Chapter 3. Microbial Kinetics

All the figures and tables in this material are from the reference below unless specified otherwise.
Reference: Bruce E. Rittmann and Perry L. McCarty, "Environmental Biotechnology: Principles and Applications", McGraw-Hill, 2001.

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3. Microbial Kinetics

- **Microorganisms fuel their lives by performing redox reaction that generate the energy and reducing power needed to maintain and construct themselves.**

- **Because redox reactions are nearly always very slow unless catalyzed, microorganisms produce enzyme catalysts that increase the kinetics of their redox reactions to exploit chemical resources in their environment.**

- **Engineers want to take advantage of these microbially catalyzed reaction because the chemical resources of the microorganisms usually are the pollutants that the engineers must control:**

  e.g., Organics (BOD, COD, e^- donor), NH_4^+ (e^- donor, nutrient), NO_3^- (e^- acceptor, nutrient), PO_4^{3-} (nutrient), etc.

3. Microbial Kinetics

- **Engineers who employ microorganisms for pollution control must recognize two interrelated principles:**

  First :

  Active microorganisms catalyze the pollutant removing reactions.
  So 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 (e^-) - generating primary substrates.
  So the rate of biomass production 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.**

3.1 Basic Rate Expressions

- **A microbial process model is based on the mass balance on**

  i) the active biomass and ii) the primary substrate that limits the growth rate of the biomass.

- **In the vast majority of cases, the rate limiting substrate is the e^- donor. So the term *substrate* now refers to the *primary e^- donor substrate.***

- ***Jacques Monod* : *Nobel prize winner, director of Pasteur Institute.***

- ***Monod equation* : relationship most frequently used to represent bacterial growth kinetics developed in the 1940s.**

  → **His original work related the specific growth rate of fast-growing bacteria to the concentration of a rate limiting, e^- donor substrate.**

3.1 Basic Rate Expressions

√ ***Monod equation*** **:**
largely empirical, but widely applied for microbial systems

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

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

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

t = Time (T)

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

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

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

3.1 Basic Rate Expressions

- **Compare Monod equation with Michaelis-Menten equation**

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

$$v = v_m \frac{S}{K_M + S}$$

→***Michaelis-Menten equation :***

- *developed in 1913*
- *theory of enzyme action and kinetics*

v **: Reaction velocity**

K_M **: Substrate concentration giving one-half of the maximum velocity**

- **Microorganisms are "bags full of enzymes" so that it is not surprising that the growth rate of microorganisms (Monod eq.) is related to the reactions of the catalysts that mediate many reactions (Michaelis and Menten eq.)**

3.1 Basic Rate Expressions

√ Endogenous decay:

1) Environmental engineers study more slowly growing bacteria that has an energy demand for maintenance.
2) Active biomass has an energy demand for maintenance of cell functions (motility, repair and re-synthesis, osmotic regulation, transport and heat loss).
3) Flow of energy and electrons are required to meet maintenance needs.
4) Cells oxidize themselves to meet maintenance- energy needs.

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

b = endogenous-decay coefficient (T^{-1})

μ_{dec} = specific growth rate due to decay (T^{-1})

3.1 Basic Rate Expressions

- **Oxidation decay rate :**

  Although most of the decayed biomass is oxidized, a small fraction accumulates as inert biomass.

$$\mu_{resp} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{decay} = -f_d b$$

f_d **:** fraction of active biomass that is biodegradable

- **The rate at which active biomass is converted to inert biomass**

$$\mu_{inert} = \mu_{dec} - \mu_{resp}$$

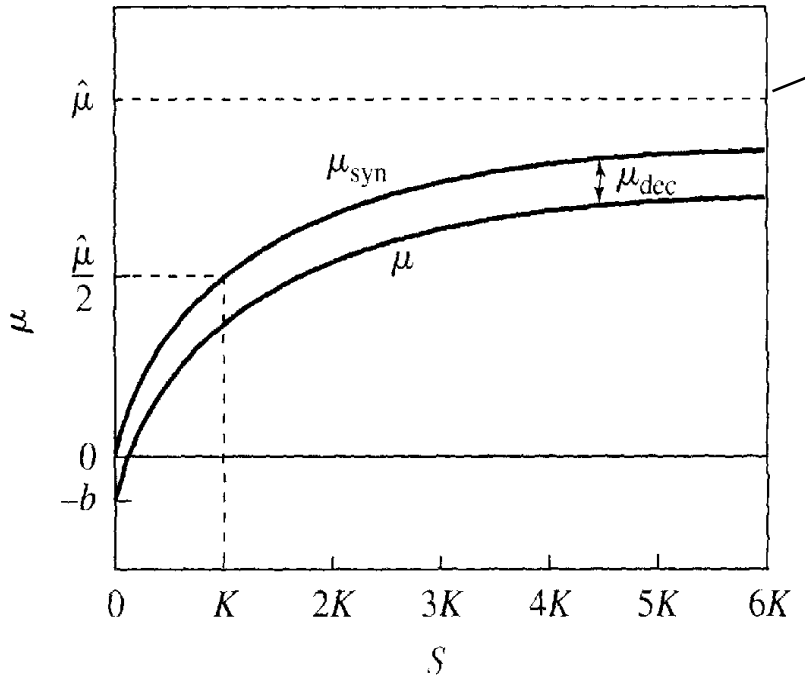
$$-\frac{1}{X_a}\frac{dX_i}{dt} = \left(\frac{1}{X_a}\frac{dX_a}{dt}\right)_{inert} = -b - (-f_d b) = -(1 - f_d)b$$

X_i **:** inert biomass concentration ($M_x L^{-3}$)

3.1 Basic Rate Expressions

- **The net specific growth rate of active biomass (μ) is the sum of synthesis and decay**

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



Maximum specific growth rate

Figure3.1 schematic of how the synthesis and net specific growth rates depend on the substrate conc entration.

At S=20K, $\mu_{syn} = 0.95\ \hat{\mu}$

If S = 0, then $\mu = \mu_{syn} + \mu_{dec} = \hat{\mu}\frac{S}{K+S} - b = 0 - b$

3.1 Basic Rate Expressions

√ Rate of substrate utilization

- **The ultimate interest is to remove substrate**

Y = g Cell produced / g Substrate consumed

$$r_{ut} = -\frac{\hat{q} S}{K + S} X_a \qquad \text{where,} \quad \hat{\mu} = \hat{q} Y$$

r_{ut} = rate of substrate utilization ($M_s L^{-3} T^{-1}$)

$\hat{q}$ = maximum specific rate of substrate utilization ($M_s M_x^{-1} T^{-1}$)

√ Net rate of cell growth

$$r_{net} = \mu X_a = \hat{\mu} \frac{S}{K + S} X_a - b X_a$$

$$r_{net} = Y \frac{\hat{q} S}{K + S} X_a - b X_a$$

r_{net} = the net rate of active-biomass growth ($M_x L^{-3} T^{-1}$)

3.1 Basic Rate Expressions

√ Starvation

Because $b = Ym$ in Ch. 2

$$\mu = Y\frac{\hat{q}S}{K+S} - b \quad \Rightarrow \quad \mu = Y\left(\frac{\hat{q}S}{K+S} - m\right)$$

$$\mu = Y\left(\frac{-dS/dt}{X_a} - m\right)$$

m: maintenance energy (M/MT)

(= the substrate utilization rate per unit mass of organisms at SS)

- **At the starvation state, the specific substrate utilization rate becomes less than m.**

When $\dfrac{-dS/dt}{X_a} < m = \dfrac{b}{Y} \quad \Rightarrow \quad \mu < 0$

$\dfrac{-dS/dt}{X_a} = m = \dfrac{b}{Y} \quad \Rightarrow \quad \mu = 0$ At steady-state ($dx_a/dt = 0$)

3.2 Parameter Values

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	$\hat{q}$	$\hat{\mu}$
Aerobic, Heterotrophs	Carbohydrate BOD	O_2	BOD	0.7	0.49 gVSS/gBOD$_L$	27 gBOD$_L$/gVSS-d	13.2
	Other BOD	O_2	BOD	0.6	0.42 gVSS/gBOD$_L$	20 gBOD$_L$/gVSS-d	8.4
Denitrifiers	BOD	NO_3^-	BOD	0.5	0.25 gVSS/gBOD$_L$	16 gBOD$_L$/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_4^+	O_2	CO_2	0.14	0.34 gVSS/gNH_4^+-N	2.7 gNH_4^+-N/gVSS-d	0.92
	NO_2^-	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/gBOD$_L$	8.4 gBOD$_L$/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_2S	O_2	CO_2	0.2	0.28 gVSS/gH_2S-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/gBOD$_L$	8.7 gBOD$_L$/gVSS-d	0.5
Fermenters	sugar BOD	sugars	sugars	0.18	0.13 gVSS/gBOD$_L$	9.8 gBOD$_L$/gVSS-d	1.2

Y is computed assuming a cellular VSS$_a$ composition of $C_5H_7O_2N$, 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 $\hat{q} = 1e^-$ eq/gVSS$_a$-d.

