7. Reaction Mechanisms, Pathways, Bioreactions and Bioreactors

○ Active Intermediates and Free Radicals
  - Pseudo-steady-state-hypothesis
  - Reaction pathway

○ Enzymes
  - Michealis-Menten enzyme kinetics
  - Enzyme inhibition

○ **Bioreactors**

○ **Pharmacokinetics**

○ **Polymerization**

2. Enzymes XVIII

○ **Types of Enzyme Inhibition 10**

- Substrate inhibition

- **most substrate acts as an inhibitor**

$$S + E \bullet S \longrightarrow S \bullet E \bullet S \quad (\text{Inactive})$$

- **incompetitive reaction, I → S**

$$-r_S = \frac{V_{\max} S}{K_M + S + \dfrac{S^2}{K_I}}$$

- **at low conc. of substrate**

$$K_M >> (S + \frac{S^2}{K_I}) \qquad\qquad -r_S \sim \frac{V_{\max} S}{K_M}$$

2. Enzymes XIX

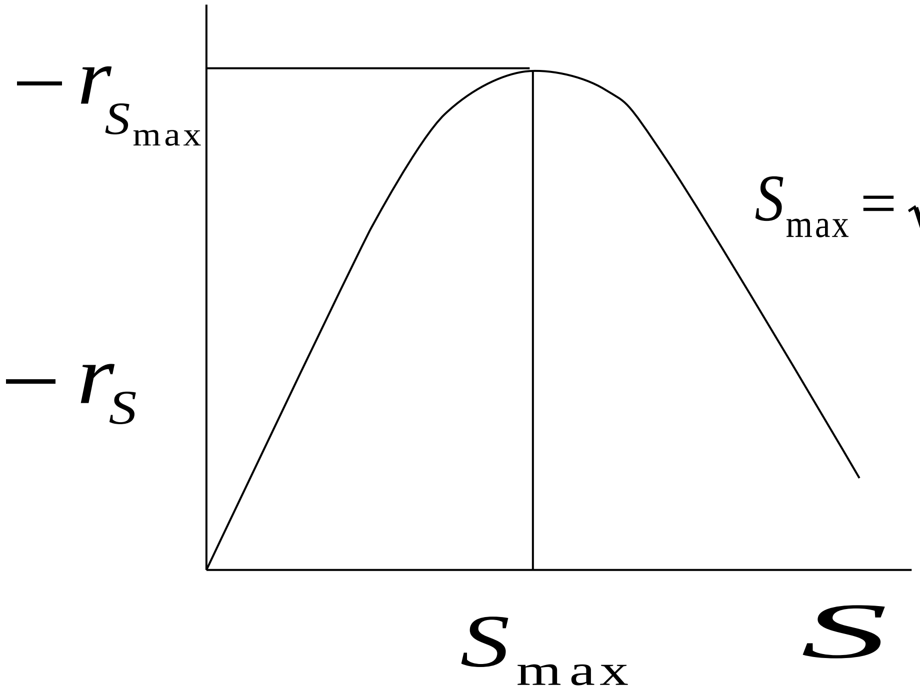
○ **Types of Enzyme Inhibition 11**

- Substrate inhibition 2

- **at high conc. of substrate**

$$K_M + S << \left(\frac{S^2}{K_I}\right)$$

$$-r_S = \frac{V_{\max} K_I}{S}$$



$$S_{\max} = \sqrt{K_M K_I}$$

3. Bioreactors I

○ **Bioreactor**

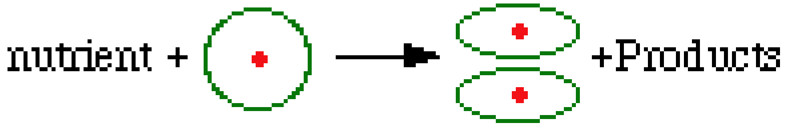
- **A reactor that sustains and supports life for cells and tissue cultures**
- **Cell metabolism**
  - **transformation of chemical energy**
  - **construction, breakdown, digestion of cellular components**
- **Microbial growth**
  - **enzyme rxns are involved during growth**
- **Monod equation ☞ similar to MM equation**

※ Bioproduct market USD 16 billion

3. Bioreactors II

○ **Bioconversion**

- **Advantages**
  - **mild reaction conditions**
  - **high yields**
  - **enzyme acts as stereo-specific catalyst**
- **Bacteria as living chemical factory**
  - **recombinant DNA**



$$\text{Nutrient} \xrightarrow{\text{cells}} \text{more cells} + \text{Product}$$

