Reaction Mechanism, Bioreaction and Bioreactors

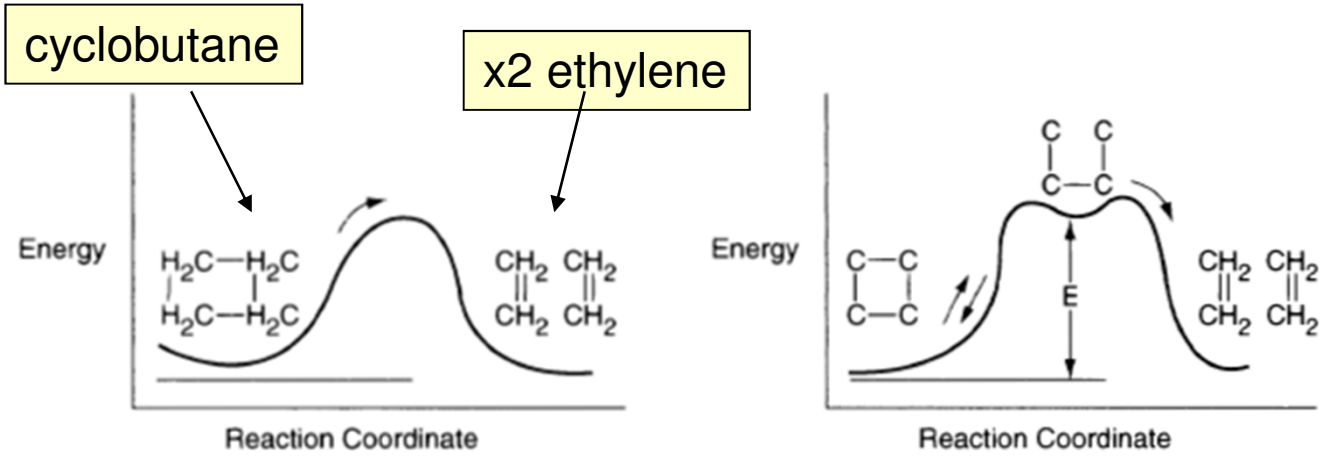
Lecture 9

Active Intermediates

- Many reaction proceed via formation of active intermediate by collision or interaction with other molecules

$$A + M \rightarrow A^{*} + M$$

- The idea was suggested in 1922 by F.A.Lindermann , acitve intermediates were experimentally observed using femtosecond spectroscopy by A.Zewail (Nobel Prize 1999)



Pseudo –Steady-State Hypothesis (PSSH)

- Decomposition of the intermediate doesn't occur instantaneously, activated species have finite life time
- Active intermediates react as fast as they are formed, so their net rate of formation is zero:

$$r_{A^*} = \sum_{i=1}^{N} r_{iA^*} \equiv 0$$

Pseudo –Steady-State Hypothesis (PSSH)

- Gas-phase decomposition of azomethane into ethane and N_2:

$$(CH_3)_2 N_2 \rightarrow C_2H_6 + N_2$$

- experimentally found to follow
  - 1st order at pressures above 1atm $r_{C_2H_6} \propto C_{AZO}$
  - 2nd order below 50mmHg $r_{C_2H_6} \propto C^2_{AZO}$

Activation (1) $A + M \xrightarrow{k_1} A^* + M$

Deactivation (2) $A^* + M \xrightarrow{k_2} A + M$

Decomposition (3) $A^* \xrightarrow{k_3} P$

Pseudo –Steady-State Hypothesis (PSSH)

- Suggested mechanism:

$$(CH_3)_2N_2 + (CH_3)_2N_2 \xrightarrow{k_{1AZO^*}} (CH_3)_2N_2 + [(CH_3)_2N_2]^*$$

$$[(CH_3)_2N_2]^* + (CH_3)_2N_2 \xrightarrow{k_{2AZO^*}} (CH_3)_2N_2 + (CH_3)_2N_2$$

$$[(CH_3)_2N_2]^* \xrightarrow{k_{3AZO^*}} C_2H_6 + N_2$$

- the rate laws

$$r_{1AZO^*} = k_{1AZO^*} C^2_{AZO}$$

$$r_{2AZO^*} = -k_{2AZO^*} C_{AZO^*} C_{AZO}$$

$$r_{3AZO^*} = -k_{3AZO^*} C_{AZO^*}$$

$$r_{AZO^*} = r_{1AZO^*} + r_{2AZO^*} + r_{3AZO^*} \equiv 0$$

Pseudo –Steady-State Hypothesis (PSSH)

- solving for C_{azo^*} and finding the rate of formation of product:

$$r_{C_rH_6} = \frac{k_1 k_3 C^2_{AZO}}{k_2 C_{AZO} + k_3}$$

- at low concentrations

$$k_2 C_{AZO} \ll k_3 \quad r_{C_rH_6} = k_1 C^2_{AZO}$$

- at high concentrations

$$k_2 C_{AZO} \gg k_3 \quad r_{C_rH_6} = \frac{k_1 k_3}{k_2} C_{AZO} = k C_{AZO}$$

