Περιβαλλοντική Βιοτεχνολογία- Environmental Biotechnology

Ενότητα 3: Microbial kinetics

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Πολυτεχνική Σχολή

Τμήμα Χημικών Μηχανικών

Introduction

The utilization of microorganisms for environmental applications

- Metabolically active microorganisms catalyze the pollutant-removing reactions. The rate of pollutant removal depends on the concentration of the catalyst, or the active biomass.

- 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.

Basic rate expressions

- In the vast majority of cases, the rate-limiting substrate is the electron donor.

- The microbial kinetic is described by at least 2 **rate expressions**:

    (α) for the growth of the biomass

    (β) for the utilization of the substrate

Specific growth rate of fast-growing bacteria (**Monod, 1949**):

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

This equation is a convenient mathematical representation for a smooth transition from a first-order relation (in low S) to a zero-order relation (in high S).

Basic rate expressions

- The active biomass has an energy demand for maintenance

- Cell functions such as motility, repair and resynthesis, osmotic regulation, transport and heat loss.

- Flow of energy and electrons required for maintenance needs is represented as **endogenous decay**.

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

f$_d$
fraction of the active biomass that is biodegradable

True respiration for energy generation

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

Convertion to inert biomass

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

Basic rate expressions

Net specific growth rate of active biomass:

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

Rate of substrate utilization:

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

Net rate of cell growth :

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

Net specific growth rate of active biomass:

$$\mu = r_{net}/X_a = Y\frac{\hat{q}\,S}{K+S} - b \quad \text{with} \quad \hat{\mu} = Y\hat{q}$$

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

Parameter values

- The parameters describing biomass growth and substrate utilization cannot be taken as "random variables." The values are constrained by the cell's stoichiometry and energetics.

- The true yield **Y** is proportional to f_s^0

- **MAX** values of f_s^0: **0.6 – 0.7 e⁻ eq. cell/ e – eq. donor** (aerobic heterotrophs)

- **MIN** values of f_s^0: **0.05 – 0.1 e⁻ eq. cell/ e – eq. donor** (autotrophs & anaerobes)

$$Y = 0.6 \frac{e^- eq.cells}{e^- eq.donor} \cdot \frac{113\,gVSS}{20\,e^- eq.cells} \cdot \frac{1\,e^- eq.donor}{8\,gBOD_L} = 0.42 \frac{gVSS}{gBOD_L}$$

- The true yield is estimated from **Y = -ΔX/ΔS** using batch experiment in rapidly growing cells, so biomass decay cannot be neglected.

Parameter values

- The maximum specific rate of substrate utilization is controlled largely by electron flow to the electron acceptor.

- For 20°C, the maximum flow to the energy reaction is: **q_e = 1 e⁻ eq/g VSS-d**.

$$\hat{q} = \hat{q}_e / f_e^0 \quad \text{and} \quad \hat{\mu} = Y \hat{q}$$

$$\hat{q}_T = \hat{q}_{20} \left(1.07\right)^{T-20} \quad \text{or} \quad \hat{q}_T = \hat{q}_{T^R} \left(1.07\right)^{T - T^R}$$

- The endogenous decay rate (b) depends on **temperature** and **species type**.

MAX values of **b : 0.1 – 0.3 1/d (at 20°C)** (aerobic heterotrophs)

MIN values of **b < 0.05 1/d** (autotrophs & anaerobes)

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

Parameter values

- The value of biodegradable fraction f_d = 0.8

- 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.

MIN values of **K : µg/l – 1 mg/l**   (for simple electron-donor substrates and when mass-transport is not included)

MAX values of **K : mg/l – 100s mg/l** (for difficult to degrade compounds and when mass-transport resistance is included)

Basic mass balances

Influent

Q , S⁰

Effluent

Q , S, Xₐ, Xᵢ

V, S, Xₐ, Xᵢ
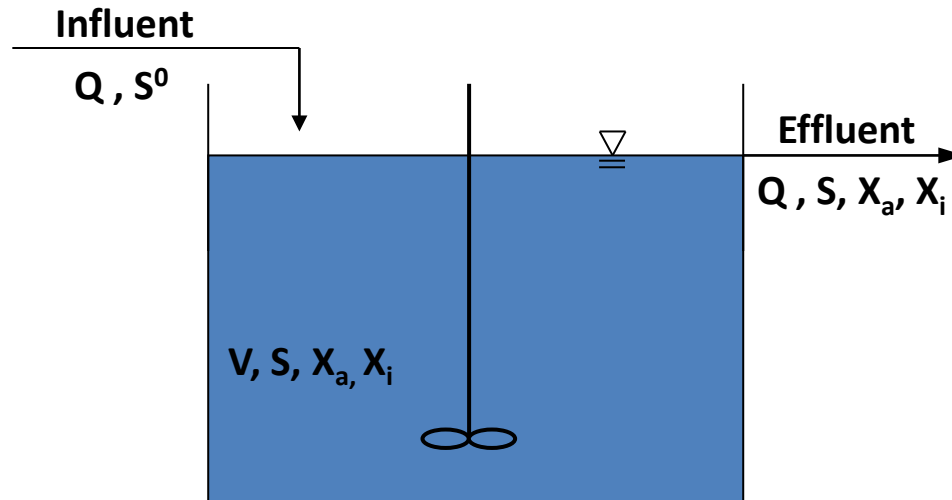
State mass balances :