3. Bioreactors III

○ Cell growth

I. Lag phase

- adjusting new environment

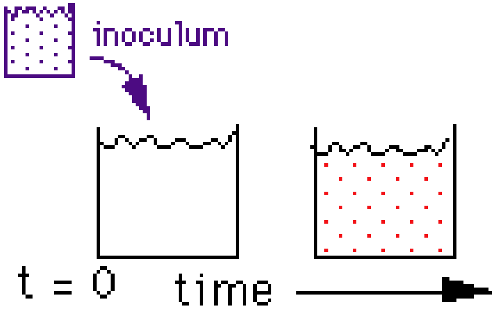
II. Exponential growth phase

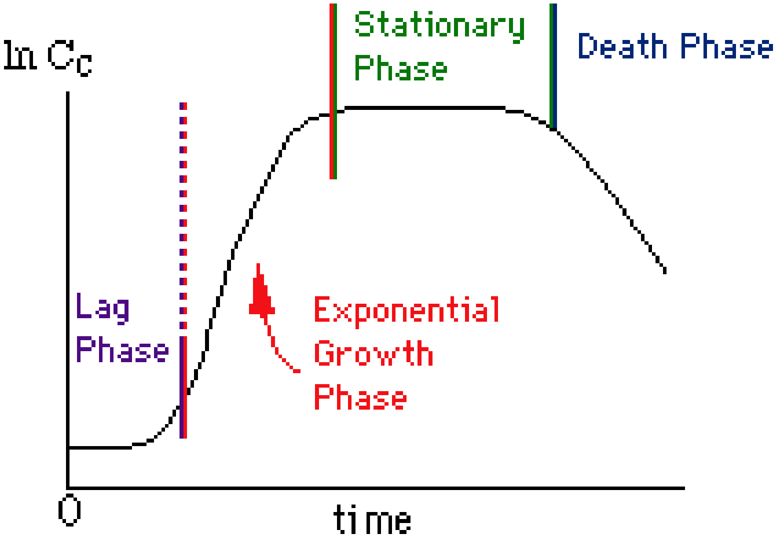
- dividing at the maximum rate

III. Stationary phase

- cells reach minimum biological space
- useful products

IV. Death phase





3. Bioreactors IV

○ **Rate Laws**

Cells + Substrate → More cells + Product

- Monod equation

$$r_g = \mu C_C$$

$$\mu = \mu_{\max} \frac{C_S}{K_S + C_S}$$

$$\boxed{r_g = \frac{\mu_{\max} C_S C_C}{K_S + C_S}}$$

- In general

$$r_g = k_{OBS} \left(\frac{\mu_{\max} C_S}{K_S + C_S} \right) C_C$$

$$k_{OBS} = \left(1 - \frac{C_P}{C_P^*} \right)^n$$

CP*= Where Product concentration at which all metabolism ceases.

3. Bioreactors V

- **Stoichiometry**

  - Yield coefficients

$$Y_{C/S} = \frac{\text{Mass of new cells formed}}{\text{Mass of substrate consumed}} = -\frac{\Delta C_C}{\Delta C_S}$$

with $Y_{C/S} = 1/Y_{S/C}$

$$Y_{P/S} = \frac{\text{Mass of product formed}}{\text{Mass of substrate consumed}} = -\frac{\Delta C_P}{\Delta C_S}$$

  - Maintenance

$$m = \frac{\text{Mass of substrate consumed for maintenance}}{\text{Mass of cells} \cdot \text{Time}}$$

3. Bioreactors VI

- **Stoichiometry 2**
  - **Rate of substrate consumption for maintenance**

$$\boxed{r_{sm} = mC_c}$$

  - **When maintenance can be neglected**

$$\boxed{C_c = Y_{c/s}[C_{s0} - C_s]}$$

  - **Total substrate consumed is to form new cells (C) and product (P)**

$$S \xrightarrow{cells} Y'_{c/s}C + Y'_{p/s}P$$

3. Bioreactors VII

○ **Stoichiometry 3**

- Substrate utilization

- Substrate balance

- **during the growth phase ⇒ lumped into** $Y_{s/c}$

$$\boxed{-r_s = Y_{s/c} r_g + mC_c}$$

3. Bioreactors VIII

○ **Stoichiometry 4**

- Substrate balance 2

- **corresponding rate of product formation**

$$\boxed{r_p = Y_{p/c} r_g}$$

- **for stationary phase, nutrient for growth virtually exhausted**
- **a different nutrient is used for cell maintenance**