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

3.2 Parameter Values

Y = g Cell produced / g Substrate consumed

√ **Aerobic heterotrophs:**

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

$$= 0.42 \text{ gVSS/gBOD}_L$$

√ **Denitrifying heterotrophs:**

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

$$= 0.25 \text{ gVSS/gBOD}_L$$

√ **H_2-Oxidizing sulfate reducers:**

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

$$= 0.28 \text{ gVSS/gH}_2$$

Table 2.4 Cell formation (R_c) and common electron acceptor half-reactions (R_a)

Reaction Number	Half-reaction	$\Delta G^{0'}$ kJ/e^- eq
Cell Synthesis Equations (R_c)		
Ammonium as Nitrogen Source		
C-1	$\frac{1}{5}\,CO_2 + \frac{1}{20}\,HCO_3^- + \frac{1}{20}\,NH_4^+ + H^+ + e^- = \frac{1}{20}\,C_5H_7O_2N + \frac{9}{20}\,H_2O$	
Nitrate as Nitrogen Source		
C-2	$\frac{1}{28}\,NO_3^- + \frac{5}{28}\,CO_2 + \frac{29}{28}\,H^+ + e^- = \frac{1}{28}\,C_5H_7O_2N + \frac{11}{28}\,H_2O$	
Nitrite as Nitrogen Source		
C-3	$\frac{5}{26}\,CO_2 + \frac{1}{26}\,NO_2^- + \frac{27}{26}\,H^+ + e^- = \frac{1}{26}\,C_5H_7O_2N + \frac{10}{26}\,H_2O$	
Dinitrogen as Nitrogen Source		
C-4	$\frac{5}{23}\,CO_2 + \frac{1}{46}\,N_2 + H^+ + e^- = \frac{1}{23}\,C_5H_7O_2N + \frac{8}{23}\,H_2O$	
Common Electron-Acceptor Equations (R_a)		
I-14 Oxygen	$\frac{1}{4}\,O_2 + H^+ + e^- = \frac{1}{2}\,H_2O$	−78.72
I-7 Nitrate	$\frac{1}{5}\,NO_3^- + \frac{6}{5}\,H^+ + e^- = \frac{1}{10}\,N_2 + \frac{3}{5}\,H_2O$	−72.20
I-9 Sulfate	$\frac{1}{8}\,SO_4^{2-} + \frac{19}{16}\,H^+ + e^- = \frac{1}{16}\,H_2S + \frac{1}{16}\,HS^- + \frac{1}{2}\,H_2O$	20.85
O-12 CO_2	$\frac{1}{8}\,CO_2 + H^+ + e^- = \frac{1}{8}\,CH_4 + \frac{1}{4}\,H_2O$	23.53
I-4 Iron (III)	$Fe^{3+} + e^- = Fe^{2+}$	−74.27

3.2 Parameter Values

√ Experimental estimation of *Y*

- A small inoculum is grown to exponential phase and harvested from batch growth. Measure the changes in biomass and substrate concentration from inoculum until the time of harvesting.

- The true yield is estimated from

$$Y = -\Delta X / \Delta S$$

- 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.

3.2 Parameter Values

√ $\hat{q}$ **is controlled largely by e⁻ flow to the electron acceptor.**

- **For 20 °C, the maximum flow to the energy reaction is about 1 *e⁻ eq / gVSS-d,* where 1 g *VSS* represents 1 g of biomass.**

$$\hat{q}_e = about\ 1\ e^- eq\ /VSS\text{-}d \quad at\ 20^0\ C$$

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

e.g., if $f_s^0 = 0.7$, then $f_e^0 = 0.3$

$$\hat{q} = \hat{q}_e / f_e^0 = 8g\ BOD_L / VSS\text{-}d\ /\ 0.3 = 27g\ BOD_L / gVSS\text{-}d$$

- $\hat{q}$ **is temperature-dependent.**

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

$$\hat{q}_T = \hat{q}_{T^R}(1.07)^{(T\text{-}T^R)}$$

3.2 Parameter Values

√ ***b* is depends on microbial species and temperature.**

- ***b* = 0.1- 0.3 /d for aerobic heterotrophs b < 0.05 /d for slower-growing species**

$$b_T = b_{T^R}(1.07)^{(T-T^R)}$$

- **Biodegradable fraction (f_d) is quite reproducible and has a value near 0.8 for a wide range of microorganisms**

√ ***K* : the *Monod* half-maximum-rate concentration**

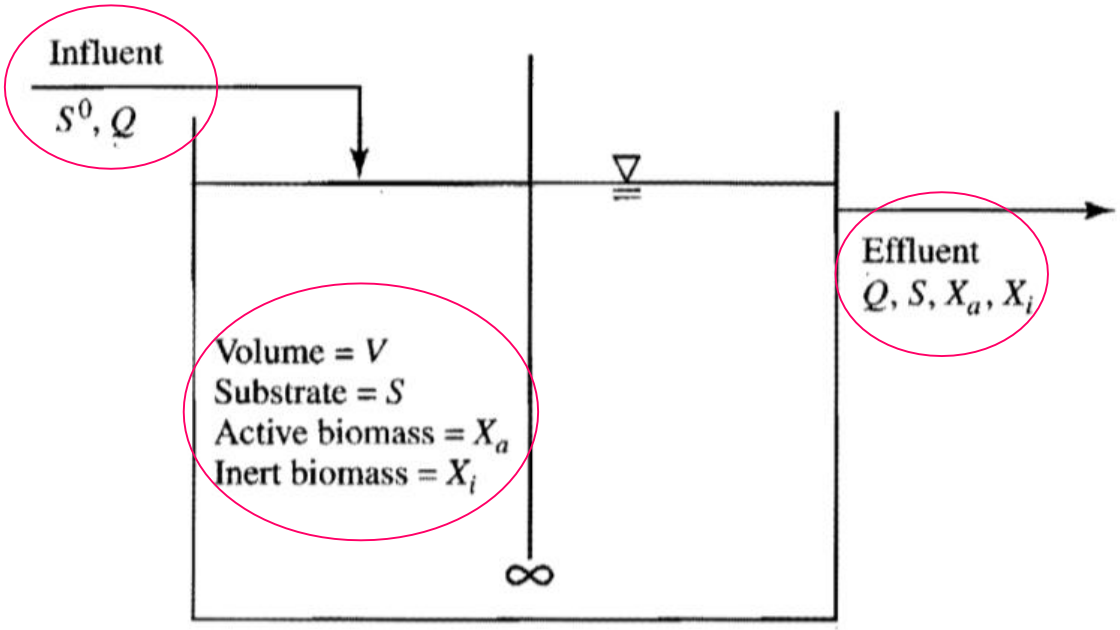
$$\mu = Y\frac{\hat{q}S}{K+S} - b$$

- Most variable and least predictable parameter
- Its value can be affected by the substrate`s affinity for transport or metabolic enzymes
- Mass-transport resistances (approaching of substrate and microorganisms to each other) ignored for suspended growth are lumped into the *Monod* kinetics by an increase in *K*

3.3 Basic Mass Balances

√ **Chemostat:**

a completely mixed reactor, having uniform and steady concentrations of active cell, substrate, inert biomass and any other constituents.