Active biomass :   **0 = μ Xₐ V - Q Xₐ**

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

Substrate: **0 = rₐ V + Q (S⁰ − S)**

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

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

$$X_{a,ss} = Y\left(S^0 - S\right)\frac{1}{1 + b\left(\dfrac{V}{Q}\right)}$$

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Basic mass balances

Hydraulic retention time : $\theta = V/Q$

Dilution rate : $D = Q/V$

Solids retention time :

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

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

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

$$X_{a,ss} = Y\,\frac{S^0 - S}{1 + b\,\theta_x}$$

for S = S⁰ | **washout**

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

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Mass balances on inert biomass and volatile solids

- Inert solids were produced from self-oxidation of active biomass. In addition real influents often contain refractory volatile suspended solids.

- A steady-state mass balance on inert biomass :

$$0 = (1 - f_d)\, b\, X_a\, V \; + \; Q\, (X_i^0 - X_i)$$

- Volatile solids : $\quad X_v = X_i + X_a$

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

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

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

- Net yield :

$$Y_n = Y \frac{1 + \left(1 - f_d\right) b\,\theta_x}{1 + b\,\theta_x} = Y_{obs} \qquad \longrightarrow \qquad f_s = f_s^0 \frac{1 + \left(1 - f_d\right) b\,\theta_x}{1 + b\,\theta_x}$$

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Mass balances on soluble microbial products

- **Soluble microbial products (SMP)** appear to be cellular components that are released during cell lysis, diffuse through the cell membrane, are lost during synthesis or are excreted for some purpose. Ευθύνονται σε μεγάλο βαθμό για το COD και BOD στην απορροή. SMP also can complex metals, foul membranes and cause color or foaming.

SMP = UAP + BAP

UAP : **substrate utilization – associated products**

Production: **r$_{UAP}$ = - k$_{1 * }$r$_{ut}$**   Utilization:

$$r_{deg-UAP} = -\frac{\hat{q}_{UAP} UAP}{K_{UAP} + UAP} X_a$$

BAP: **biomass – associated products**

Production: **r$_{BAP}$ = k$_{2 * }$X$_a$**   Utilization:

$$r_{deg-BAP} = -\frac{\hat{q}_{BAP} BAP}{K_{BAP} + BAP} X_a$$

Mass balances on soluble microbial products

• **Mass balances for soluble microbial products (SMP)** :

$$0 = -k_1 r_{ut} V - \frac{\hat{q}_{UAP} \, UAP}{K_{UAP} + UAP} X_a V - Q \cdot UAP \qquad 0 = k_2 X_a V - \frac{\hat{q}_{BAP} \, BAP}{K_{BAP} + BAP} X_a V - Q \cdot BAP$$

$$UAP = -\frac{(\hat{q}_{UAP} X_a \vartheta + K_{UAP} + k_1 r_{ut} \vartheta)}{2}$$

$$+ \frac{\sqrt{(\hat{q}_{UAP} X_a \vartheta + K_{UAP} + k_1 r_{ut} \vartheta)^2 - 4 K_{UAP} k_1 r_{ut} \vartheta}}{2}$$

$$BAP = \frac{-(K_{BAP} + (\hat{q}_{BAP} - k_2) X_a \vartheta)}{2}$$

$$+ \frac{\sqrt{(K_{BAP} + (\hat{q}_{BAP} - k_2) X_a \vartheta)^2 + 4 K_{BAP} k_2 X_a \vartheta}}{2}$$

$$r_{ut} = -(S^0 - S) / \vartheta = -\frac{\hat{q} S}{K + S} X_a$$

Mass balances on soluble microbial products

Information to define the soluble microbial products (SMP) kinetic parameters is sparse. Noguera (1991) analyzed aerobic data and obtained the following best-fit values:

