Advanced Reactor Design

Week 11
Bioreactors

Saba A. Gheni, Ph.D.
Chemical Engineering Department
ghenis@tu.edu.iq

COLLEGE OF ENGINEERING - كلية الهندسة
Tikrit University - جامعة تكريت

Introduction

- Definition of Bioreactors
- Importance in Biotechnology and Industrial Applications
- Role in Scaling Up Biological Processes
- Real-world Examples (e.g., pharmaceuticals, biofuels, waste treatment)

Topics to be Covered

- 1. Basics of Bioreactors
- 2. Types of Bioreactors
- 3. Design and Operating Principles
- 4. Factors Affecting Bioreactor Performance
- 5. Applications in Various Industries

Objectives

- Understand the fundamentals of bioreactors
- Explore different types and their applications
- Learn about key design and operational factors
- Analyze case studies to understand real-world usage

Review: Nonelementary Reaction Kinetics

Nonelementary reaction kinetics

- No direct correspondence between reaction order and stoichiometry
- Result of multiple elementary reaction steps and reactive intermediates (an intermediate so reactive it is consumed as fast as it is formed)

How do we determine the reaction mechanism?

1. Postulate a reaction mechanism that is a series of elementary reactions
2. Derive a rate equation for the postulated mechanism
3. Determine whether the rate eq for the postulated mechanism consistent with the experimental results. If it is, you're done. If they are not consistent, go back to step 1.

Review: Postulating a Reaction Mechanism Based on an Experimentally Observed Rate Law

1. If C_B appears in the denominator of the experimentally observed rate law, then one elementary reaction step is probably:

$$B + A^* \longrightarrow \text{Collision products}$$

where A^* is a reactive intermediate

2. If the denominator contains a constant (by itself, not multiplied by a concentration), then one reaction step is probably:

$$A^* \longrightarrow \text{Decomposition products}$$

3. If the numerator contains a species concentration, then one rxn step is probably:

$$C_{species} [+ \text{ other species?}] \longrightarrow A^* [+ \text{ other products?}]$$

Derive a rate equation for the postulated mechanism and check if it describes the experimentally observed rate equation

This will definitely be on quiz 3!

Review: Deriving a Rate Equation for a Postulated Mechanism

1) Write rate equation for postulated mechanism

$$r_A = (\text{rxns that form A}) - (\text{rxns that consume A})$$

$$-r_A = -\left[(\text{rxns that form A}) - (\text{rxns that consume A})\right]$$

2) For concentrations of reactive intermediates C_{I*} that appear in the rate equation $-r_A$

a) Write out the rate equation for reactive intermediates r_{I*}

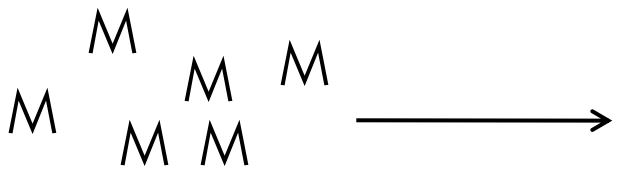
b) Apply Pseudo-Steady State Hypothesis, which states that the net formation of reactive intermediates is zero ($r_{I*}=0$)

c) Solve for C_{I*} in terms of measurable species

d) Substitute the new expression for C_{I*} in terms of measurable species back into $-r_A$

3) Rearrange $-r_A$ to check if it matches the experimentally observed rate equation

Review: Free Radical Polymerizations

monomer  polymer

1. Initiation: $I_2 \xrightarrow{k_0} 2I\bullet$ Initiator (I) decomposes to 2 free radicals

$I\bullet + M \xrightarrow{k_i} R_1\bullet$ ← Radical (1)

2. Propagation: $R_1\bullet + M \xrightarrow{k_p} R_2\bullet$ Chain elongation, new monomers add to chain

$R_j\bullet + M \xrightarrow{k_p} R_{j+1}\bullet$

3. Chain transfer: $R_j\bullet + M \xrightarrow{k_m} P_j + R_1\bullet$

"Live" polymer chain transfers radical to monomer. Polymer chain is no longer reactive (dead). Can also transfer to solvent or other species

4a. Termination by addition: $R_j\bullet + R_k\bullet \xrightarrow{k_{add}} R_{j+k}$

4b. Termination by disproportionation: $R_j\bullet + R_k\bullet \xrightarrow{k_{dis}} R_j + R_k$

Review: PSSH Applied to Thermal Cracking of Ethane

The thermal decomposition of ethane to ethylene, methane, butane and hydrogen is believed to proceed in the following sequence:

Initiation:

$$C_2H_6 \xrightarrow{k_1} 2CH_3\bullet \qquad -r_{1,C_2H_6} = k_1 C_{C_2H_6}$$

Propagation:

$$CH_3\bullet + C_2H_6 \xrightarrow{k_2} CH_4 + C_2H_5\bullet \qquad -r_{2,C_2H_6} = k_2 C_{CH_3\bullet} C_{C_2H_6}$$

$$C_2H_5\bullet \xrightarrow{k_3} C_2H_4 + H\bullet \qquad \boxed{r_{3,C_2H_4} = k_3 C_{C_2H_5\bullet}}$$

$$H\bullet + C_2H_6 \xrightarrow{k_4} C_2H_5\bullet + H_2 \qquad -r_{4,C_2H_6} = k_4 C_{H\bullet} C_{C_2H_6}$$

Termination:

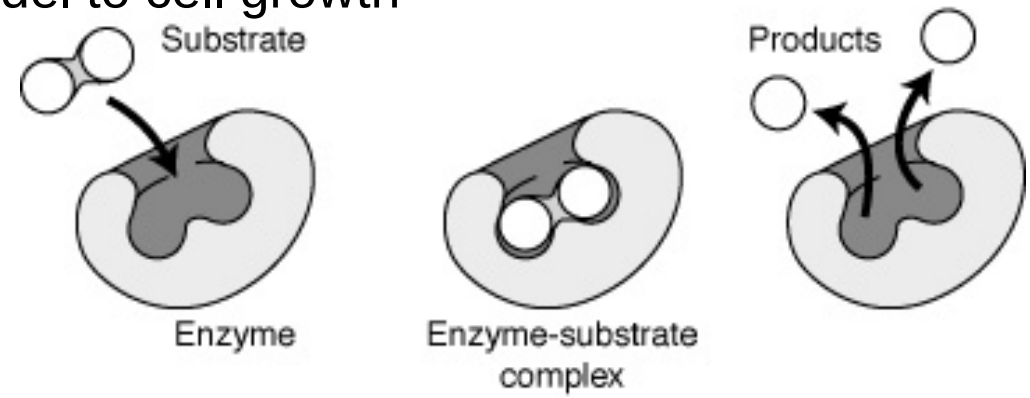
$$2C_2H_5\bullet \xrightarrow{k_5} C_4H_{10} \qquad -r_{5,C_2H_5\bullet} = k_5 \left(C_{C_2H_5\bullet}\right)^2$$