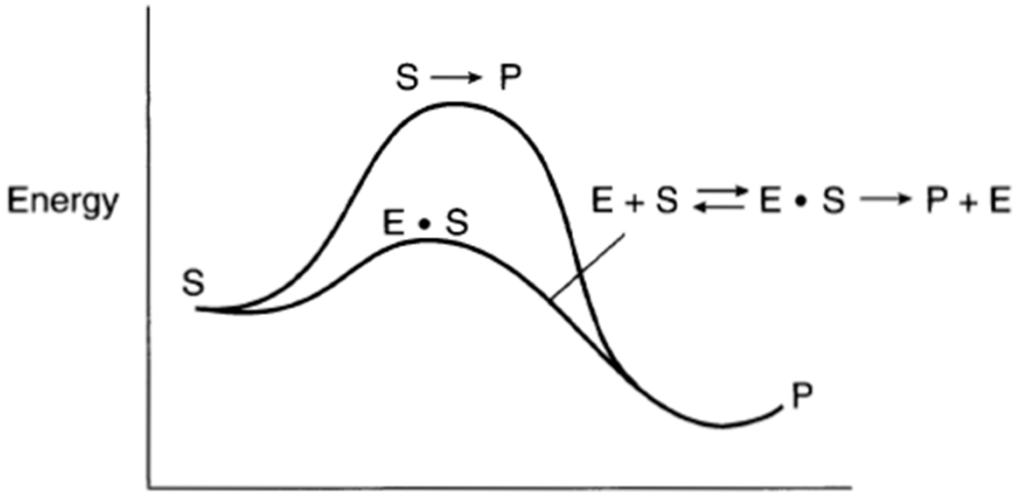
Pseudo –Steady-State Hypothesis (PSSH)

- Experimentally finding the mechanism:

$$r_{C_rH_6} = \frac{k_1 C^2_{AZO}}{k' C_{AZO} + 1}$$

- Rule of thumb for developing the mechanism:
  - species having the concentration appearing in the denominator probably collide with the active intermediate
  - species having the concentration appearing in the numerator probably produce the active intermediate

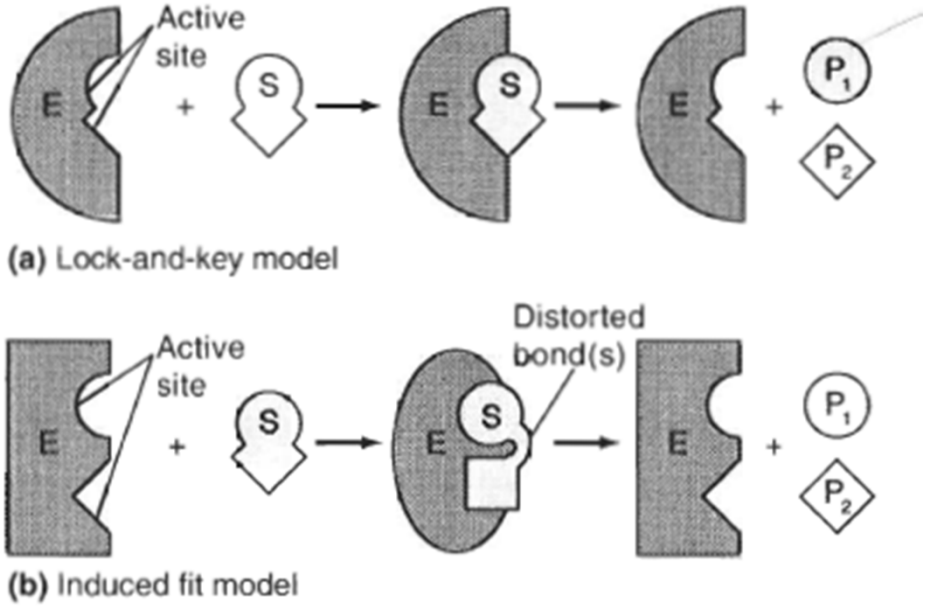
Enzymatic reactions



- lower activation energies for enzymatic pathways lead to enormous enhancement in reaction rates
- enzymes are highly specific: one enzyme can usually catalyze only one type of reaction
- enzymes usually work at mild conditions; at extreme temperatures or pH may unfold losing its activity

Enzymatic reactions

- Two models for enzyme-substrate interaction: the ***lock-and-key*** and the ***induced fit***:



both the enzyme molecule and the substrate molecule are distorted therefor **stressing** and **weakining** the bond for rearrengement