⇒ produce the desired product

$$\boxed{r_p = \frac{k_p C_{sn} C_c}{K_{sn} + C_{sn}}}$$

3. Bioreactors IX

- Stoichiometry 5
  - Substrate balance 3
    - net rate of secondary nutrient consumption

$$-r_{sn} = mC_c + Y_{sn/p} r_p$$

$$= mC_c + \frac{Y_{sn/p} k_p C_{sn} C_c}{K_{sn} + C_{sn}}$$

- the desired product can be obtained with no cell growth stage ⇒ concern about the change of secondary nutrient

$$C_p = Y_{p/s}[C_{sn0} - C_{sn}]$$

3. Bioreactors X

○ **Stoichiometry 6**

- **Two limiting situations for substrate consumption to cell growth and product formation**
  - **product formed only during growth phase**
  - **product formed only during stationary phase**

☞ **Luedeking-Piret equation**

$$q_p = \underset{\text{growth}}{\underset{\downarrow}{\alpha}}\mu_g + \beta \rightarrow \text{non-growth}$$

with $r_p = q_p C_c$

3. Bioreactors XI

○ **Mass Balance**

- Counting the number of living cells

- Counting the mass of living cells

3. Bioreactors XII

○ **Mass Balance**

- Batch operation

- **cell** $V\dfrac{dC_c}{dt}=r_gV-r_dV$ $\qquad \dfrac{dC_c}{dt}=r_g-r_d$

- **substrate** $V\dfrac{dC_c}{dt}=r_sV=Y_{s/c}(-r_g)V-mC_cV$

$$\boxed{\frac{dC_c}{dt}=Y_{s/c}(-r_g)-mC_c}$$

- **in stationary phase, no growth**

$$\boxed{V\frac{dC_s}{dt}=-mC_cV+Y_{s/p}(-r_p)V}$$

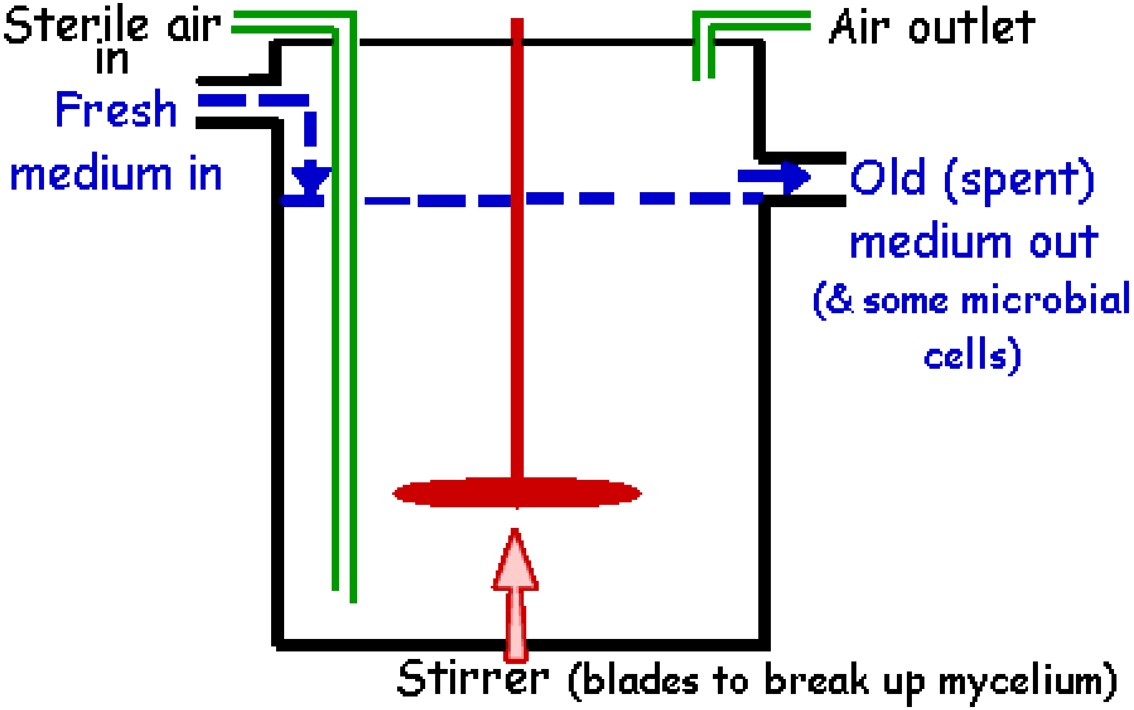
3. Bioreactors XIII

- Mass Balance 2
  - Batch operation
    - product

$$V\frac{dC_p}{dt} = r_p V = Y_{p/s}(-r_s)V$$

3. Bioreactors XIV

- **Chemostats**



3. Bioreactors XV

- **Design Equations**
  - **Dilution rate** $D = \frac{v_0}{V}$