(a) Use the PSSH to derive a rate law for the rate of formation of ethylene

(b) Compare the PSSH solution in Part (a) to that obtained by solving the complete set of differential balances

Bioreactors

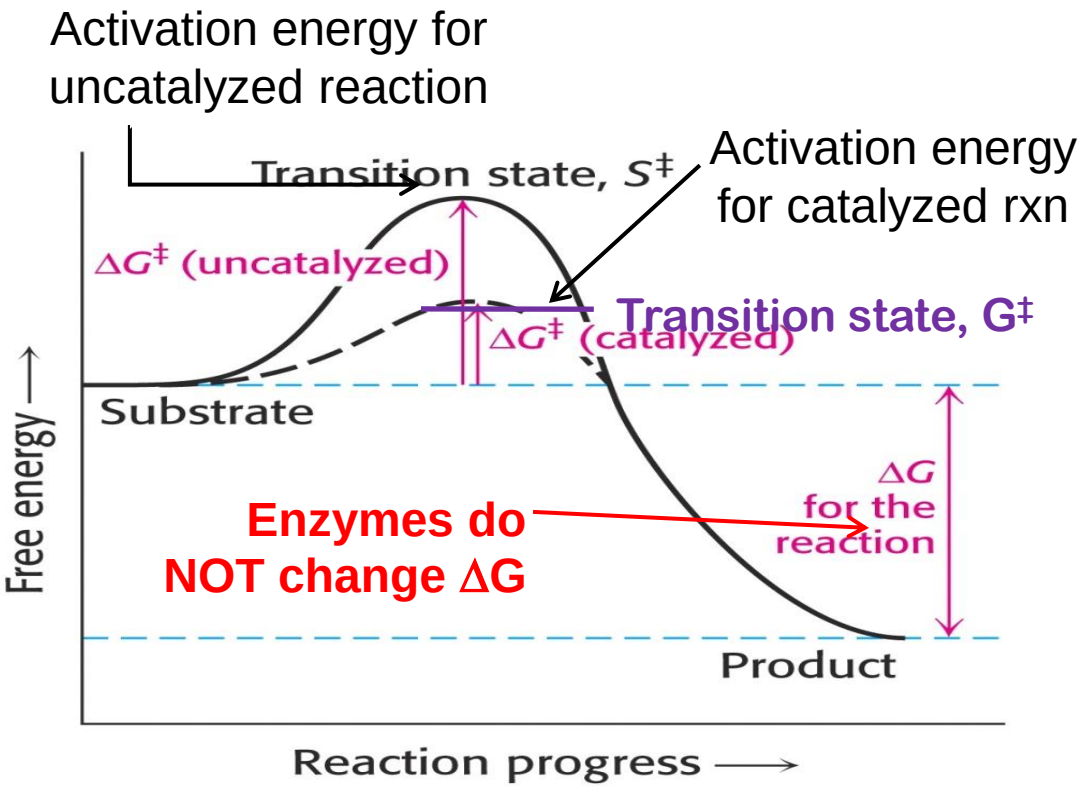
- Today's goals:
  - Predict rates of enzyme-catalyzed reactions
  - Determine effect of chemical inhibitors on rxn rate
  - Develop mathematical expression based on fundamental steps of rxn
  - Apply model to cell growth



- Enzymes: Protein catalyst that execute complex biochemical reactions- all synthetic and degradative reactions in living organisms!
- Increases the rate of reaction without undergoing permanent chemical change – not used up (consumed) by the reaction
- Substrate: the reactant that the enzyme acts on

Enzymes Increase Reaction Rate

- **Effects the reaction rate (kinetics), NOT equilibrium (thermo)**
- Lower activation energy $\Delta G^{\ddagger}$ increases reaction rate, reach equilibrium faster
- ΔG is unchanged, so ratio of products to reactants at equilibrium is the same



$$\text{Kinetic}: A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

$k_{1,cat} > k_{1,uncat}$

$\Delta G^{\ddagger}$ determines rxn rate

$\Delta G^{\ddagger} = -RT \ln(k)$

Enzymes change $\Delta G^{\ddagger}$

Thermodynamic:

$$K = \frac{[C]}{[A][B]} = \frac{k_1}{k_{-1}}$$

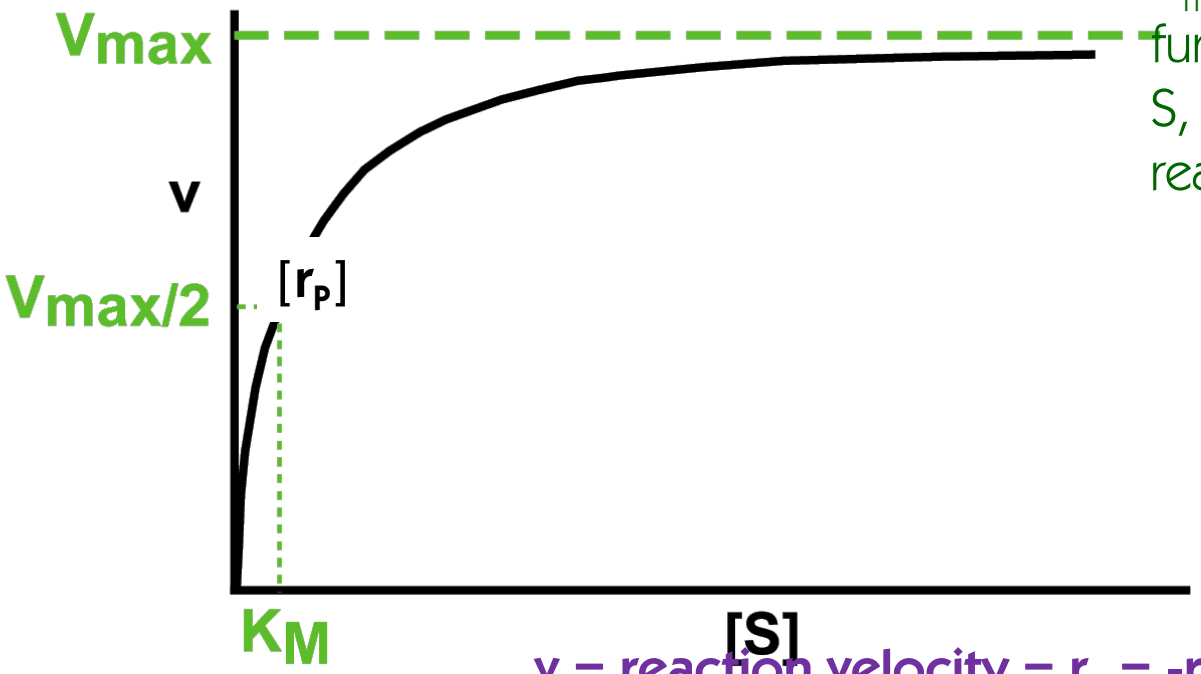
$K_{cat} = K_{uncat}$

ΔG determines equilibrium

$\Delta G = -RT \ln(K)$

Enzymes do NOT change ΔG

Michaelis-Menten (M-M) Equation



V_{max}: maximum reaction rate, further increases in substrate, S, no longer increase the reaction velocity, v