Enzymatic reactions

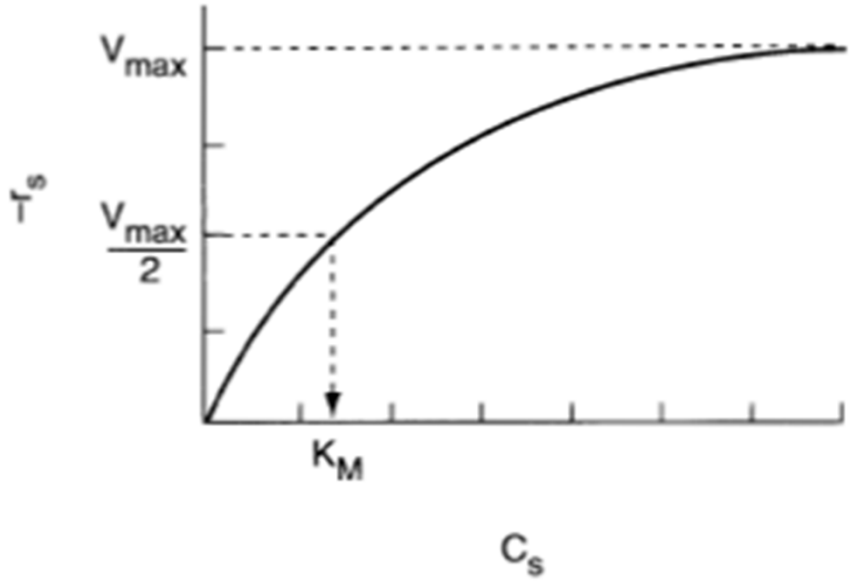
- There are six classes of enzymes
  1. Oxidoreductases $AH_2 + B + E \rightarrow A + BH_2 + E$
  2. Transferases $AB + C + E \rightarrow AC + B + E$
  3. Hydrolases $AB + H_2O + E \rightarrow AH + BOH + E$
  4. Isomerases $A + E \rightarrow isoA + E$
  5. Lyases $AB + E \rightarrow A + B + E$
  6. Ligases $A + B + E \rightarrow AB + E$

Michaelis-Menten equation

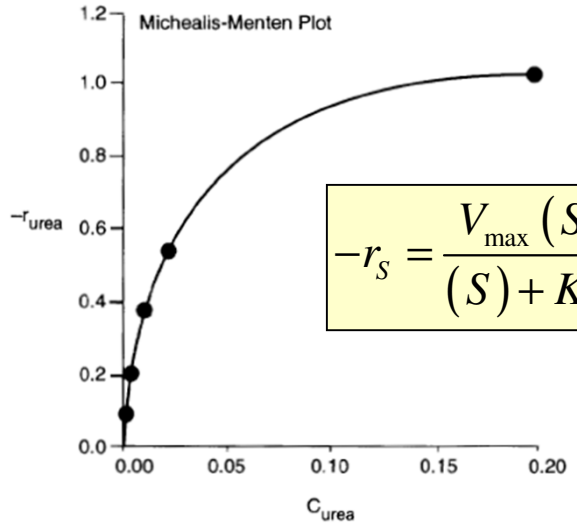
$$-r_S = \frac{k_{cat}(E_t)(S)}{(S)+K_M}$$

- k_{cat} (s^{-1})– the turnover number: the number of substrate molecules converted in a given time on a single enzyme molecule when saturated with the substrate
- K_m (mol/l) – Michaelis constant or affinity constant: measure of attraction of the enzyme to the substrate

