
\[ X_v = X_i + X_a \quad [1] \]
\[ X_v: \text{ volatile suspended solids concentration (VSS)} \]

\[ X_a = \gamma \left( \frac{S^0 - S}{1 + b\theta_x} \right) \quad [2] \]

\[ Y_a = \gamma \cdot \frac{1 + (1 - f_d) \cdot b \cdot \theta_x}{1 + b \cdot \theta_x} \quad [3] \]

\( f_d \): fraction of active biomass that is biodegradable

Solution:

A **steady-state** mass balance on inert biomass, \( X_i \), is:

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

where

- \( f_d \) = fraction of the active biomass that is biodegradable
- \( b \) = endogenous-decay coefficient (T^{-1})
- \( X_a \) = concentration of active biomass (M\_xL^{-3})
- \( V \) = reactor volume (L^{3})
- \( Q \) = influent (ML^{-3})
- \( X_i \) = the concentration of inert biomass in the chemostat (M\_xL^{-3}) and \( X_i^0 \) = the input concentration of inert biomass (M\_xL^{-3}).

The first term on the right is the production of inert biomass in the reactor while the second term denotes exiting inert biomass. Hence, addition of these two terms
should equal zero in a steady-state process. Solving for \( X_i \) yields

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

Assuming chemostat, hydraulic retention time (HRT) \( \theta \) equals solids retention time (SRT) \( \theta_x \) because any biomass produced in the system must exit in the effluent (\( QX_a \)) and total active biomass is \( VX_a \). This can be written as:

\[
\theta_x = \frac{VX_a}{QX_a} = \theta
\]

Using this equality and equations [1] & [2], \( X_v \) can be computed as

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

The second term on the right-hand side represents the net accumulation of biomass from synthesis and decay which is constituted by the multiplication between the difference in substrate concentration (\( S^0 - S \)) and the net yield (\( Y_n \)) whose definition is given as:

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

II. You are interested in estimating the maximum specific growth rate for bacteria (\( \mu \)) for aerobic oxidation of acetate. You have found that the yield of bacteria is 45 g per gram of acetate. Also, you have determined that when the acetate concentration is 10mg/l the rate of utilization is 4g acetate/g bacteria-d and when the concentration is 15mg/l, the rate of utilization is 6g acetate/g bacteria-d. Estimate \( \mu \). (10 points)

Solution:

\[
r_{ut} = -\frac{\dot{q}S}{K + S} X_a
\]

\[
-r'_{ut} = \frac{r_{ut}}{X_a} = \frac{\dot{q}S}{K + S}
\]

\[
\text{(unit of } r'_{ut} \text{: } \frac{g \text{ acetate}}{l \times d} \div \frac{g \text{ bacteria}}{l} = \frac{g \text{ acetate}}{g \text{ bacteria} \times d})
\]

\[
4 = \dot{q} \frac{10}{K + 10} = \dot{q} \frac{15}{K + 15}
\]
1) Factors on which the success of environmental biotechnology depends
   i) How individual microorganisms with desired characteristics can survive in competition with other organisms.
   ii) How desired functions can be maintained in complex ecosystems.
   iii) How the survival and proliferation of undesired microorganisms can be prevented.

2) Non-destructive analysis techniques of EPS
   i) FT-IR allows users to record the appearance of certain chemical groups on the surface of an internal reflection element exposed to the aqueous phase. This technique is used to study adhesion and biofilm development of bacteria as well as the adsorption of isolated polysaccharides.
   ii) NMR is capable of determining the number of carbohydrate monomers as well as certain non-carbohydrate components in the repeating unit of EPS.
   iii) CLSM employs various types of probes to monitor protein, polysaccharide, and nucleic acid in the biofilm and allows users to study charge distribution, hydrophilicity/phobicity, permeability, and EPS bound molecules in biofilm. Most importantly, 3D imaging helps study detailed structure of biofilm.

3) loosely bound EPS, tightly bound EPS and soluble EPS
   Loosely bound EPS is a loose and dispersible slime layer of extracellular polymeric substances without an obvious edge and typically occupies the outer layer of cell surface.
   Tightly bound EPS is typically found in the inner layer of cell surface which has a certain shape and is bound tightly and stably with the cell
Soluble EPS are weakly bound with cells or dissolved into the solution. Generally, above bound EPSs and soluble EPS can be separated by centrifugation, with those remaining in the supernatant being soluble EPS and those forming microbial pellets being bound EPS.