v = reaction velocity = $r_P = -r_S$

K_m = substrate concentration where reaction velocity v = $V_{max}/2$

[S] = substrate concentration [P]: product concentration

Empirically found the Michaelis-Menten equation:

$$v = \frac{V_{max} C_S}{K_m + C_S}$$

Where V_{max} depends on the amount of enzyme

Rate Equation for Enzymatic Reaction

$$v = r_P = \frac{V_{max} S}{K_m + S}$$ Goal: derive this experimentally determined reaction rate

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

E: enzyme S: substrate

ES: enzyme-substrate complex

$$\text{rate of product formation}: \quad v = r_P = \frac{dC_P}{dt} = k_2 C_{ES}$$

We cannot measure C_{ES}, so we need to get C_{ES} in terms of species we can measure. Start by writing the rate equation for C_{ES} :

$$\frac{dC_{ES}}{dt} = k_1 C_S C_E - (k_{-1} + k_2) C_{ES}$$

The free enzyme concentration C_E is also difficult to measure. Use the mass balance to get C_E in terms of C_{ES} and C_{E0}.

$$C_E = C_{E0} - C_{ES} \qquad \text{where } C_{E0} = C_{E,t=0}$$

Substitute into rate eq for C_E:

$$\rightarrow \frac{dC_{ES}}{dt} = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$

C_{ES} in Measurable Quantities

$$\rightarrow \frac{dC_{ES}}{dt} = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$

Pseudo-steady state assumption: ES is a reactive intermediate, so $\frac{d[ES]}{dt} = 0$

$$\frac{dC_{ES}}{dt} = 0 = k_1 C_S (C_{E0} - C_{ES}) - (k_{-1} + k_2) C_{ES}$$ Now solve for C_{ES}

Multiply out and rearrange $\rightarrow k_{-1} C_{ES} + k_2 C_{ES} = k_1 C_S C_{E0} - k_1 C_S C_{ES}$

Bring C_{ES} to left side of equation $\rightarrow k_{-1} C_{ES} + k_2 C_{ES} + k_1 C_S C_{ES} = k_1 C_S C_{E0}$

Factor out C_{ES} $\rightarrow C_{ES} (k_{-1} + k_2 + k_1 C_S) = k_1 C_S C_{E0}$

Divide by quantity in bracket $\rightarrow C_{ES} = \frac{k_1 C_S C_{E0}}{k_{-1} + k_2 + k_1 C_S}$

Divide top & bottom by k_1 $\rightarrow C_{ES} = \frac{C_S C_{E0}}{\frac{k_{-1} + k_2}{k_1} + C_S}$

Plug this expression for C_{ES} into dC_p/dt

Derivation of the M-M Equation

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

E: enzyme S: substrate
ES: enzyme-substrate complex

$$\text{rate of product formation: } v = r_P = \frac{dC_P}{dt} = k_2 C_{ES}$$

$$\rightarrow C_{ES} = \frac{C_S C_{E0}}{\frac{k_{-1} + k_2}{k_1} + C_S}$$

Plug this expression for C_{ES} into dC_p/dt

$$r_P = \frac{dC_P}{dt} = \frac{k_2 C_{E0} C_S}{\frac{k_{-1} + k_2}{k_1} + C_S}$$

Compare to experimentally observed rate eq:

$$v = r_P = \frac{V_{max} C_S}{K_m + C_S}$$

$$V_{max} = k_2 C_{E0}$$

V_{max} occurs when enzyme is fully saturated with S (in ES form)

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

When $C_S >> K_m$, then:

$$r_P = -r_S \approx V_{max}$$

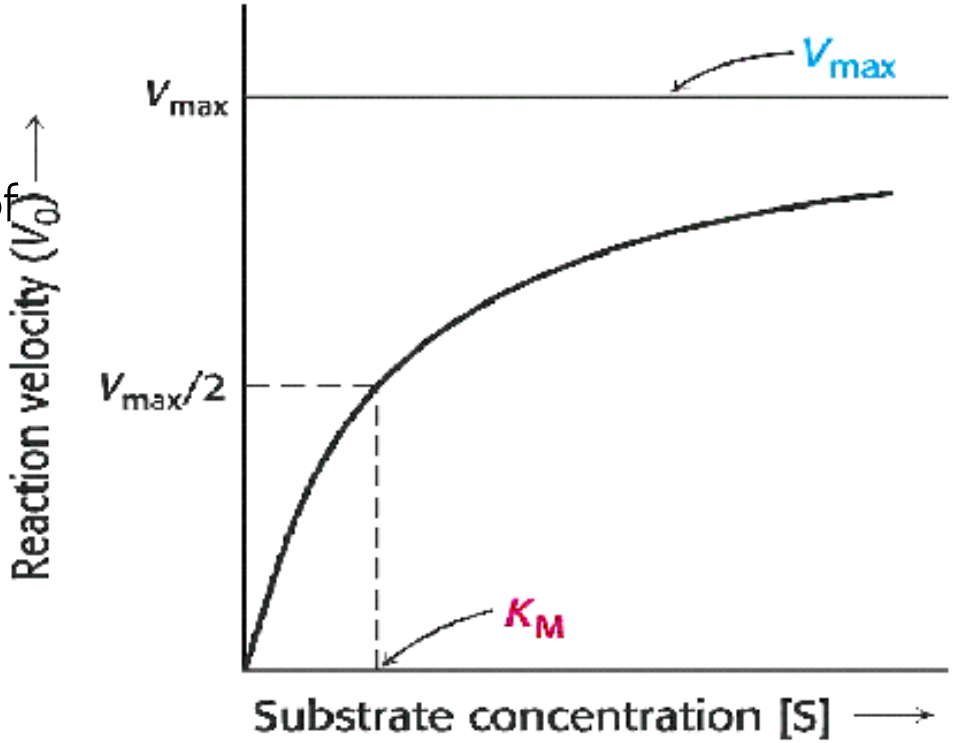
When $C_S << K_m$, then:

$$r_P = -r_S = \frac{V_{max} C_S}{K_m}$$

Complications with Measuring Rates with the M-M Equation

In practice, V_{max} can be difficult to estimate using the MM equation. Everyone reported different values of V_{max}.