$$-r_S = \frac{V_{\max}(S)}{(S)+K_M}$$

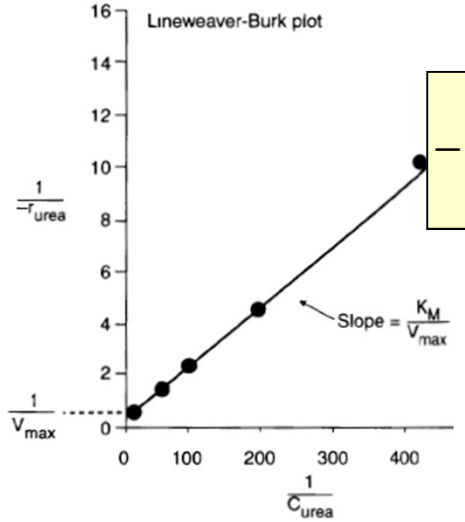


Evaluation of Michaelis-Menten parameters



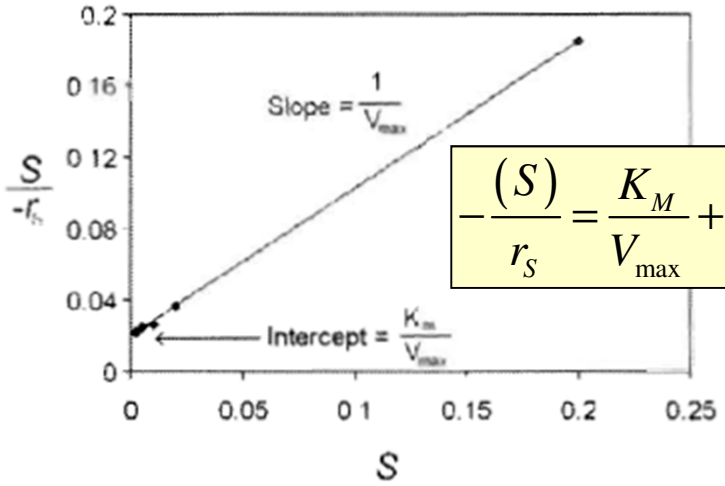
$$-r_S = \frac{V_{\max}(S)}{(S)+K_M}$$

- Michaelis-Menten plot



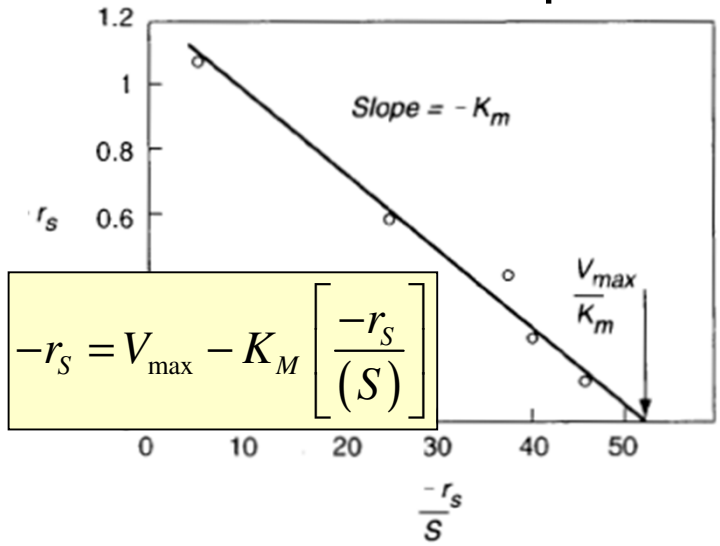
$$-\frac{1}{r_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\left[\frac{1}{(S)}\right]$$

- Lineweaver-Burk plot



$$-\frac{(S)}{r_S} = \frac{K_M}{V_{\max}} + \frac{1}{V_{\max}}(S)$$

- Hanes-Woolf plot
(better V_{max})



$$-r_S = V_{\max} - K_M\left[\frac{-r_S}{(S)}\right]$$

- Eadie-Hofstee plot
(doesn't bias low concentraion points

Batch reactor calculations

- mole balance on urea:

$$-\frac{dN_{urea}}{dt} = -r_{urea}V \quad \Rightarrow \text{in liquid} \quad -\frac{dC_{urea}}{dt} = -r_{urea}$$

- combining with the Michaelis-Menten law

$$-r_{urea} = \frac{V_{\max} C_{urea}}{C_{urea} + K_M} \quad \Rightarrow \quad t = \int_{C_{urea}}^{C_{urea0}} \frac{dC_{urea}}{-r_{urea}} = \int_{C_{urea}}^{C_{urea0}} \frac{C_{urea} + K_M}{V_{\max} C_{urea}} dC_{urea}$$

- after integration, in terms of conversion

$$C_{urea} = C_{urea0}(1 - X) \qquad t = \frac{K_M}{V_{\max}} \ln \frac{1}{1 - X} + \frac{C_{urea0} X}{V_{\max}}$$

Briggs-Haldane equation

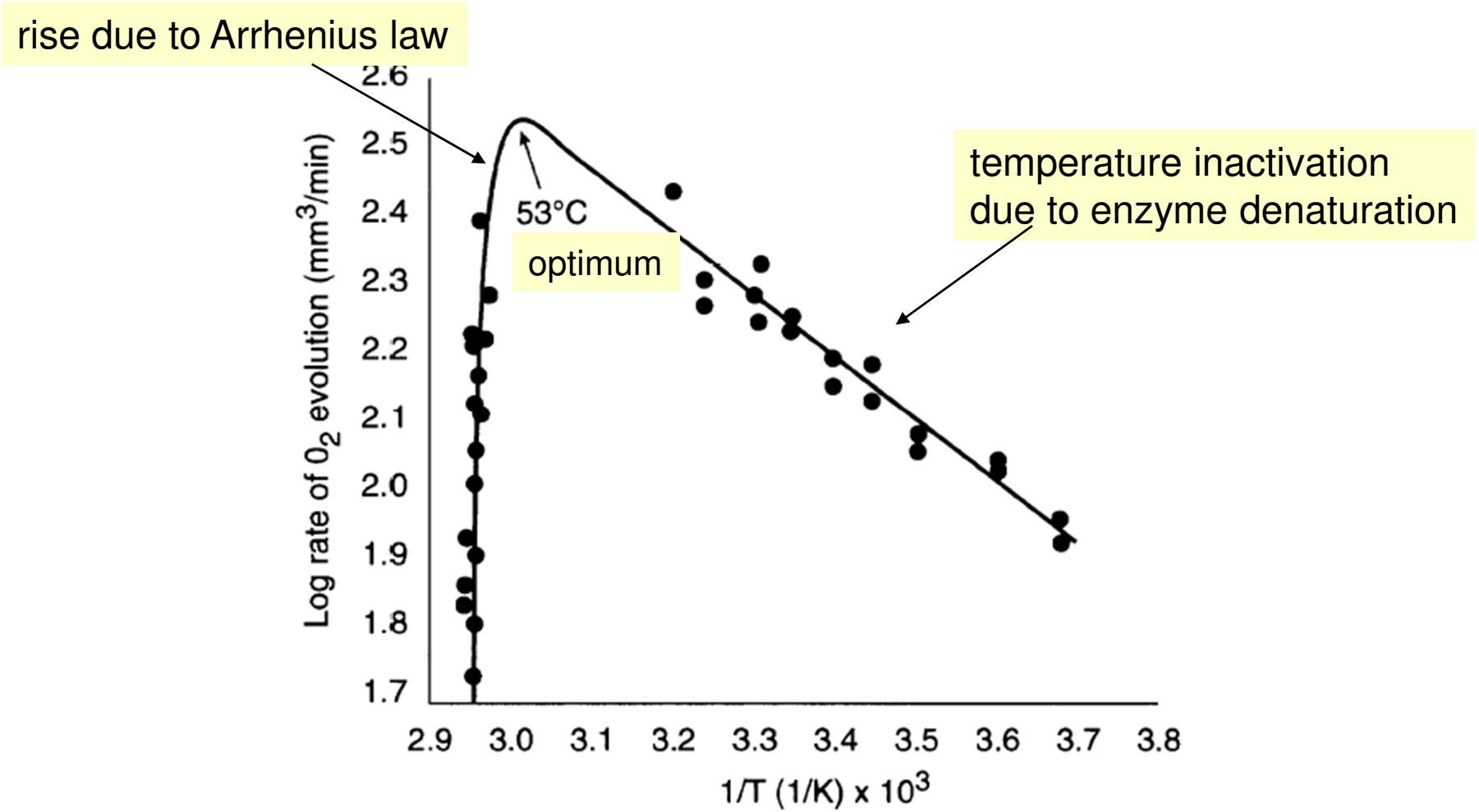
- If the reaction of forming the product from the enzyme-substrate complex is reversible