$$k_1 = 0.12\, g\, COD_p\, /\, g\, COD_s$$

$$k_2 = 0.09\, g\, COD_p\, /\, g\, VSS_a \cdot d$$

$$\hat{q}_{UAP} = 1.8\, g\, COD_p\, /\, g\, VSS_a \cdot d$$

$$K_{UAP} = 100\, mg\, COD_p\, /\, l$$

$$\hat{q}_{UAP}\, /\, K_{UAP} = 18\, l\, /\, g\, VSS_a \cdot d$$

$$\hat{q}_{BAP} = 0.1\, g\, COD_p\, /\, g\, VSS_a \cdot d$$

$$K_{BAP} = 85\, mg\, COD_p\, /\, l$$

$$\hat{q}_{BAP}\, /\, K_{BAP} = 1.2\, l\, /\, g\, VSS_a \cdot d$$

Noguera et al. (199a) estimated SMP parameters for a methanogenic system:

$$k_1 = 0.21\, g\, COD_p\, /\, g\, COD_s$$

$$k_2 = 0.035\, g\, COD_p\, /\, g\, VSS_a \cdot d$$

$$\hat{q}_{UAP}\, /\, K_{UAP} = 2.4\, l\, /\, g\, VSS_a \cdot d$$

$$\hat{q}_{BAP}\, /\, K_{BAP} = 0.31\, l\, /\, g\, VSS_a \cdot d$$

Nutrients and electron acceptors

A process in environmental biotechnology 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. The electron acceptor is consumed in proportion to electron-donor utilization multiplied by the sum of exogenous and endogenous flows of electron to the terminal acceptor.

Nutrient and acceptor requirements can be determined from the stoichiometric equations developed in previous class. The net yield fraction, f_s, is used to construct the overall reaction including synthesis and endogenous decay. Another approach obtains nutrient requirements directly from the reactor mass balances. This approach is advantageous when materials other than biomass and substrate are important. SMP are key examples of other important materials. In terms of our chemostat model, the rate of nutrient consumption is:

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

Nutrients and electron acceptors

Recall that r_{ut} is the rate of substrate utilization and has a negative sense, which also makes r_n negative. The most important nutrients are N and P. The empirical formula for bacterial VSS, $C_5H_7O_2N$, has γ_N = 14 g N/113 g VSS = 0.124 g N/g VSS. Generally, the P requirement is 20% of the N requirement, which makes γ_P = 0.025 g P/g VSS. An overall mass balance on a nutrient is then:

$$0 = QC_n^0 - QC_n + r_n V$$

in which C_n and C_n^0 are the effluent and influent nutrient concentrations ($M_n L^{-3}$), respectively. Solution for C_n gives:

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

Nutrients and electron acceptors

A parallel means to compute the use rate of electron acceptor (denoted $\Delta S_\alpha / \Delta t$ and having units $M_a T^{-1}$) is by a mass balance on electron equivalents expressed as oxygen demand. For the chemostat, the input of oxygen equivalents or oxygen demand (O.D) includes the electron-donor substrate and the input VSS:

$$O.D.\ inputs = QS^0 + 1.42 \frac{g\ COD}{g\ VSS} X_v^0 Q$$

The outputs are residual substrate, SMP, and all VSS:

$$O.D.\ outputs = QS + Q(SMP) + 1.42 \frac{g\ COD}{g\ VSS} X_v Q$$

Nutrients and electron acceptors

The acceptor consumption as O_2 equivalents is the difference between the inputs and the outputs.

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

in which γ_α is the stoichiometric ratio of acceptor mass to oxygen demand. For oxygen, γ_α = 1 g O_2/g COD; for NO_3^--N, γ_α is 0.35 g NO_3^--N/g COD.
The acceptor can be supplied in the influent flow or by other means, such as aeration to provide oxygen. This can be expressed as:

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

in which S_a and S_α^0 are the effluent and influent concentrations of the acceptor, and R_α is the required mass rate of acceptor supply ($M_\alpha T^{-1}$).

Input active biomass

Whether by design or by happenstance, some biological processes receive significant inputs of biomass active in degradation of the substrate. **Three circumstances** provide practical examples. **First**, when microbial processes are operated in series, the downstream process often receives significant biomass from the upstream process. **Second**, microorganisms may be discharged in a waste stream or grown in the sewers. **Third**, bioaugmentation is the deliberate addition of microorganisms to improve some aspect of process performance.