- **Active biomass**

$$0 = \mu X_a V - Q X_a$$

- **Substrate**

$$0 = r_{ut} V + Q\left(S^0 - S\right)$$

Q = feed flow rate

S^o = feed substrate concentration

3.3 Basic Mass Balances

- **From the mass-balance equation of active biomass**

$$0 = \mu X_a V - Q X_a$$

$$\mu = Y \frac{\hat{q} S}{K + S} - b$$

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

$$\frac{\hat{q} S}{K + S} X_a V = \frac{b X_a V + Q X_a}{Y}$$

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

- **From the mass-balance equation of substrate**

$$0 = r_{ut} V + Q\left(S^0 - S\right)$$

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

$$0 = -\frac{\hat{q} S}{K + S} X_a V + Q\left(S^0 - S\right)$$

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

3.3 Basic Mass Balances

√ **HRT : Hydraulic Retention (or residence, detention) Time**

$$hydraulic\ \ retention\ time\ (T) = \theta = V/Q$$

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

√ **SRT : Solids Retention Time**
= MCRT : Mean Cell Residence Time
= Sludge age

$$\theta_\chi = \frac{Active\ biomass\ in\ the\ system}{Production\ rate\ of\ active\ biomass} = \mu^{-1}$$

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

3.3 Basic Mass Balances

√ **SRT with quantitative parameters**

$$\theta_x = \frac{VX_a}{QX_a} = \frac{V}{Q} = \theta = \frac{1}{D} \Rightarrow \theta_\chi = \theta$$

SRT = HRT in our chemostat

$$S = K\frac{1 + b\left(\frac{V}{Q}\right)}{Y\hat{q}\left(\frac{V}{Q}\right) - \left(1 + b\left(\frac{V}{Q}\right)\right)} \Rightarrow S = K\frac{1 + b\theta_\chi}{Y\hat{q}\theta_\chi - (1 + b\theta_\chi)}$$

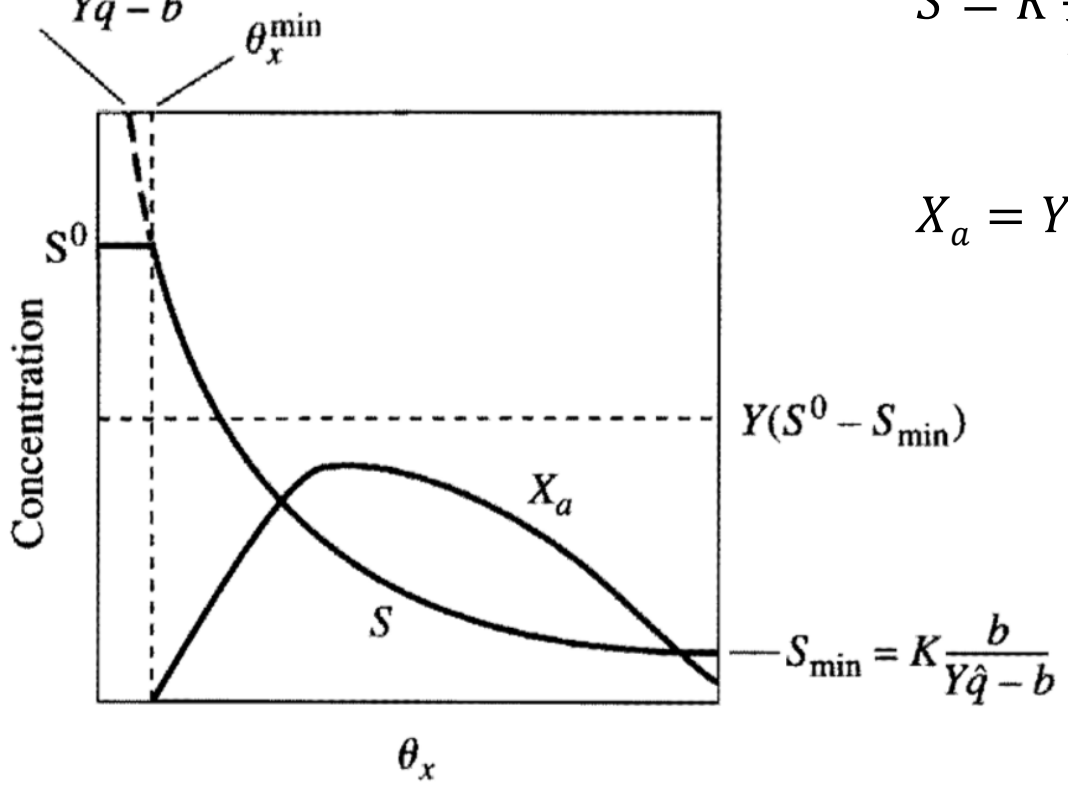
$$\frac{V}{Q} = \theta = \theta_x$$

$$X_a = Y(S^0 - S)\frac{1}{1 + b\left(\frac{V}{Q}\right)} \Rightarrow X_a = Y\left(\frac{S^0 - S}{1 + b\theta_\chi}\right)$$

3.3 Basic Mass Balances

√ S and X_a are controlled by SRT (θ_x)

$$[\theta_x^{min}]_{lim} = \frac{1}{Y\hat{q} - b}$$



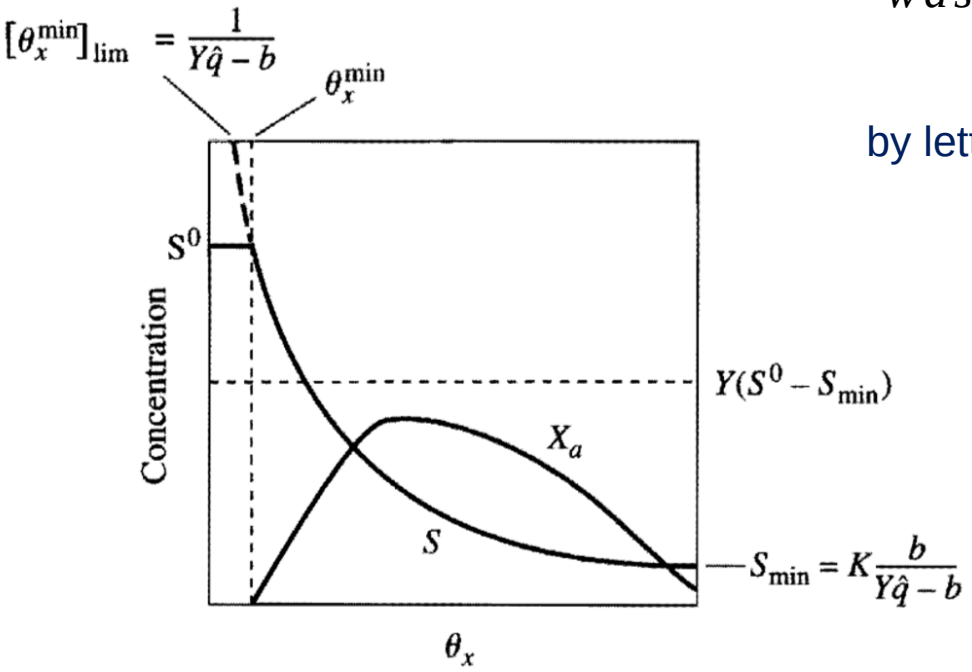
$$S = K\frac{1 + b\theta_x}{Y\hat{q}\theta_x - (1 + b\theta_x)}$$

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

$\theta_x = \theta = V/Q$, $\theta_x = \uparrow$ *then* $Q \downarrow (V = const.)$

3.3 Basic Mass Balances

- **At very small** θ_χ ($S = S^0$, $X_a = 0$)



θ_χ^{min} : θ_χ value at which washout begins

$$washout \quad : \theta_\chi < \theta_\chi^{min}$$

by letting $S = S^0$ in the eq below and solving for θ_χ

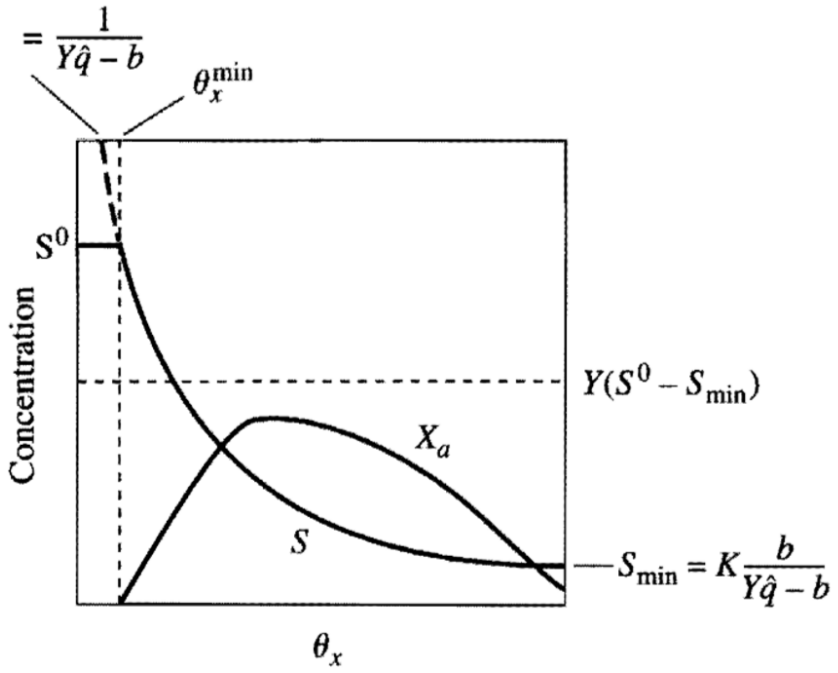
$$S = K\frac{1 + b\theta_\chi}{Y\hat{q}\theta_\chi - (1 + b\theta_\chi)}$$

$$\theta_\chi^{min} = \frac{K + S^0}{S^0(Y\hat{q} - b) - bK}$$

3.3 Basic Mass Balances

- **Absolute minimum θ_χ** ($S^0 \rightarrow \infty$)

$$\theta_\chi^{\min} = \frac{K + S^0}{S^0\left(Y\hat{q} - b\right) - bK}$$



$\theta_\chi^{\min}$ decreases with increasing S^0

$$[\theta_\chi^{\min}]_{\lim} = \lim_{s^o \rightarrow \infty} \frac{K + S^0}{S^0\left(Y\hat{q} - b\right) - bK}$$

$$= \lim_{s^o \rightarrow \infty} \frac{K / S^0 + 1}{\left(Y\hat{q} - b\right) - bK / S^0}$$