Since a solution with infinite concentration of substrate is impossible to make, a different equation was needed.



Lineweaver-Burk Equation

Lineweaver & Burk inverted the MM equation

$$r_P = \frac{V_{max} C_S}{K_m + C_S}$$

$$\rightarrow \frac{1}{r_P} = \frac{K_m + C_S}{V_{max} C_S}$$

$$\rightarrow \frac{1}{r_p} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{C_S}\right) + \frac{1}{V_{max}}$$

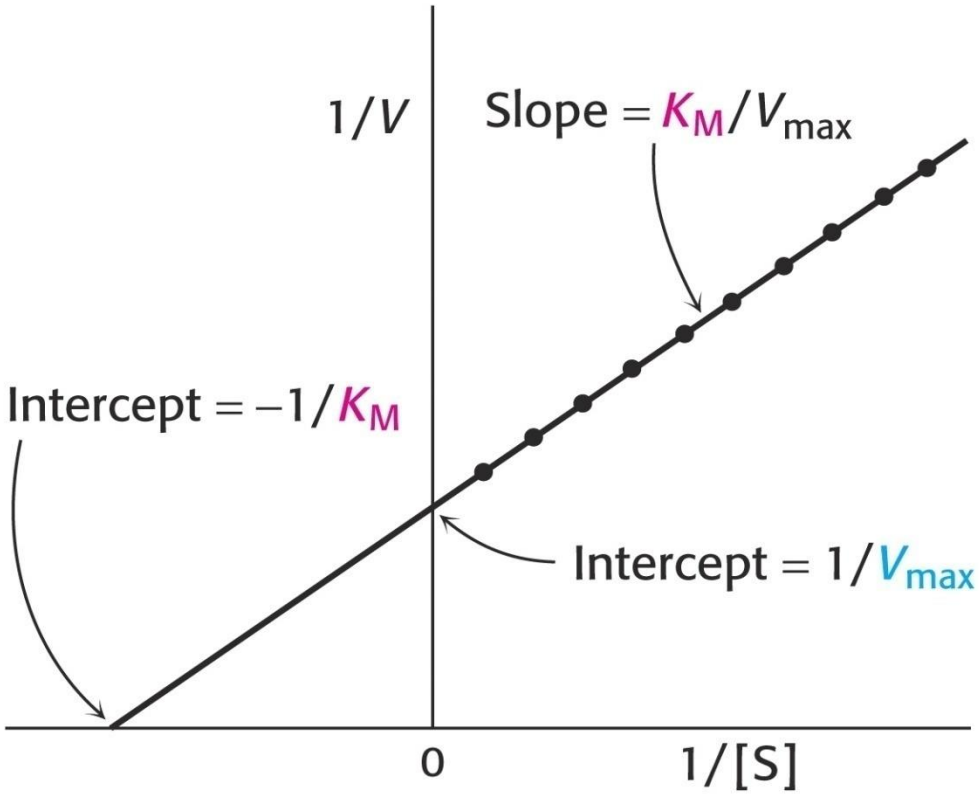
$$y = \quad (m)\ (x) + b$$

By plotting 1/ v vs $1/C_S$, a linear plot is obtained:

Slope = K_m/V_{max}

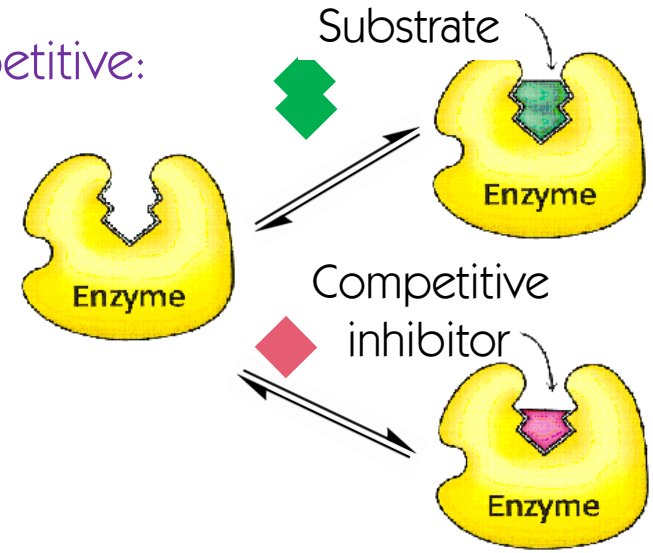
y-intercept = $1/V_{max}$

x-intercept= $-1/K_m$



Types of Reversible Inhibition

1. Competitive:



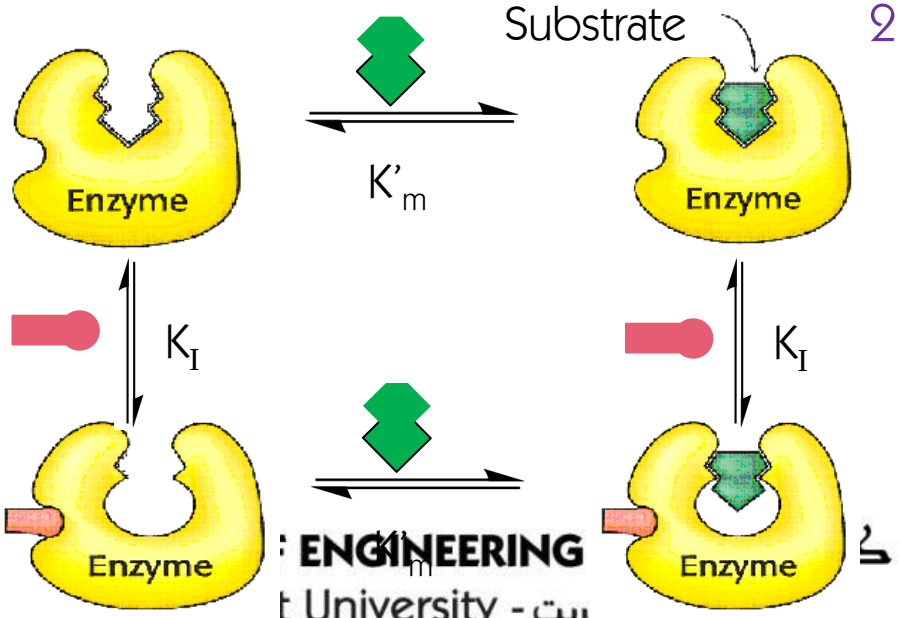
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \rightleftharpoons EI \quad (K_I)$$