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

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

The other mass balances remain the same. The above equation can be solved for *S* when the SRT is redefined mathematically to maintain its definition as the reciprocal of the net specific growth rate:

$$S = K\frac{1+b\vartheta_x}{Y\hat{q}\vartheta_x - (1+b\vartheta_x)} \quad where \quad \vartheta_x = \mu^{-1} = \frac{X_aV}{QX_a - QX_a^0}$$

Input active biomass

Although the mass balances for substrate, inert, and volatile biomass remain the same as before, their solutions differ, due to the change in definition of ϑ_x. In particular, mass balance, combining different equations, give:

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

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

$$X_\upsilon = X_i + X_a = X_i^0 + (1 + (1 - f_d)b\vartheta)X_a$$

Input active biomass

The concentration of active biomass is "built up" by input of active biomass, and this "build up" is quantified by the ratio ϑ_x/ϑ.

$$\frac{\vartheta_x}{\vartheta} = \frac{1}{1 - X_a^0 / X_a}$$

When X_α^0 approaches, but is less than X_α, ϑ_x is substantially greater than ϑ. It is even possible to have $X_\alpha^0 > X_\alpha$, if X_α^0 is very large. In such a case, ϑ_x is negative, and the process is in net biomass decay. Supplying a very large X_α^0 is a means to make the steady-state μ negative and sustain $S < S_{min}$.

Once S and X_α are determined from the proper definition of ϑ_x, UAP and BAP concentrations are computed from previous equations, as usual.

Hydrolysis of particulate substrates

Despite its great importance in many situations, hydrolysis has not been thoroughly researched. The best way to represent hydrolysis kinetics is not settled. Part of the problem comes about because the hydrolytic enzymes are not necessarily associated with or proportional to the active biomass, although the active biomass produces them. Exactly what controls the level of hydrolytic enzymes is not established, and their measurement is neither simple, nor has it been carried out frequently in systems relevant to environmental biotechnology. A simple, but reasonably reliable approach for describing hydrolysis kinetics is a first-order relationship with respect to the particulate (or large polymer) substrate:

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

in which

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})

Hydrolysis of particulate substrates

When previous equation is used for the hydrolysis rate, the steady-state mass balance on particulate substrate in a chemostat is:

$$0 = Q(S_p^0 - S_p) - k_{hyd} S_p V$$

in which S_p^0 = the effluent concentration of particulate substrate ($M_s L^{-3}$). Solving the above equation gives:

$$S_p = \frac{S_p^0}{1 + k_{hyd} \vartheta}$$

Here, ϑ represents the liquid detention time and should not be substituted by ϑ_x. The destruction of particulate substrate results in the formation of soluble substrate with conservation of the electron equivalents, or BOD_L. When both substrate types have the same mass measure (generally oxygen demand as a surrogate for electron equivalents), the formation rate of soluble substrate is simply $k_{hyd} S_p$.

Hydrolysis of particulate substrates

Then, the steady-state mass balance on soluble substrate is:

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

Because its shows an additional source of substrate from hydrolysis, S^0 effectively is increased by $k_{hyd}S_p V/Q$; the amount of biomass accumulated should be augmented. Other constituents of particulate substrates also are conserved during hydrolysis. Good examples are the nutrients nitrogen, phosphorus, and sulfur. The formation rate for soluble forms of these nutrients is:

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

in which

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 ($M_n M_s^{-1}$).

Inhibition

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, but the effective kinetic parameters depend on the concentration of the inhibitor.

$$r_{ut,eff} = -\frac{\hat{q}_{eff} S}{K_{eff} + S} X_a$$

$$\mu_{eff} = Y_{eff}(-r_{ut,eff}) - b_{eff}$$

Inhibition

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:

$$\hat{q}_{eff} = \frac{\hat{q}}{1 + \dfrac{S}{K_{IS}}}, \quad K_{eff} = \frac{K}{1 + S / K_{IS}}$$

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.

Inhibition

The second type of inhibition is competitive, and a separate inhibitor is present at concentration I ($M_T L^{-3}$). 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}:

$$K_{eff} = K(1 + \frac{I}{K_I})$$

where K_I = an inhibition concentration of the competitive inhibitor ($M_T L^{-3}$). 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 q_{eff} remains equal to q. Competitive inhibitors usually are substrate analogues.

Inhibition

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 q_{eff}:

$$\hat{q}_{eff} = \frac{\hat{q}}{1 + I / K_I}$$

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.

Inhibition

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

$$\hat{q}_{eff} = \frac{\hat{q}}{1 + I / K_I} \qquad K_{eff} = K(1 + \frac{I}{K_I})$$

Mixed inhibition is a more general form of uncompetitive inhibition in which the K_I values 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:

$$Y_{eff} = \frac{Y}{1 + I / K_I}, \quad b_{eff} = b(1 + I / K_I)$$

References

The images where their origin is not mentioned are derived from the book:

Environmental Biotechnology : Principles and Applications,

Bruce E. Rittmann and Perry L. McCarty,

McGraw-Hill Series in Water Resources and Environmental Engineering

Τέλος Ενότητας

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- Το παρόν εκπαιδευτικό υλικό έχει αναπτυχθεί στο πλαίσιο του εκπαιδευτικού έργου του διδάσκοντα.

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https://eclass.upatras.gr/courses/CMNG2145

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