$$E + S \rightleftarrows E \bullet S \rightleftarrows E + P$$

- The Briggs-Haldane equation can be derived applying PSSH to the enzyme kinetics:

$$-r_S = \frac{V_{\max}\left(C_S - C_P / K_C\right)}{C_S + K_{\max} + K_p C_p}$$

Effect of Temperature



- catalytic breakdown of H_2O_2 vs temperature

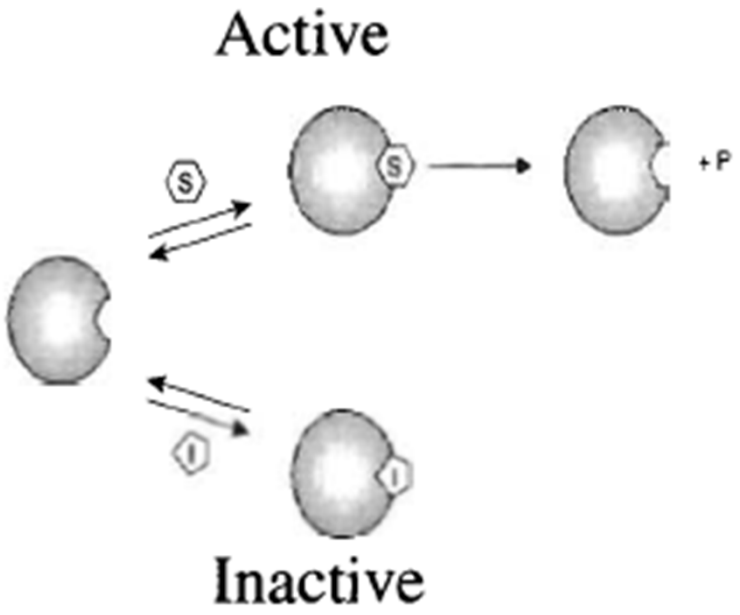
Enzyme inhibition

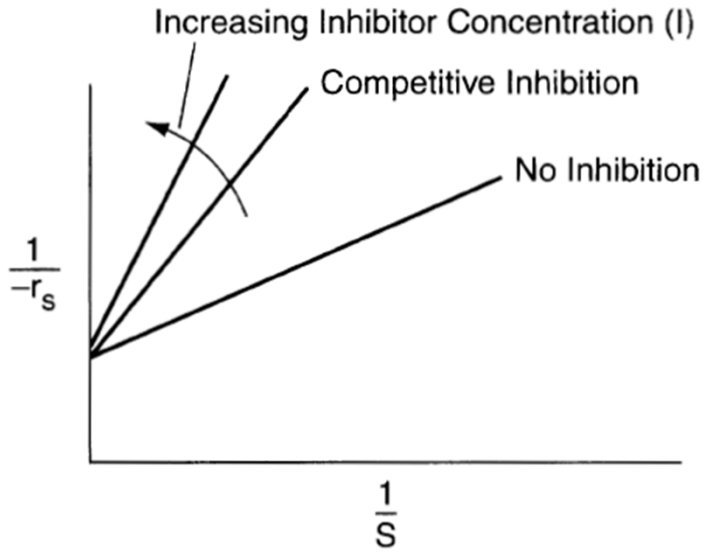
- **Competitive:** substrate and inhibition compete for the same site on the enzyme
- **Uncompetitive:** inhibitor deactivates enzyme-substrate complex (doesn't allow to form the product)
- **Noncompetitive**: inhibitor attaches to a different site on the enzyme

Inhibition of Enzyme reactions

- Competitive inhibition

$$E + S \xrightarrow{k_1} E \bullet S$$
$$E \bullet S \xrightarrow{k_2} E + S$$
$$E \bullet S \xrightarrow{k_3} P + E$$
$$I + E \xrightarrow{k_4} E \bullet I \text{ (inactive)}$$
$$E \bullet I \xrightarrow{k_5} E + I$$