I is the inhibitor

- Binds to active site & blocks substrate binding
- Reduces the C_{Enzyme} available for binding

2. Noncompetitive



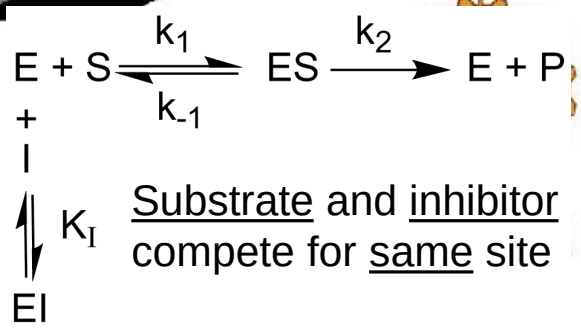
$$E + S \underset{}{\overset{K'_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \rightleftharpoons EI \quad (K_I) \qquad ES + I \rightleftharpoons ESI \quad (K_I)$$

$$EI + S \overset{K'_m}{\rightleftharpoons} ESI$$

- Inhibitor binds to some other site
- Does not affect substrate binding

1. Competitive Inhibition



Substrate and inhibitor compete for same site

Competitive inhibition

$$r_p = \frac{V_{max} C_S}{K_m\left(1+\frac{C_I}{K_I}\right)+C_S}$$

vs **No inhibition**

$$r_p = \frac{V_{max} C_S}{K_m + C_S}$$

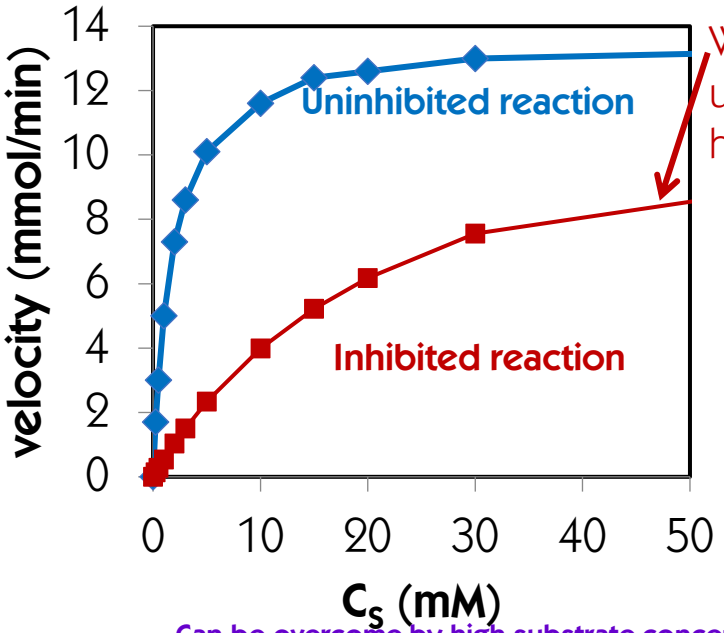
$K_{m,\,app} > K_m$

$V_{max,\,app} = V_{max}$

K_m observed w/ competitive inhibitor →

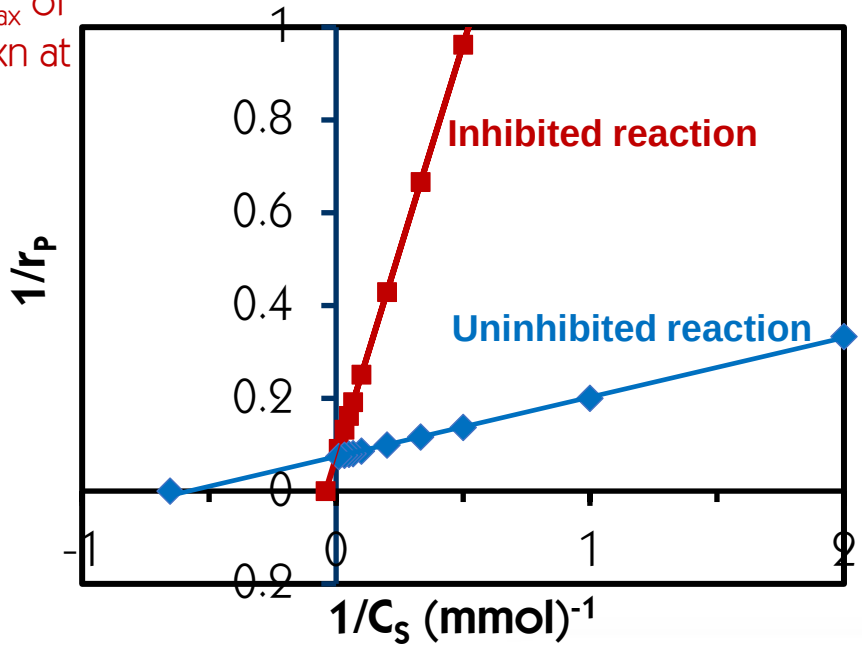
$$K_{m,app} = K_m\left(1+\frac{[I]}{K_I}\right)$$

$$\frac{1}{r_P} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{C_S}\right) + \frac{1}{V_{max}}$$