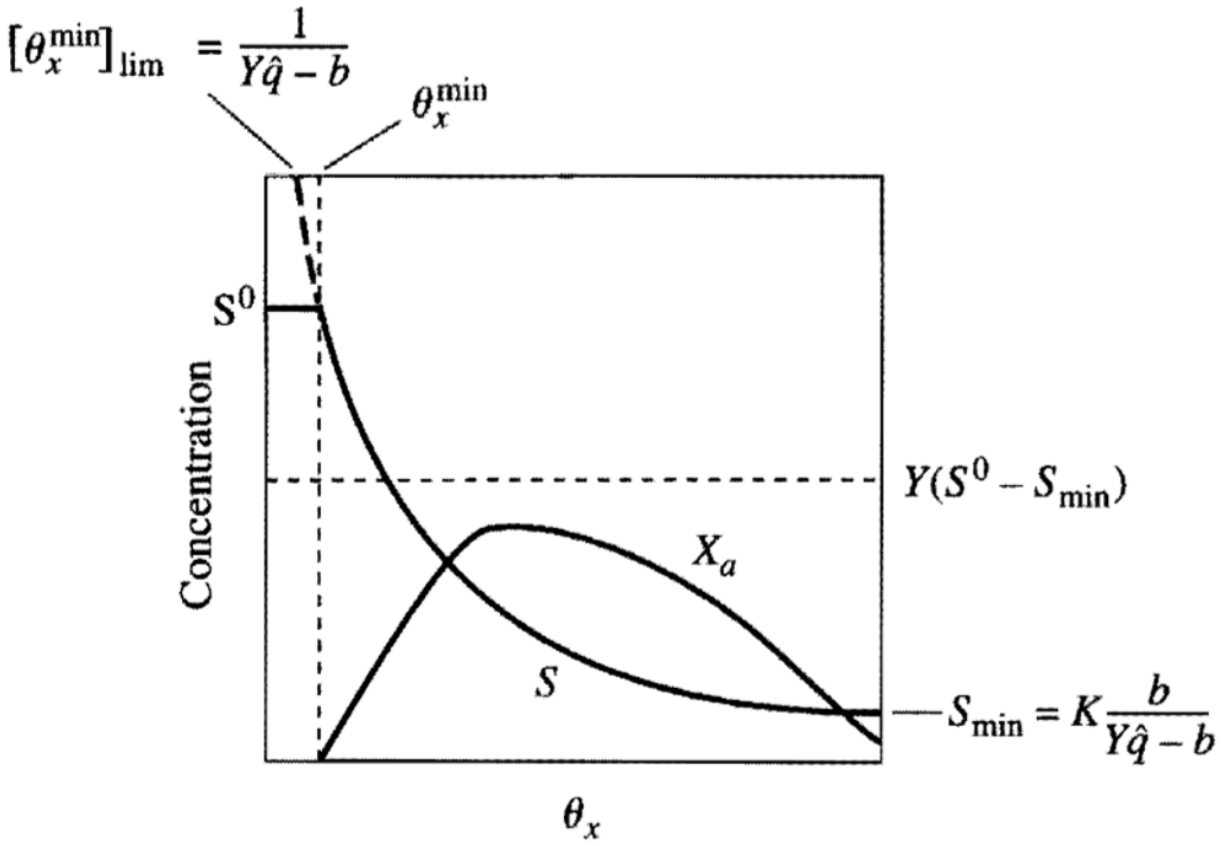
$$= \frac{1}{Y\hat{q} - b}$$

$[\theta_\chi^{\min}]_{\lim}$ defines an absolute minimum θ_χ (ormaximum μ) boundary *for having steady-state biomass.* It is a fundamental delimiter of a biological process.

3.3 Basic Mass Balances

- **For all $\theta_\chi > \theta_\chi^{min}$**

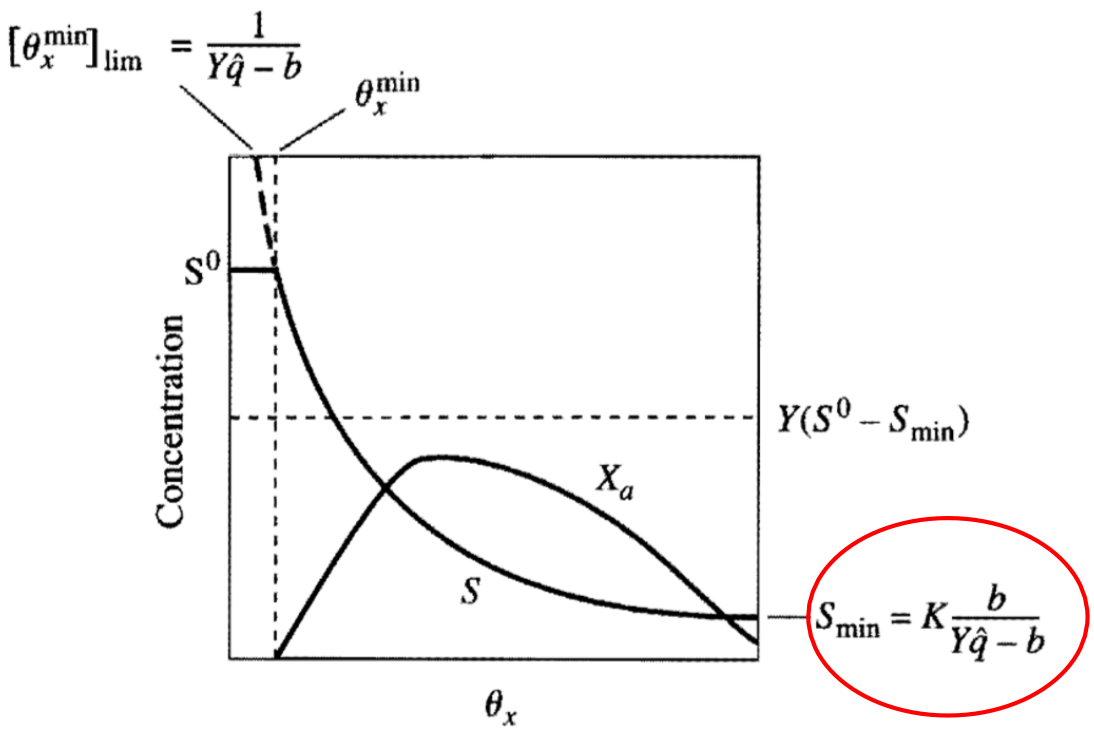
  S declines monotonically with increasing θ_χ



3.3 Basic Mass Balances

- **For very large θ_χ**

  S approaches another key limiting value (S_{min}).



$$S = K\frac{1+ b\theta_\chi}{Y\hat{q}\theta_\chi - (1+b\theta_\chi)}$$

$$S_{min} = \lim_{\theta x \to \infty} K\frac{1+ b\theta_\chi}{Y\hat{q}\theta_\chi - (1+b\theta_\chi)}$$

$$= \lim_{\theta x \to \infty} K\frac{1/\theta_\chi + b}{Y\hat{q} - 1/\theta_\chi - b}$$