4) Psychrophile, Barophile

Psychrophile: extremophilic organisms that are capable of growth and reproduction in cold temperatures, ranging from $-15^\circ C$ to $+10^\circ C$.
Barophile: organisms which thrives at high pressures such as deep sea bacteria or archaea.

5) Physical meaning of $K_M$ of the Michaelis-Menten equation.
affinity between the substrate and the enzyme:
- a low value of $K_M$: very strong affinity
- a large value of $K_M$: poor affinity

6) Seven inorganic or organic electron acceptors except oxygen.
nitrate, nitrite, Fe(III), sulfate, carbon dioxide, chlorate, perchlorate,
chromate, selenate, and chlorinated organics (tetrachloroethylene, chlorobenzoate)

7) Aerobic organisms have smaller $f_e^0$, but larger $f_s^0$ than anaerobic organisms. What are the differences between aerobic and anaerobic processes which can be originated from this factor?
In aerobic process, the microorganisms use oxygen for synthesis easily (more spontaneous reaction). Because the Gibbs free energy gap between electron donating and accepting is more than that of anaerobic process. So to get same $f_s$, anaerobic process should need more reactor volume or less HRT.

IV. The following figure shows the effect of influent active biomass and sludge age on effluent substrate concentration for a chemostat at steady state. **(10 points)**
Extract as much of useful information as possible from the figure.
1) $X_a^0 = 0$, washout occurs for $\theta_x$ of about 0.6 d.

2) As $X_a^0$ Increases, complete washout is eliminated, because the reactor always contains some biomass.

3) Increasing $X_a^0$ also makes $S$ lower, and the effect is most dramatic near washout.
2.3 Organic matter is converted in sequential steps by different bacterial species to methane in anaerobic methanogenesis of organic wastes. One important step is the conversion of the intermediate butyrate to acetate, for which the following electron donor and acceptor half-reactions apply: (10 points)

Electron donor:

\[
\frac{1}{2}CH_3CHOO^- + \frac{1}{4}CO_2 + H^+ + e^- = \frac{1}{4}CH_3CH_2CH_2COO^- + \frac{1}{4}HCO_3^- + \frac{1}{4}H_2O
\]

Electron acceptor half-reaction:

\[
H^+ + e^- = \frac{1}{2}H_2
\]

Determine \( \Delta G_r \) for the resulting energy reaction under the following conditions:

1. (a) All constituents are at unit activity
2. (b) All constituents are at unit activity, except pH=7.0
3. (c) The following typical activities under anaerobic conditions apply:

- \([CH_3COO^-]=10^{-3}M\), \([CO_2]=0.3\ \text{atm}\), pH = 7.0
- \([CH_3CH_2CH_2COO^-]=10^{-2}M\), and \([HCO_3^-]=10^{-1}M\).
- \(PH_2(g) = 10^{-6}\ \text{atm}\).

2. Under which of the above three conditions is it possible for bacteria to obtain energy for growth?

Solution:

1. (a) assume unit activity

\[
R_a \rightarrow H^+ + e^- = \frac{1}{2}H_2(g), \Delta G_a^0 = 0\ kJ/e^\text{-eq}
\]

\[
R_d \rightarrow \frac{1}{2}CH_3COO^- + \frac{1}{4}CO_2 + H^+ + e^- = \frac{1}{4}CH_3CH_2CH_2COO^- + \frac{1}{4}HCO_3^- + \frac{1}{4}H_2O
\]
\[ \Delta G^0_d = \frac{1}{4} \Delta G_{\text{butyrate}} + \frac{1}{4} \Delta G_{\text{HCO}_3^-} + \frac{1}{4} \Delta G_{\text{H}_2\text{O}} - \frac{1}{2} \Delta G_{\text{acetate}} - \frac{1}{4} \Delta G_{\text{CO}_2} - \Delta G_{\text{H}^+} - \Delta G_{e^-} \]
\[ = \frac{1}{4} [-352.63] + \frac{1}{4} [-586.85] + \frac{1}{4} [-237.178] - \frac{1}{2} [-369.41] \]
\[ - \frac{1}{4} [-394.359] - 0 - 0 = -10.87 \text{kJ}/e^-\text{eq} \]
\[ \Delta G^0_r = \Delta G^0_d - \Delta G^0_d = 0 - (-10.87) = 10.87 \text{kJ}/e^-\text{eq} \]

