 3.9), and biomass will not accumulate or will gradually disappear. Therefore, steady-state biomass can be sustained only when $S > S_{\min}$. S_{\min} is a fundamental delimiter of biological process performance for large θ_X .

4. When $\theta_x > \theta_x^{\min}$, X_a rises initially, because $S^0 - S$ increases as θ_x becomes larger. However, X_a reaches a maximum value in a chemostat and then declines as decay becomes dominant for large θ_x . If θ_x were to extend to infinity, X_a would approach zero.

In summary, the simple graph in Figure 3.3 tells us that we can reduce S from S^0 to S_{\min} as we increase θ_x from θ_x^{\min} to infinity. The exact value of θ_x we pick depends on a balancing of substrate removal, biomass production (equals QX_a), and other

factors we will discuss later. In practice, engineers often specify a microbiological safety factor, which is defined as θ_x/θ_x^{min} . Safety factors, which typically range from around five to hundreds, are discussed in Chapter 5.

3.4 Mass Balances on Inert Biomass and Volatile Solids

Because some fraction of newly synthesized biomass is refractory to self-oxidation, endogenous respiration leads to the accumulation of inactive biomass. In addition, real influents often contain refractory volatile suspended solids that we cannot differentiate easily from inactive biomass. So, we must expand our chemostat analysis to account for inactive biomass (and other nonbiodegradable volatile suspended solids).

A steady-state mass balance on inert biomass is

$$0 = (1 - f_d)bX_aV + Q(X_i^0 - X_i)$$
 [3.29]

in which

 $X_i = \text{concentration of inert biomass in the chemostat } (M_x L^{-3})$

 X_i^0 = input concentration of inert biomass (or indistinguishable refractory volatile suspended solids) (M_xL^{-3}).

Thus, we are relaxing the original requirement that only substrate enters in the influent. Also, the first term in Equation 3.29, the formation rate of inert biomass from active-biomass decay, comes from Equation 3.4.

Solution of Equation 3.29 is

$$X_i = X_i^0 + X_a(1 - f_d)b\theta$$
 [3.30]

Equation 3.30 emphasizes that X_i is comprised of influent inerts (X_i^0) and inerts formed from decay of X_a (i.e., $X_a(1 - f_d)b\theta$). Figure 3.4 shows that X_i increases monotonically from X_i^0 to a maximum of $X_i^0 + Y(S^0 - S_{\min})(1 - f_d)$. Thus, operation at a large θ results in greater accumulation of inert biomass.

The sum of X_i and X_a is called X_v , the volatile suspended solids concentration, or VSS. Letting $\theta_x = \theta$ for the chemostat, X_v can be computed directly as

$$X_v = X_i^o + X_a(1 + (1 - f_d)b\theta_x) = X_i^0 + Y(S^0 - S)\frac{1 + (1 - f_d)b\theta_x}{1 + b\theta_x}$$
 [3.31]

Figure 3.4 demonstrates that X_v generally follows the trend of X_a , but it does not equal zero; when X_a goes to zero, X_v equals X_i .

The second term on the right-hand side of Equation 3.31 represents the net accumulation of biomass from synthesis and decay. It is constituted by the change in substrate concentration $(S^0 - S)$ multiplied by the net yield (Y_n) :

$$Y_n = Y \frac{1 + (1 - f_d)b\theta_x}{1 + b\theta_x}$$
 [3.32]