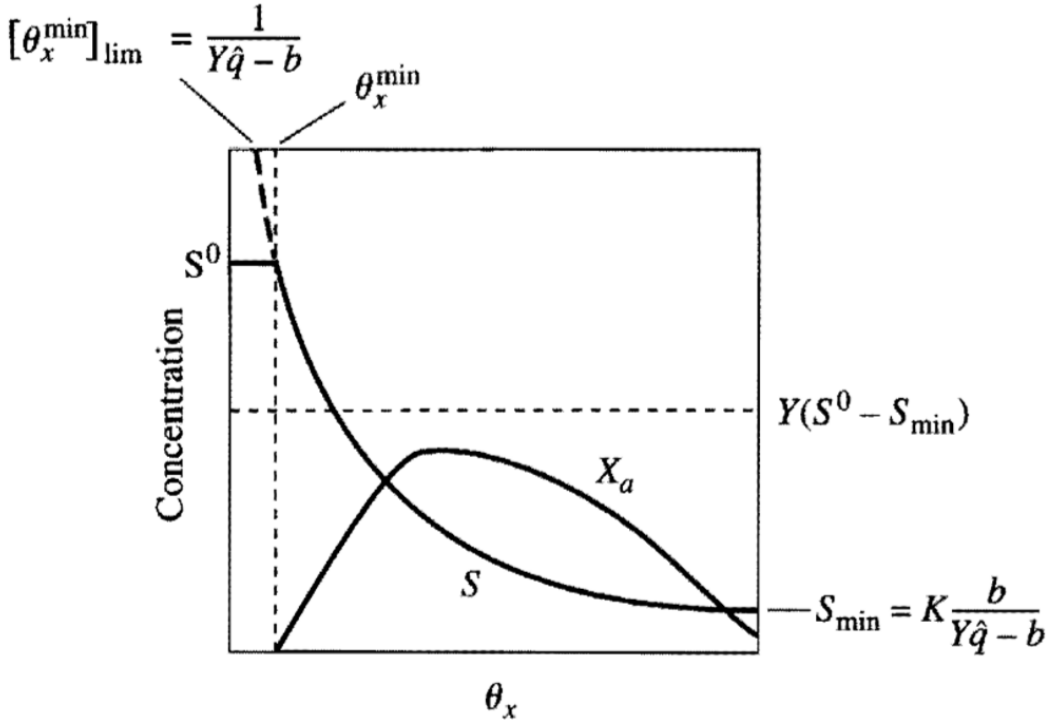
$$= K\frac{b}{Y\hat{q} - b}$$

- S_{min} is the minimum concentration capable of supporting steady-state biomass.
- If S < S_{min}, the cells net specific growth rate becomes negative (Biomass will not accumulate and gradually disappear.
- Therefore, steady-state biomass can be sustained only when S > S_{min}.

3.3 Basic Mass Balances

- **When $\theta_\chi > \theta_\chi^{min}$, X_a = ?**

- X_a rises initially because (S^0 – S) increases with increasing θ_χ
- However, X_a reaches a maximum and declines.
  as the decay ($b\theta_\chi$) becomes dominant, and the increase in (S^0 – S) slows down.
- If θ_χ were to extend to infinity, X_a would approach zero.



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

3.3 Basic Mass Balances

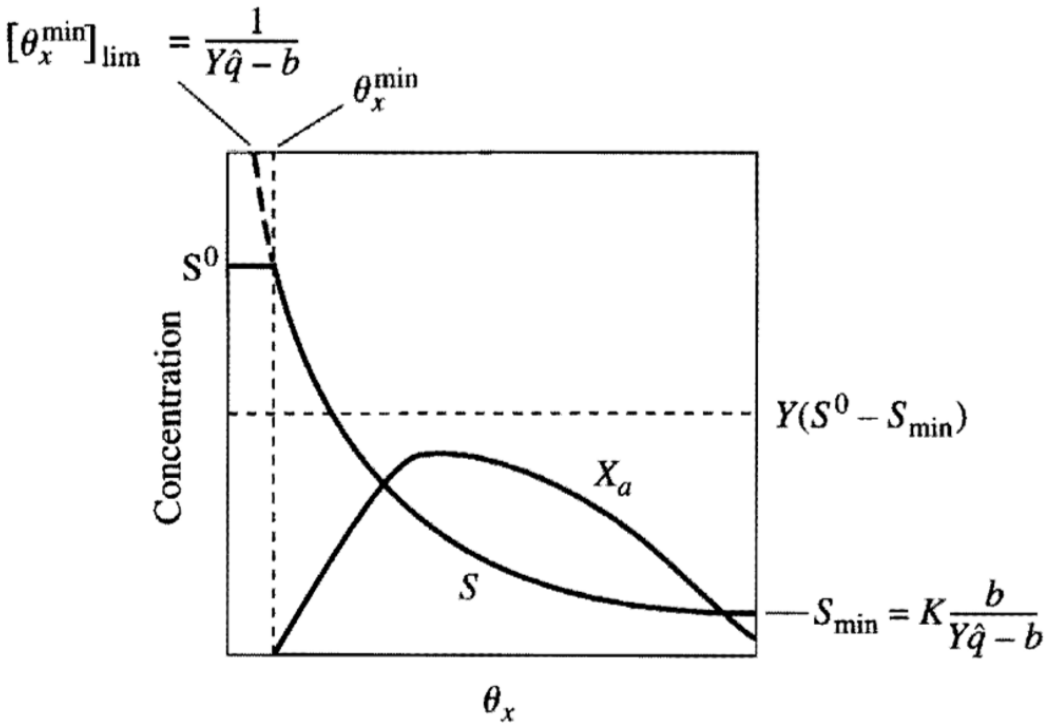
- **Microbiological safety factor**

- We can reduce S from S^o to S_{min} as we increase θ_χ from $\theta_\chi^{\min}$ to infinity.

- The exact value of θ_χ we pick depends on a balancing of substrate removal ($S^0 - S$), biomass production (= $Q\,X_a$), reactor volume (V) and other factors.

- In practice, engineers often specify a microbiological safety factor, defined by $\theta_\chi / \theta_\chi^{\min}$

- Typical safety factor = 5 ~ 100



3.4 Mass Balances on Inert Biomass and Volatile Solids

√ Inert biomass (X_i) and volatile solids (X_v)

- **Because some fraction of newly synthesized biomass is refractory to self-oxidation, endogenous respiration leads to the accumulation of inactive biomass ($(1-f_d)bX_aV$).**
- **Real influents often contain refractory volatile suspended solids (X_i^0) that we cannot differentiate easily from inactive biomass.**
- **Steady-state mass balance on inert biomass**

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

Formation rate of inert biomass from active biomass decay

X_i = concentration of inert biomass

X_i^0 = input concentration of inert biomass

$\theta = V/Q$

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

Inert biomass formed from active biomass decay
(Inert biomass represents a fraction of active biomass)

Influent inert biomass

3.4 Mass Balances on Inert Biomass and Volatile Solids

$$X_i = X_i^{\,0} + X_a(1 - f_d)b\theta$$

$\theta = \theta_x$ → ← $X_a = Y\left(\dfrac{S^0 - S}{1 + b\theta_x}\right)$

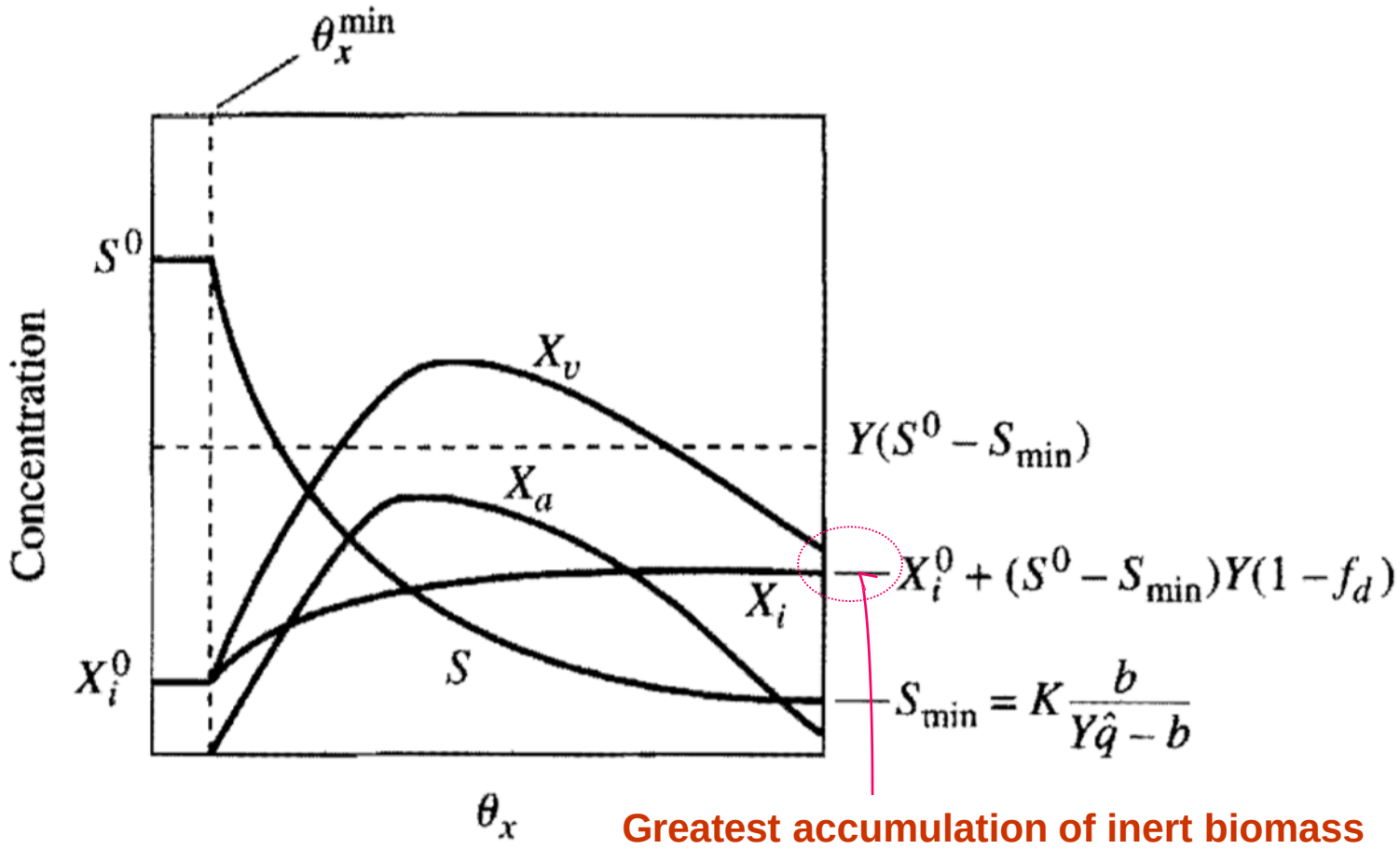
$$X_i = X_i^{\,0} + Y(S^0 - S)\frac{(1 - f_d)b\theta_x}{1 + b\theta_x}$$