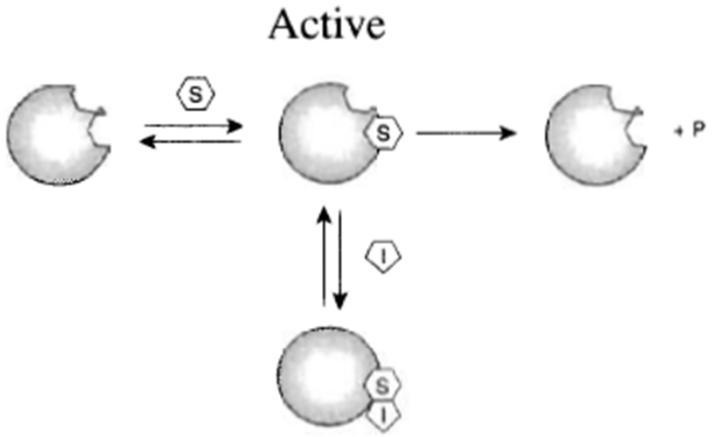




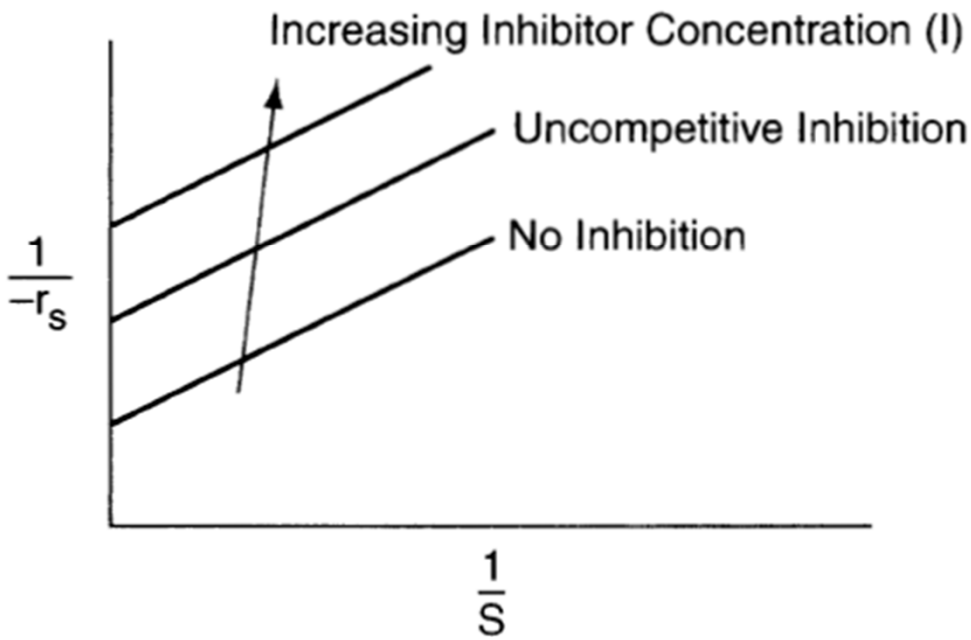
Inhibition of Enzyme reactions

- Uncompetitive inhibition: inhibitor is forming inactive I·E·S complex

$$E + S \xrightarrow{k_1} E \bullet S$$
$$E \bullet S \xrightarrow{k_2} E + S$$
$$E \bullet S \xrightarrow{k_3} P + E$$
$$I + E \bullet S \xrightarrow{k_4} I \bullet E \bullet S \text{ (inactive)}$$
$$I \bullet E \bullet S \xrightarrow{k_5} I + E \bullet S$$



$$\boxed{\frac{1}{-r_s} = \frac{1}{(S)}\frac{K_M}{V_{max}} + \frac{1}{V_{max}}\left(1 + \frac{(I)}{K_I}\right)}$$



Inhibition of Enzyme reactions

- Non-competitive (mixed) inhibition: inhibitor and the substrates react with the different sites on the enzyme

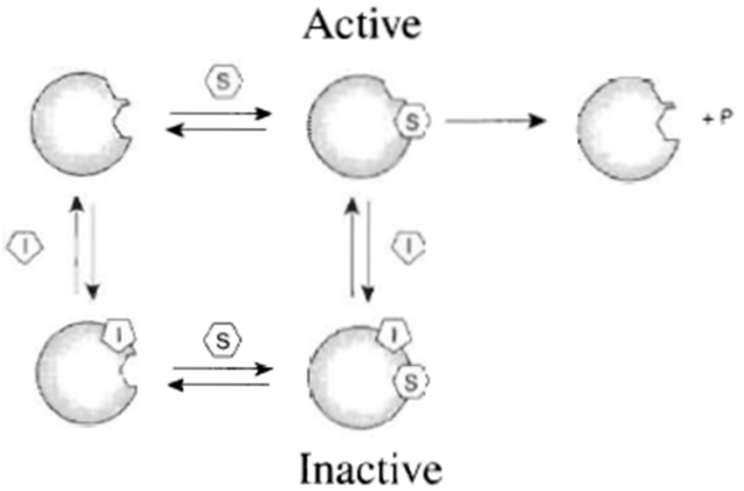
(1) $E + S \rightleftharpoons E \cdot S$

(2) $E + I \rightleftharpoons I \cdot E$ (inactive)

(3) $I + E \cdot S \rightleftharpoons I \cdot E \cdot S$ (inactive)