Will reach V_{max} of uninhibited rxn at high C_S

Can be overcome by high substrate concentration

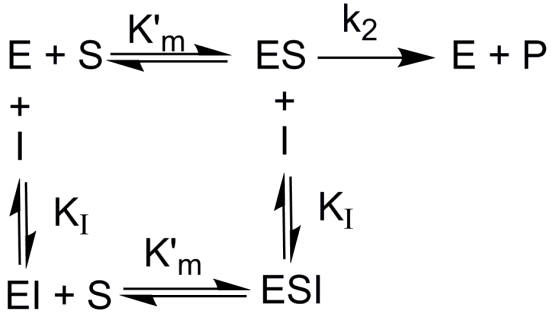


Slope = K_m/V_{max} y-int = $1/V_{max}$ x-int= $-1/K_m$

COLLEGE OF ENGINEERING - كلية الهندسة

Tikrit University - جامعة تكريت

2. Noncompetitive Inhibition

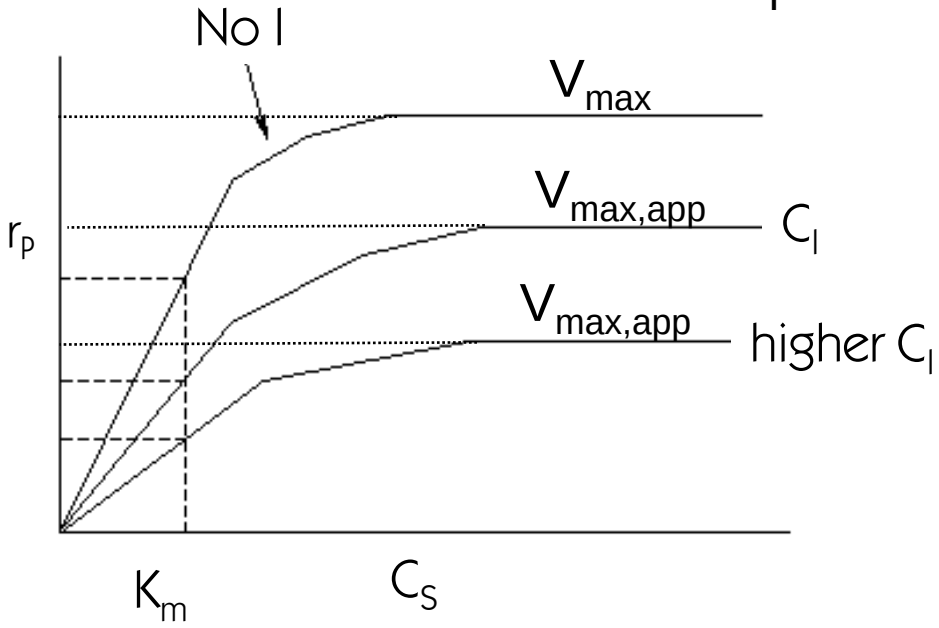


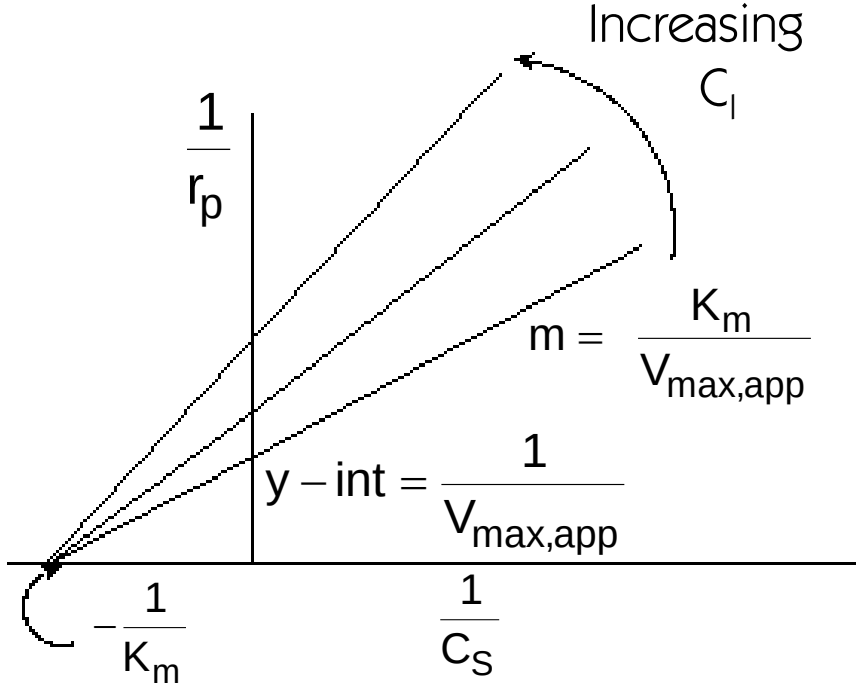
Competitive inhibition **No inhibition**

$$v = r_P = \frac{(V_{max}/(1+C_I/K_I))C_S}{C_S + K_m} \quad \text{vs} \quad r_p = \frac{V_{max}C_S}{K_m + C_S}$$

V_{max} observed w/ noncompetitive inhibitor → $V_{max,app} = \dfrac{V_{max}}{1+\dfrac{C_I}{K_I}}$

substrate and inhibitor bind different sites



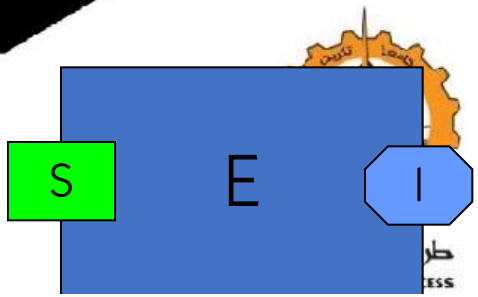


$V_{max,app} < V_{max}$

$K_{m,app} = K_m$

$$\frac{1}{r_P} = \frac{K_m}{V_{m,app}}\left(\frac{1}{C_S}\right) + \frac{1}{V_{m,app}}$$

3. Uncompetitive Inhibition



$$v = r_p = \frac{(V_{max}/(1+C_I/K_I))C_S}{C_S + [K_m/(1+C_I/K_I)]} \text{ vs } r_p = \frac{V_{max}C_S}{K_m + C_S}$$

substrate & inhibitor bind different sites but I only binds after S is bound

$$V_{max,app} = \frac{V_{max}}{\left(1+\frac{C_I}{K_I}\right)}$$

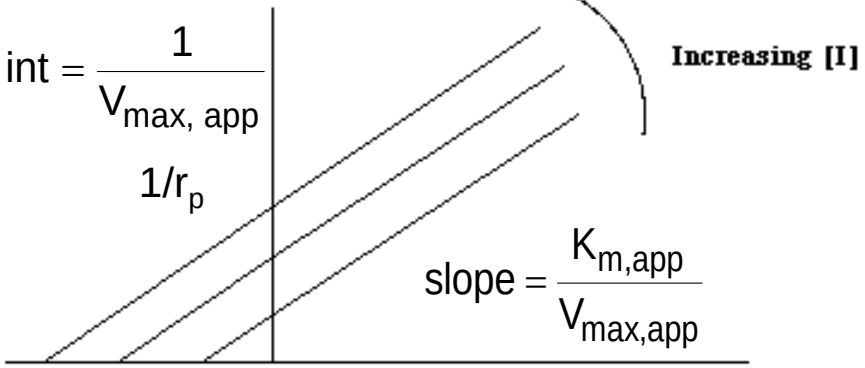
$$K_{m,app} = \frac{K_m}{\left(1+\frac{C_I}{K_I}\right)}$$

$$E + S \underset{}{\overset{K'_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$ES + I \overset{K_I}{\rightleftharpoons} ESI$$