$$X_i^{\,max} = \lim_{\theta_x \to \infty} X_i^{\,0} + Y\left(\frac{S^0 - S}{1 + b\theta_x}\right)(1 - f_d)b\theta_x$$

$$= \lim_{\theta_x \to \infty} X_i^{\,0} + Y\left(\frac{S^0 - S}{\frac{1}{b\theta_x} + 1}\right)(1 - f_d)$$

$$X_i^{\,max} = X_i^{\,0} + Y(S^0 - S_{min})(1 - f_d)$$

3.4 Mass Balances on Inert Biomass and Volatile Solids



- X_i increases monotonically from X_i^0 to a maximum X_i^{max}
- Operation at a large θ_x results in greater accumulation of inert biomass

3.4 Mass Balances on Inert Biomass and Volatile Solids

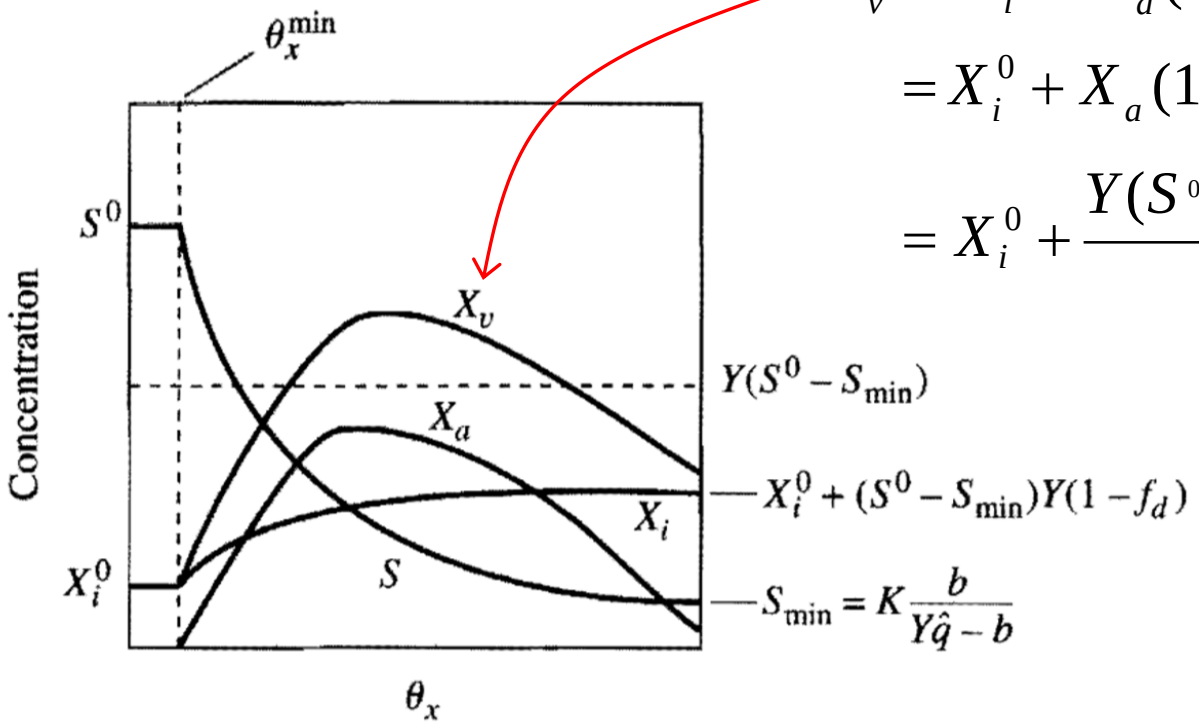
$$X_v = X_i + X_a$$

X_v: volatile suspended solids concentration (VSS)

$$X_v = X_i^0 + X_a(1-f_d)b\,\theta_\chi + X_a$$

$$= X_i^0 + X_a(1+(1-f_d)b\,\theta_\chi)$$

$$= X_i^0 + \frac{Y(S^0-S)\cdot[1+(1-f_d)b\theta_\chi]}{1+b\theta_\chi}$$



- X_v generally follow the trend of X_a, but it is higher than X_a (When X_a goes to zero, X_v equals X_i).
- If $\theta_x < \theta_x^{min}$, $X_v = X_i^0$, $S = S^0$

3.4 Mass Balances on Inert Biomass and Volatile Solids

√ Net yield (Y_n)

Net accumulation of biomass from synthesis and decay

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

$$= X_i^0 + Y_n(S^0 - S)$$

Y_n: net yield or observed yield (Y_{obs})

$$\therefore Y_n = Y \cdot \frac{1 + (1 - f_d) \cdot b \cdot \theta_x}{1 + b \cdot \theta_x}$$

- **The above equation is parallel to the relationship between f_s and f_s^0:**

$$\therefore f_s = f_s^0 \cdot \frac{1 + (1 - f_d) \cdot b \cdot \theta_x}{1 + b \cdot \theta_x}$$

3.5 Soluble Microbial Products

√ SMP (Soluble Microbial Products)

- Much of the soluble organic matter in the effluent from a biological reactor is of microbial origin.
- It is produced by the microorganisms as they degrade the organic substrate in the influent to the reactor.
- The major evidence for this phenomenon comes from experiments;

"*single soluble substrates of known composition were fed to microbial cultures and the resulting organic compounds in the effluent were examined for the presence of the influent substrate*."

"The bulk of the effluent organic matter was not the original substrate and was of high molecular weight, suggesting that it was of microbial origin."

- It is called "Soluble Microbial Products (SMP)".

3.5 Soluble Microbial Products

√ SMP

- Biodegradable, although some at a very low rate
- Moderate formula weights (100 to 1000)
- The majority of the effluent COD and BOD
- They can complex metals, foul membranes and cause color or foaming.
- They are thought to arise from two processes:

1) growth associated (UAP), 2) non-growth associated (BAP)

SMP = UAP + BAP

√ UAP (Substrate-Utilization-Associated Products)

- Growth associated
- They are generated from substrate utilization and biomass growth
- They are not intermediates of catabolic pathways

√ BAP (Biomass-Associated Products)

- Non-growth associated
- Related to decay and lysis of cell
- Release of soluble cellular constituents through lysis and solubilization of particulate cellular components

3.5 Soluble Microbial Products

√ **UAP formation kinetics :**

$$r_{UAP} = - k_1 r_{ut}$$

r_{UAP} = rate of UAP-formation ($M_p L^{-3} T^{-1}$)

k_1 = UAP- formation coefficient ($M_p M_s^{-1}$)

√ **BAP *formation kinetics :***

$$r_{BAP} = k_2 X_a$$

r_{BAP} = rate of BAP-formation ($M_p L^{-3} T^{-1}$)

k_2 = BAP- formation coefficient ($M_p M_x^{-1} T^{-1}$)

3.6 Nutrients and Electron Acceptors

- Biotechnological Process must provide sufficient nutrients and electron acceptors to support biomass growth and energy generation.
- Nutrients, being elements comprising the physical structure of the cells, are needed in proportion to the net production of biomass.
- Active and inert biomass contain nutrients, as long as they are produced microbiologically.
- e^- acceptors are consumed in proportion to e^- donor utilization multiplied by the sum of exogenous and endogenous flows of e^- to the terminal acceptor.