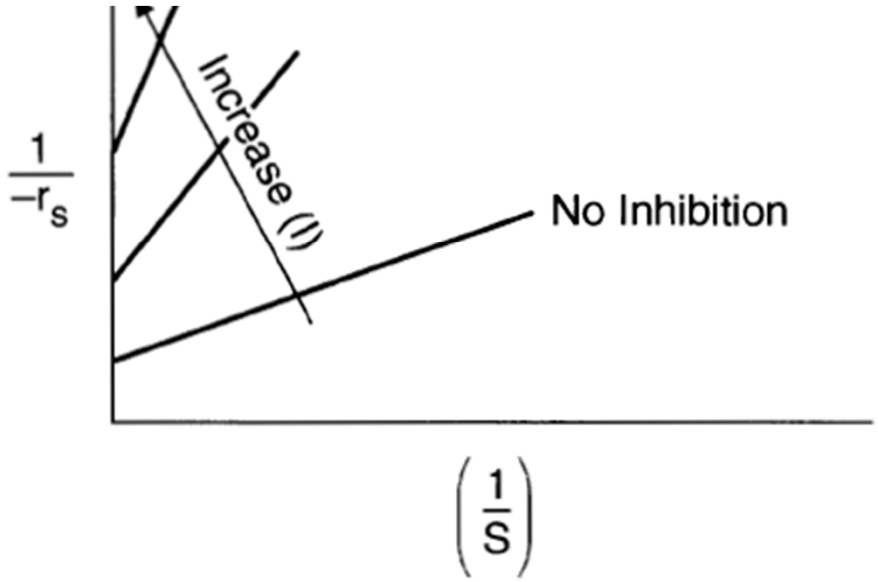
(4) $S + I \cdot E \rightleftharpoons I \cdot E \cdot S$ (inactive)

(5) $E \cdot S \longrightarrow P + E$



$$-r_s = \frac{V_{max}(S)}{((S) + K_M)\left(1 + \frac{(I)}{K_I}\right)}$$

$$\frac{1}{-r_s} = \frac{1}{V_{max}}\left(1 + \frac{(I)}{K_I}\right) + \frac{1}{(S)}\frac{K_M}{V_{max}}\left(1 + \frac{(I)}{K_I}\right)$$

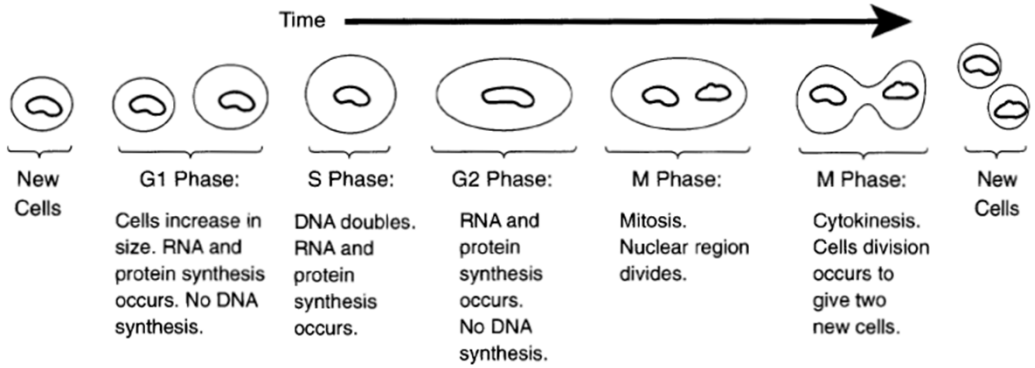


Bioreactors

- Advantages of bioconversion:
  - mild reaction conditions
  - high yields approaching 100%
  - stereospecific synthesis

- Cells consume nutrients to grow, to produce more cells and to produce the product in question:
  - (I) fueling reactions (nutrient degradation)
  - (II) synthesis of small molecules (amino acids)
  - (III) synthesis of large molecules (proteins, DNA, RNA)