$V_{max,\,app} < V_{max}$

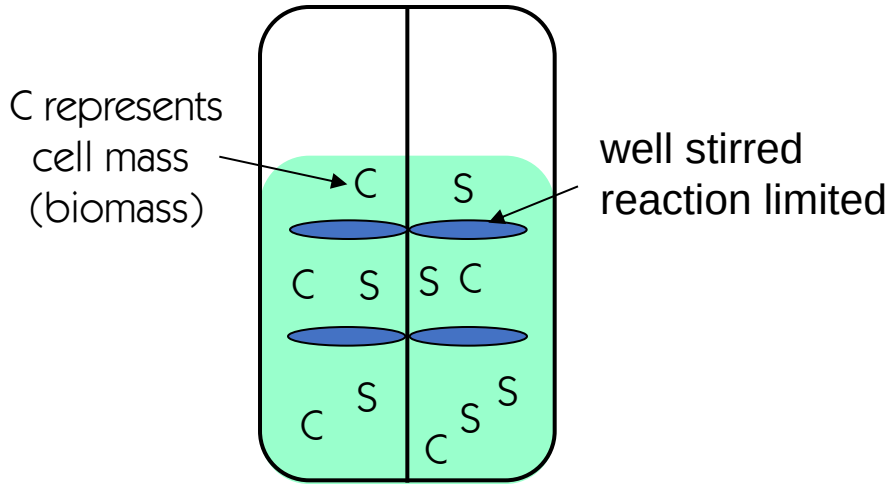
$K_{m,\,app} < K_m$

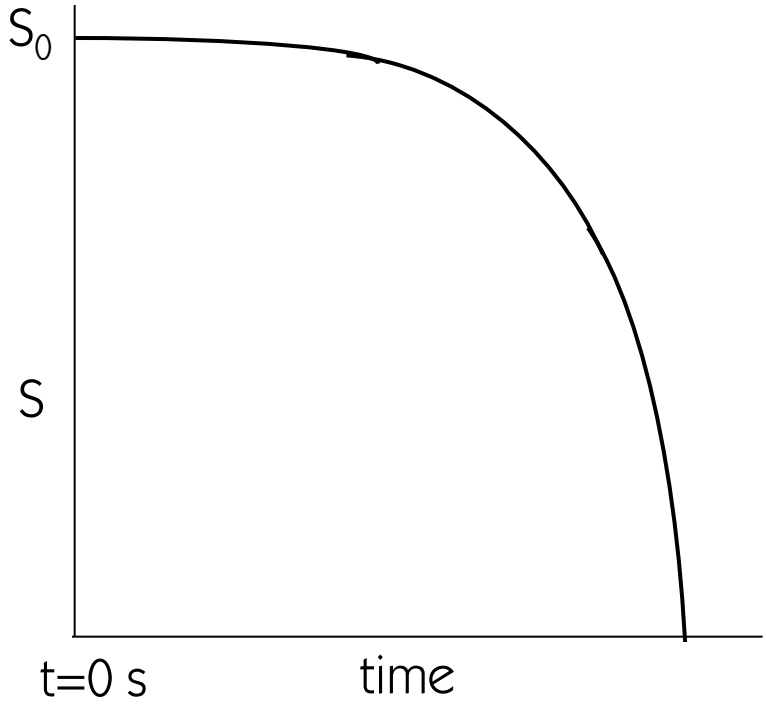


$$\frac{1}{r_P} = \frac{K_{m,app}}{V_{max,app}}\left(\frac{1}{C_S}\right) + \frac{1}{V_{max,app}}$$

$$\text{x-int} = -\frac{1}{K_{m,\,app}}$$

Batch Bioreactor or Fermentor





Batch Reactor

In a reactor with substrate at concentration C_{S0} and C_C =0

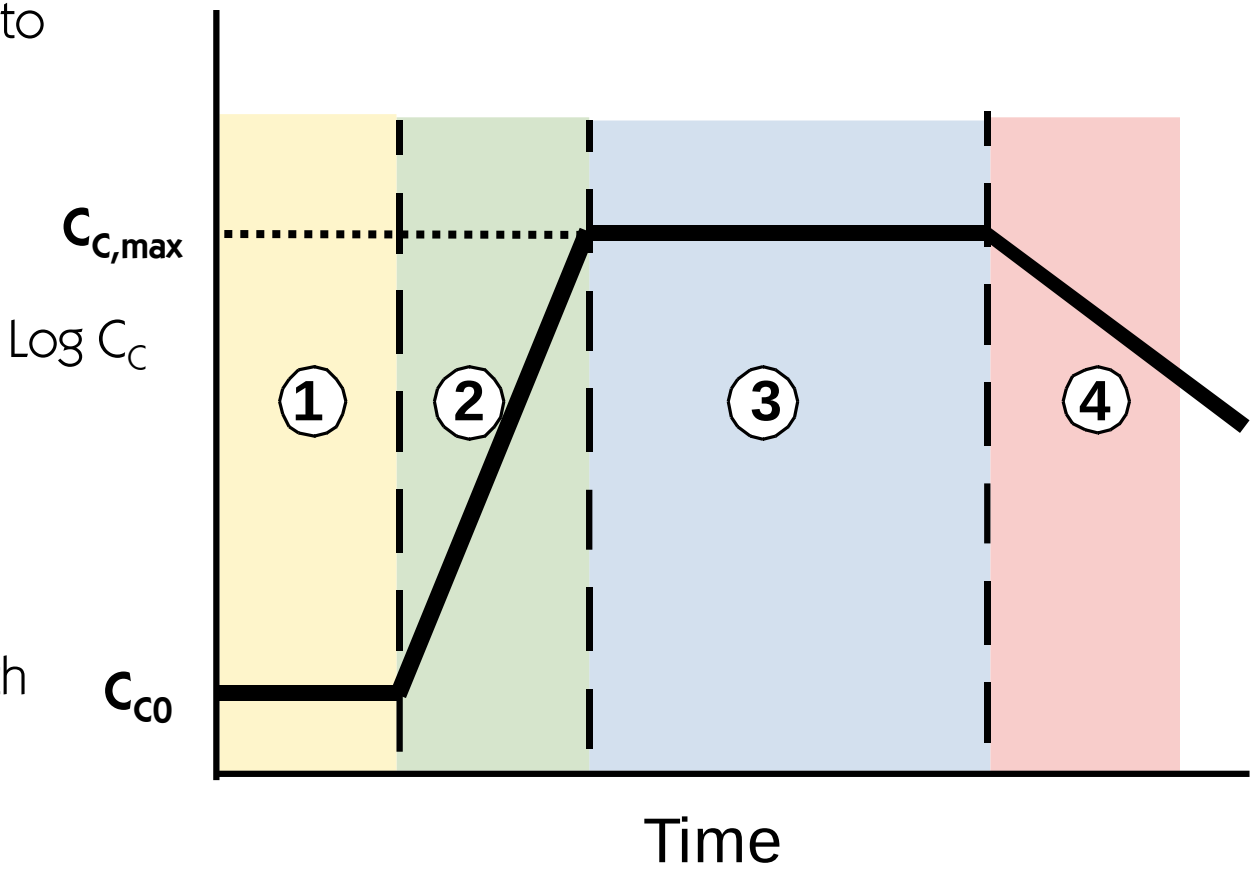
add cells at concentration C_{C0} at t = 0

add no more S or cells after t = 0

monitor C_S and C_C with time

Kinetics of Microbial Growth (Batch or Semi-Batch)