  - **Mass balance (CSTR)**
  - **Monod equation**

$$r_g = \mu C_c = \frac{\mu_{\max} C_s C_c}{K_s + C_s}$$

3. Bioreactors XVI

○ **Design Equations 2**

- **Steady state operation**

$$DC_c = r_g - r_d$$

$$D(C_{s0} - C_s) = -r_s$$

- **Neglecting the death rate**

$$F_c = C_c v_0 = r_g V = \mu C_c V$$

- **Divide by C_cV** $\boxed{D = \mu}$

☞ **specific growth rate of the cell can be controlled by varying D**

3. Bioreactors XVII

- Design Equations 3
  - Steady state operation 2
    - solving for steady state substrate conc.

$$C_c = \frac{DK_s}{\mu_{\max} - D}$$

- single nutrient limiting, substrate consumed to cell growth only, cell maintenance neglected

$$-r_s = r_g Y_{s/c} \qquad C_c = Y_{c/s}(C_{s0} - C_s)$$

$$C_c = Y_{c/s}\left[C_{s0} - \frac{DK_s}{\mu_{\max} - D}\right]$$

3. Bioreactors XVIII

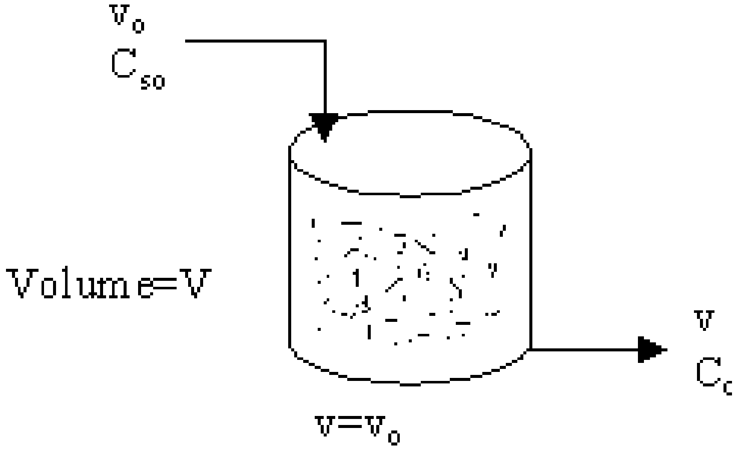
- **Wash out**
  - **Neglect death rate and cell maintenance**
  - **Steady state**

$$0 = -\upsilon C_C + r_g V$$

$$D C_C = r_g = \frac{\mu_{max} C_S}{K_S + C_S} C_C = \mu C_C$$

$$D = \mu = \frac{\mu_{max} C_S}{K_S + C_S}$$

$$C_S = \frac{D K_S}{\mu_{max} - D}$$



$$C_C = Y_{C/S}\left[C_{SO} - C_S\right] = Y_{C/S}\left[C_{SO} - \frac{D K_S}{\mu_{max} - D}\right]$$

3. Bioreactors XIX

- **Wash out 2** $$D_W = \frac{\mu_{max} C_{SO}}{K_S + C_{SO}}$$

- Maximum production rate

- **dividing by the reactor volume, V, which is constant**

production rate = $\dot{m}_c = v_o C_C$

$$\frac{\dot{m}}{V} = DC_C$$

- **substituting for C_C**

$$DC_C = DY_{C/S}\left(C_{SO} - \frac{DK_s}{\mu_{max} - D}\right)$$