3.6 Nutrients and Electron Acceptors

√ Nutrient consumption rate (r_n)

- Nutrients are needed in proportion to the net production of biomass ($r_{ut} Y_n$)
- In chemostat model, rate of nutrients consumption (r_n)

$$r_n = \gamma_n r_{ut} Y_n$$

$$\Leftarrow \quad Y_n = Y \cdot \frac{1 + (1 - f_d) \cdot b \cdot \theta_x}{1 + b \cdot \theta_x}$$

$$r_n = \gamma_n r_{ut} Y \frac{1 + (1 - f_d) b \theta_x}{1 + b \theta_x}$$

r_n = rate of nutrient consumption ($M_n L^{-3} T^{-1}$) (negative value)

γ_n = the stochiometric ratio of nutrient mass to VSS for the biomass [$M_n M_x^{-1}$]

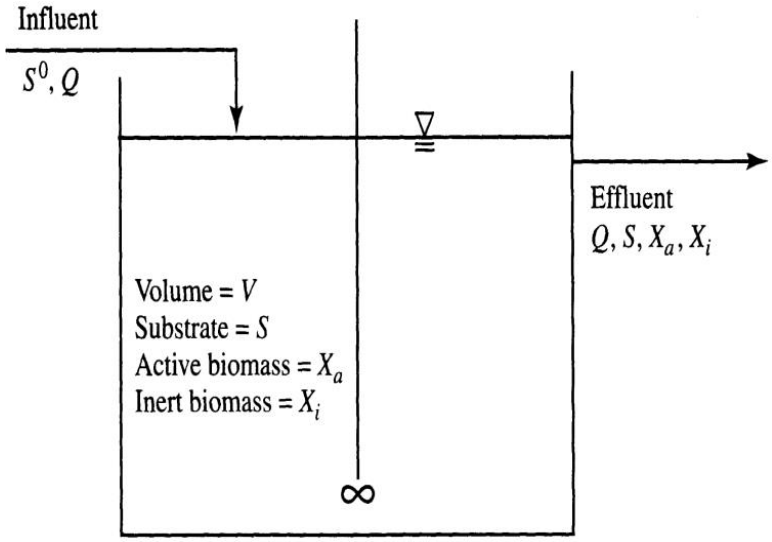
e.g., $< C_5H_7O_2N >$

γ_N = 14 g N/113 g cell VSS = 0.124 g N/g VSS

$\gamma_P = 0.2 * \gamma_N$ =0.025 g P/g VSS

3.6 Nutrients and Electron Acceptors

√ **Mass balance for nutrients in a chemostat**



$$0 = Q C_n^0 - Q C_n + r_n V$$

$$C_n = C_n^0 + r_n \theta$$

C_n^0 : influent concentration of nutrient ($M_n L^{-3}$)

C_n : effluent concentration of nutrient ($M_n L^{-3}$)

r_n : rate of nutrient consumption ($M_n L^{-3} T^{-1}$) (negative value)

- **The supply of nutrients must be supplemented if *Cn* becomes negative.**

3.6 Nutrients and Electron Acceptors

√ Use rate of electron acceptors ($\Delta S_a/\Delta t$)

- Mass balance on electron equivalents expressed as oxygen demand (OD)

$$OD\ inputs = QS^0 + 1.42\frac{gCOD}{gVSS}X_v^0 Q$$

$$OD\ \ outputs = QS + Q(SMP) + 1.42\frac{gCOD}{gVSS}X_v\ Q$$

Table 2.1 Empirical chemical formulas for prokaryotic cells

Empirical Formula	Formula Weight	COD′ Weight	% N	Referer
Mixed Cultures				
$C_5H_7O_2N$	113	1.42	12	1
$C_7H_{12}O_4N$	174	1.33	8	2
$C_9H_{15}O_5N$	217	1.40	6	2
$C_9H_{16}O_5N$	218	1.43	6	2
$C_{4.9}H_{9.4}O_{2.9}N$	129	1.26	11	3

- The e^- acceptor consumption rate as e^- acceptor *equivalents*

$\Delta S_a/\Delta t$ = *OD inputs – OD outputs*

$$\frac{\Delta S_a}{\Delta t} = \gamma_a Q\left[S^0 - S - SMP + 1.42(X_v^0 - X_v)\right]$$

γ_a = 1 g O_2/g COD for oxygen

γ_a = 0.35 g NO_3^--N/g COD for NO_3^--N

O_2 (32 g) + $4H^+$ + $4e^-$ → $2H_2O$

NO_3^- (14 g) + $6H^+$ + $5e^-$ → $1/2N_2$ + $3H_2O$

(14 g /5e^-) / (32 g /4e^-) = 0.35 g NO_3^--N/g O_2

3.6 Nutrients and Electron Acceptors

- The e^- acceptors can be supplied in the influent flow or by other means, such as aeration to provide oxygen (Ra).

$$\frac{\Delta S_a}{\Delta t} = Q[S_a^0 - S_a] + R_a$$

Acceptor consumption rate

R_a: the required mass rate of e^- acceptor supply ($M_a T^{-1}$)
S_a^0 : influent concentration of e^- acceptor ($M_a L^{-3}$)
S_a : effluent concentration of e^- acceptor ($M_a L^{-3}$)

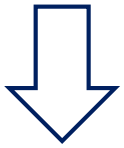
3.7 Input Active Biomass

√ **Three circumstances for inputs of active biomass:**

- **When processes are operated in series, the downstream process often receives biomass from the upstream process.**
- **Microorganisms may be discharged in a waste stream or grown in the sewers.**
- **Bioaugmentation is the deliberate addition of microorganisms to improve performance.**

√ **When active biomass is input, the previous steady-state mass balance for active biomass must be modified.**

$$0 = Y\frac{\hat{q}S}{K+S}X_aV - bX_aV - QX_a$$



$$0 = QX_a^0 - QX_a + Y\frac{\hat{q}S}{K+S}X_aV - bX_aV$$

3.7 Input Active Biomass

$$0 = QX_a^0 - QX_a + Y\frac{\hat{q}S}{K+S}X_aV - bX_aV$$

- The above equation can be solved for S.

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

- The solution for S is exactly the same as the one without X_a^0 input.

However, θ_X is now redefined.
When the SRT is redefined as the reciprocal of the net specific growth rate,

$$\theta_x = \mu^{-1} = \frac{X_aV}{QX_a - QX_a^0}$$

Without X_a^0 input, it was $\theta_x = \frac{VX_a}{QX_a}$

3.7 Input Active Biomass

√ **How does X_a^0 affect S and washout?**

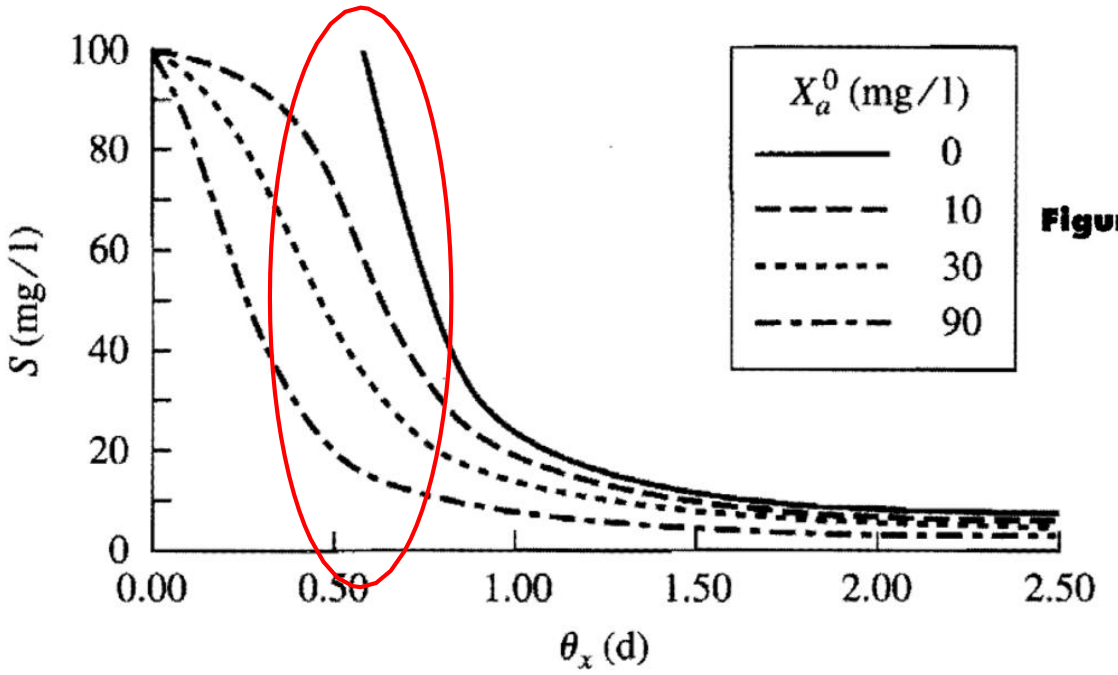


Figure 3.5 Effect of influent active biomass and θ_x on effluent substrate concentration for a chemostat at steady state. The parameters used are $Y = 0.44$ mg VSS_a/mg, $\hat{q} = 5$ mg/mg VSS_a-d, $K = 20$ mg/l, and $b = 0.2$/d.