- **Region 1: Lag phase**
  - microbes are adjusting to the new substrate
- **Region 2: Exponential growth phase**
  - microbes have acclimated to the conditions
- **Region 3: Stationary phase**
  - limiting substrate or oxygen limits the growth rate
- **Region 4: Death phase**
  - substrate supply is exhausted



Quantifying Growth Kinetics

- Relationship of the specific growth rate to substrate concentration exhibits the form of saturation kinetics
- Assume a single chemical species, S, is growth-rate limiting
- Apply Michaelis-Menten kinetics to cellular system→ called the **Monod equation**

$$\text{Monod equation: } r_g = C_C \frac{\mu_{max} C_S}{K_s + C_S}$$

- μ_{max} is the maximum specific growth rate when $S >> K_s$
- C_S is the substrate concentration
- C_C is the cell concentration
- K_s is the saturation constant or half-velocity constant. Equals the rate-limiting substrate concentration, S, when the specific growth rate is ½ the maximum
- Semi-empirical, experimental data fits to equation, assumes that a single enzymatic reaction, and therefore substrate conversion by that enzyme, limits the growth-rate

Monod Model (1942) – Nobel Prize

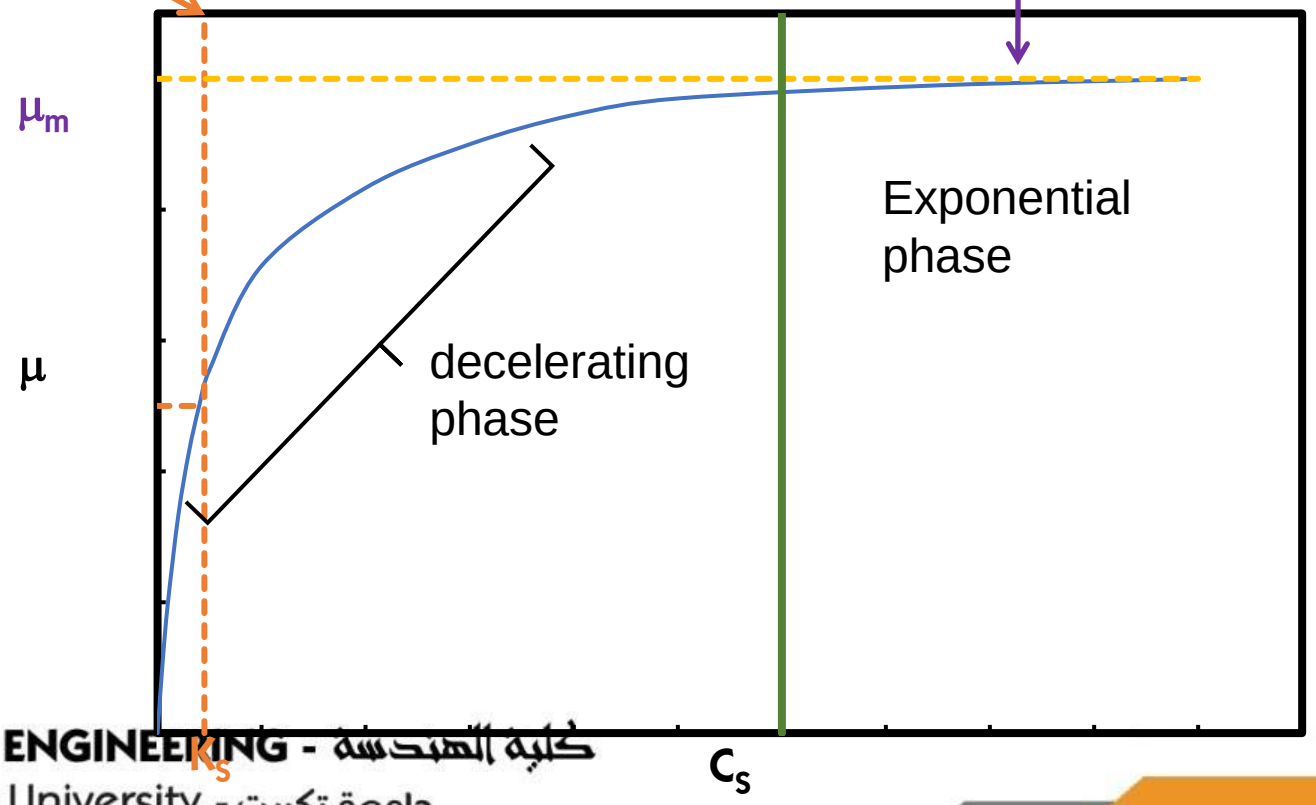
First-order kinetics:

$C_S \ll K_S \rightarrow r_g = C_C \frac{\mu_m C_S}{K_S}$

$r_g = C_C \frac{\mu_m C_S}{K_S + C_S}$

Zero-order kinetics:

$C_S >> K_S \rightarrow r_g = \mu_m$



Overall balance for cells growing on carbohydrate with products:

$$CH_mO_n + a\,O_2 + b\,NH_3 \rightarrow c\,CH_\alpha O_\beta N_\delta + d\,CH_xO_yN_z + e\,H_2O + f\,CO_2$$

Carbohydrate (can be any organic material)

Nitrogen source

Cell material (biomass)

Product

Individual elemental balances:

1) Carbon: $1 = c + d + f$
2) Hydrogen: $m + 3b = c\alpha + dx + 2e$
3) Oxygen: $n + 2a = c\beta + dy + e + 2f$
4) Nitrogen: $b = c\delta + dz$

Yield Coefficients

Cell yield for substrate: $Y_{C/S} = -\frac{\Delta C}{\Delta S}$ (ΔC ← cell mass formed; ΔS ← substrate consumed)

Cell yield for O_2: $Y_{C/O_2} = -\frac{\Delta C}{\Delta O_2}$ (ΔC ← cell mass formed; ΔO_2 ← oxygen consumed)

Product yield for substrate: $Y_{P/S} = -\frac{\Delta P}{\Delta S}$ (ΔP ← product mass formed; ΔS ← substrate consumed)

Summary

- Bioreactors are essential in scaling biological processes
- Various types and designs suit different industrial needs
- Understanding operational factors ensures efficiency
- Critical in pharmaceuticals, biofuels, and environmental management