(b) at pH = 7, \( \Delta G_{a0'} \) for H\(^+\) is \(-39.87 \text{KJ}/e^-\text{eq} \)
\[ \Delta G_{a0'}^0 = \frac{1}{2} \Delta G_{\text{H}_2} - \Delta G_{\text{H}^+} - \Delta G_{e^-} = 0 - (-39.87) = 39.87 \text{KJ}/e^-\text{eq} \]
\[ \Delta G^0_r = \Delta G^0_{a0'} - \Delta G^0_d = -10.87 - (-36.87) = 29 \text{ KJ}/e^-\text{eq} \]
\[ \Delta G^0_r = \Delta G^0_{a0'} - \Delta G^0_d = 39.87 - (29) = 10.87 \text{KJ}/e^-\text{eq} \]

(c) assume room temp. = 25 oC
\[ \Delta G^0_a = \Delta G^0_a - RT \ln Q = 10.87 + 8.314 \times 289 \times \ln Q \]
\[ Q = \frac{[\text{CH}_3\text{COO}^-]^1[\text{CO}_2]^1[\text{H}_2]^1}{[\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^-]^1[\text{HCO}_3^-]^1[\text{H}_2\text{O}]^4} = 1.315 \times 10^{-4} \]
\[ \Delta G^0_a = \Delta G^0_a - RT \ln Q = -11.20 \text{ KJ}/e^-\text{eq} \]

2. energy can only be obtained under condition (c)
2.14 Write the balanced, overall reaction for the situation in which acetate is the donor and carbon source, nitrate is the acceptor and nitrogen source, and fs = 0.333. (15 points)

Solution:

\[
\begin{align*}
\text{Rd:} & \quad \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- \\
\text{Rc:} & \quad \frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_2H_7O_2N + \frac{11}{28}H_2O \\
\text{Ra:} & \quad \frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O
\end{align*}
\]

\[R = f_eRa + f_s Rc - Rd; \quad f_e = 0.667\]

\[
\begin{align*}
\text{feRa:} & \quad 0.133NO_3^- + 0.800H^+ + 0.667e^- = 0.067N_2 + 0.400H_2O \\
+fsRc & \quad 0.0119NO_3^- + 0.0595CO_2 + 0.345H^+ + 0.333e^- = 0.0119C_2H_7O_2N + 0.131H_2O \\
-Rd & \quad 0.125CH_3COO^- + 0.375H_2O = 0.125CO_2 + 0.125HCO_3^- + H^+ + e^- \\
R: & \quad 0.145NO_3^- + 0.145H^+ + 0.125CH_3COO^- = 0.067N_2 + 0.0119C_2H_7O_2N + 0.156H_2O + 0.0655CO_2 + 0.125HCO_3^-
\end{align*}
\]

3.14 Assume you are treating a wastewater and that q̂ = 10 g COD/g VSSa-d, K = 10 mg COD/l, and b = 0.08 d-1. Say that when operating at θx of 4 d the efficiency of substrate removal is 99 percent and oxygen consumption by the microorganisms is found to be 5,000 kg/d, which is determined to be equivalent to an fe of 0.55. Estimate the oxygen consumption if θx is doubled to 8 d. (15 points)

Solution:

Given:

\[
\hat{q} = 10 \frac{g\text{ COD}}{g\text{ VSS d}} \quad K = 10 \frac{mg\text{ COD}}{L} \quad b = 0.08/d
\]

When θx = 4d, 99% removal

Oxygen consumption = 5000 kg/d (fe = 0.55) & assume f_d = 0.8
\[ O_2 \text{ consumption} = f_e Q(S^0 - S) \approx f_e Q S^0 = \frac{5000 \text{ kg}}{d} \cdot S^0 \gg S = 0.01 S^0 \]

\[ f_s = 1 - f_e = 1 - 0.55 = 0.45 \]

\[ f_s = f_s^0 \frac{1 + (1-f_d) b \theta_x}{1 + b \theta_x} = f_s^0 \frac{1 + 0.2 b \theta_x}{1 + b \theta_x} = 0.45 \]

\[ f_s^0 = \frac{(0.45) \times (1 + 0.08 \times 4)}{1 + 0.2 \times 0.08 \times 4} = 0.56 \]

When \( \theta_x = 8 \),

\[ f_s = 0.56 \frac{1 + 0.2 \times 0.08 \times 8}{1 + 0.08 \times 8} = 0.39 \]

\[ f_e = 0.61 \]

\[ \therefore O_2 \text{ consumption} = 0.61 \times \frac{5000}{0.55} = 5540 \text{ kg/d} \]