- X_a^0= 0, washout occurs for θ_x of about 0.6 d.
- As X_a^0 increases, complete washout is eliminated, because the reactor always contains some biomass.
- Increasing X_a^0, also makes S lower, and the effect is most dramatic near washout.

3.7 Input Active Biomass

- **For active biomass,** $0 = QX_a^0 - QX_a + Y\frac{\hat{q}S}{K+S}X_aV - bX_aV$
- **For substrate,** $0 = -\frac{\hat{q}S}{K+S}X_aV + Q(S^0 - S)$
- **For inert biomass** $0 = (1-f_d)bX_aV + Q(X_i^0 - X_i)$

- **Due to the change in definition of θ_x, solutions of the mass balances differ.**

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

$$X_i = X_i^0 + (1-f_d)bX_a\theta$$

$$X_v = X_i + X_a = X_i^0 + (1 + (1-f_d)b\theta)X_a$$

$$\left\{ \frac{\theta_x}{\theta} = \frac{1}{1 - X_a^0/X_a} \right.$$

Homework

Derive X_a, X_i, and X_v as a function of θ_x (or θ_x and S).

3.8 Hydrolysis of Particulate and Polymeric Substrates

√ Organic substrate of particles (large polymers)

- Organic substrates that originate as particles or large polymers take important application in environmental biotechnology.

√ Effect of hydrolysis

- Before the bacteria can begin the oxidation reaction, the particles or large polymers must be hydrolyzed to smaller molecules.
- Extracellular enzymes catalyze these hydrolysis reactions.
- Hydrolysis kinetics is not settled, because the hydrolytic enzymes are not necessarily associated with or proportional to the active biomass.
- The level of hydrolytic enzymes is not established, and the measurement is neither simple.

3.8 Hydrolysis of Particulate and Polymeric Substrates

√ Kinetics of hydrolysis

- A reliable approach is the first-order kinetics.

$$r_{hyd} = -k_{hyd} S_p$$

r_{hyd}: rate of accumulation of particulate substrate due to hydrolysis ($M_s L^{-3} T^{-1}$)
S_p : concentration of the particulate (or large-polymer) substrate ($M_s L^{-3}$)
k_{hyd} : first-order hydrolysis rate coefficient (T^{-1})

- k_{hyd} is proportional to the concentration of hydrolytic enzymes as well as to the intrinsic hydrolysis kinetics of the enzymes
- Some researchers include the active biomass concentration as part of k_{hyd}

$$k_{hyd} = k'_{hyd} X_a$$

(k'_{hyd} = specific hydrolysis rate coefficient)

3.8 Hydrolysis of Particulate and Polymeric Substrates

- **Mass balance on S_p**

$$0 = Q\left(S_p^0 - S_p\right) - k_{hyd} S_p V \quad \Rightarrow \quad S_p = \frac{S_p^0}{1 + k_{hyd}\theta}$$

- θ should not be substituted by θ_x

- The destruction of particulate substrate results in the formation of soluble substrate.
- The steady-state mass balance on soluble substrate

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

Mass balance for substrate

$$0 = (S^0 - S) - \frac{\hat{q}S}{K+S} X_a V/Q + k_{hyd} S_p V/Q$$

S^0 effectively is increased by $k_{hyd} S_p V/Q$

3.8 Hydrolysis of Particulate and Polymeric Substrates

- Other constituents of particulate substrate are also conserved during hydrolysis.
- Good examples of particulate substrate: nutrient nitrogen, phosphate, sulfur

$$r_{hydn} = \gamma_n k_{hyd} S_p$$

r_{hydn}: rate of accumulation of a soluble form of nutrient *n* by hydrolysis ($M_n L^{-3} T^{-1}$)

γ_n: stoichiometric ratio of nutrient *n* in the particulate substrate ($M_n M_s^{-1}$)

3.8 Hydrolysis of Particulate and Polymeric Substrates

√ Example 3.6

EFFECT OF HYDROLYSIS Example 3.1 showed that a chemostat fed 500 mg BOD_L/l with a liquid detention time of 2 d produced an effluent quality of:

$$S = 1.7 \text{ mg } BOD_L/l$$
$$X_a = 161 \text{ mg } VSS_a/l$$
$$X_v = 221 \text{ mg VSS/l}$$
$$\text{SMP} = 32 \text{ mg } COD_p/l$$
$$\text{Soluble } BOD_L = 33.5 \text{ mg } BOD_L/l$$
$$\text{Total COD} = 348 \text{ mg COD/l}$$

We consider now that the influent also contains biodegradable particulate organic matter with a concentration of 100 mg COD/l, and the hydrolysis rate coefficient is $k_{hyd} = 3/d$.

The computations to predict the new effluent quality proceed with the following steps:

1. S_p is computed from Equation 3.59:

$$S_p = \frac{100 \text{ mg COD/l}}{1 + (3/\text{d})(2 \text{ d})}$$
$$= 14 \text{ mg COD/l}$$

2. Since $\theta_x = \theta$ remains 2 d, and no active biomass is input, $S = 1.7$ mg BOD_L/l.
3. The effluent and reactor biomass concentrations are determined in parallel to example 3.1, except that the effective S^0 is now:

$$S^0 = 500 \text{ mg/l} + (100 - 14)\text{mg/l}$$
$$= 586 \text{ mg } BOD_L/l$$

3.8 Hydrolysis of Particulate and Polymeric Substrates

Y of casein (pp. 170)

$$X_a = 0.42 \frac{\text{g VSS}_a}{\text{g BOD}_L}\left((586 - 1.7)\frac{\text{mg BOD}_L}{1}\right)\frac{1}{1.3}$$

$$= 189 \text{ mg VSS}_a/\text{l}$$

$$\leftarrow \quad X_a = \left[Y(S^0 - S)\frac{1}{1 + b\theta_x}\right]\frac{\theta_x}{\theta} \qquad [3.53]$$

$$X_i = 50 \text{ mg VSS}_i/\text{l} + 189\frac{\text{mg VSS}_a}{1}(1 - 0.8)(0.15/\text{d})(2 \text{ d})$$

$$= 61 \text{ mg VSS}_i/\text{l};$$

$$\leftarrow \quad X_i = X_i^0 + (1 - f_d)bX_a\theta \qquad [3.54]$$

$$X_v = X_a + X_i + S_p = 189 + 61 + \frac{14}{1.42} = 264 \text{ mg VSS/l}$$

$$\leftarrow \quad X_v = X_i + X_a \quad [3.55] \quad \rightarrow \quad X_v = X_i + X_a + S_p \quad [3.55]'$$

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 = 9.8 mg COD_p/l
   BAP = 25.3 mg COD_p/l
   SMP = 35.1 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.

1

$$\text{Soluble COD and BOD}_L = S + \text{SMP}$$
$$= 1.7 + 35.1$$
$$= 36.8 \text{ mg COD/l}$$

COD'/weight = the conversion factor (pp. 129, 181)

2

$$\text{Total COD} = S + \text{SMP} + 1.42 \cdot X_v$$
$$= 1.7 + 35.4 + 1.42 \cdot 264$$
$$= 412 \text{ mg COD/l}$$

The biodegradable fraction = 0.8 (pp. 171)

3

$$\text{Total BOD}_L = S + \text{SMP} + 1.42 \cdot f_d \cdot X_a + S_p$$
$$= 1.7 + 35.4 + 1.42 \cdot 0.8 \cdot 189 + 14$$
$$= 266 \text{ mg BOD}_L/\text{l}$$