- Cell growth and division



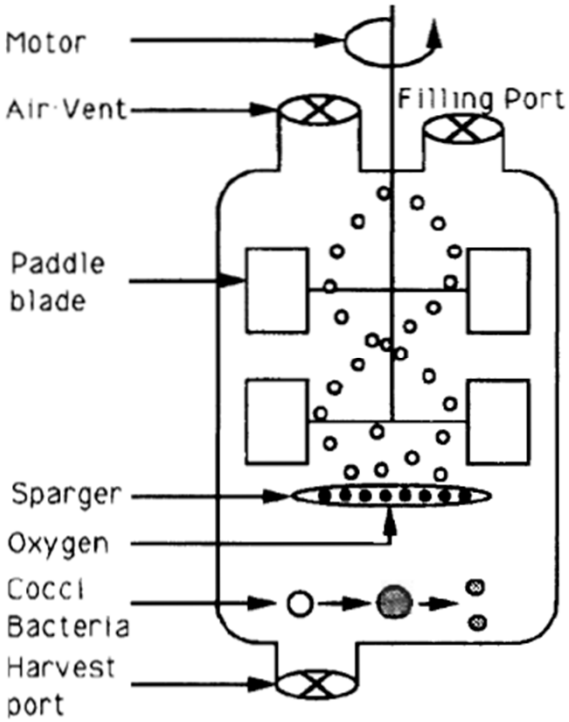
Bioreactors

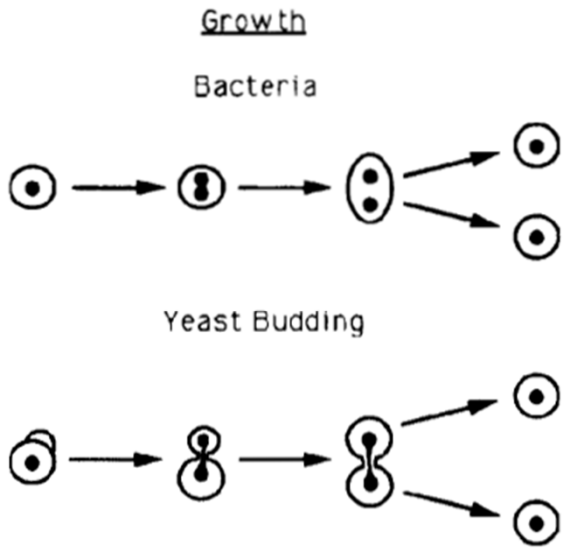
- Growth in the reactor

$$\text{Substrate} \xrightarrow{\textit{Cells}} \text{More Cells} + \text{Product}$$

e.g. CO2, water, proteins etc.

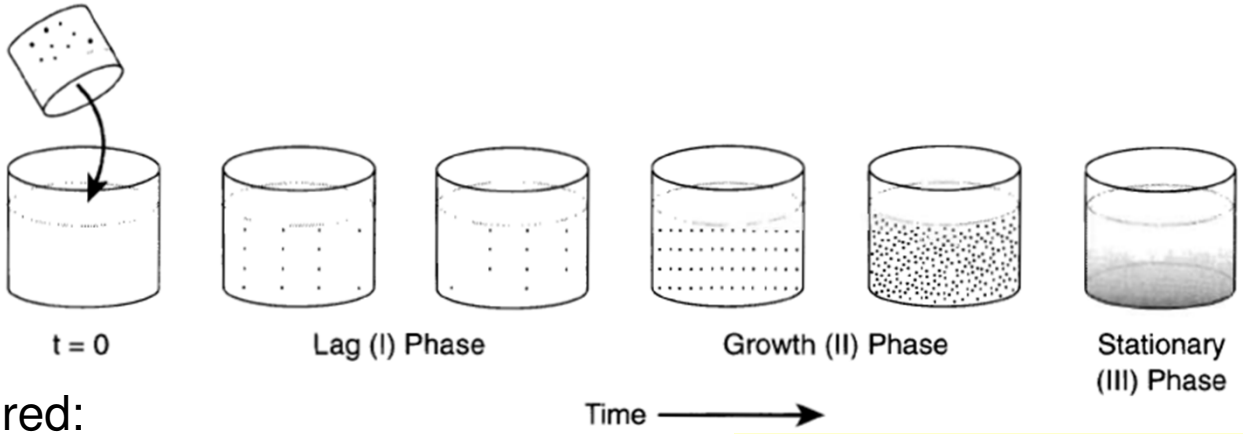
- Batch bioreactor





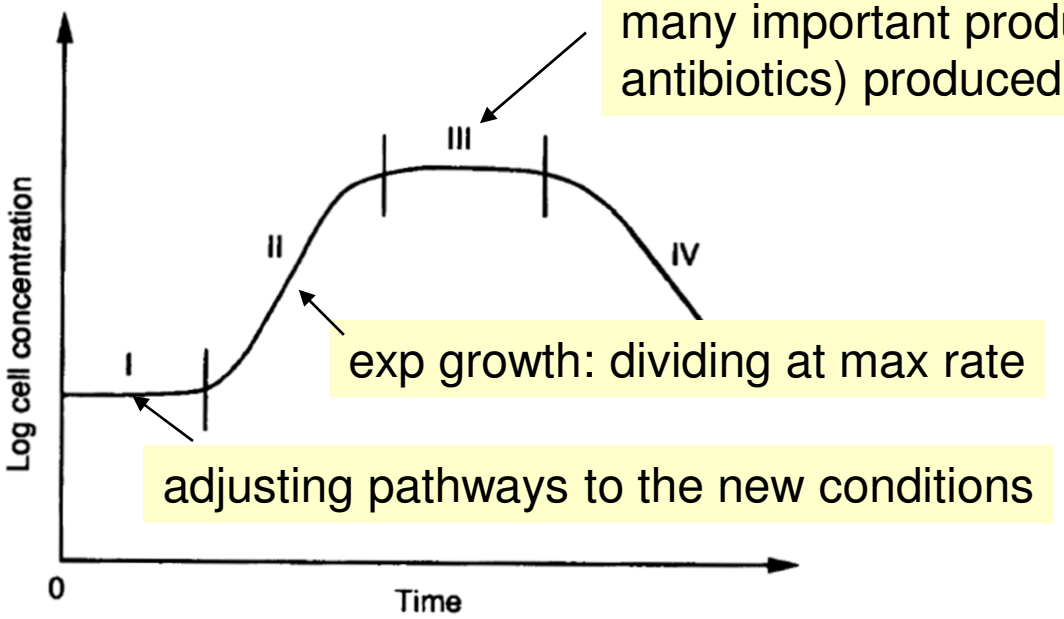
Bioreactors

- Cell growth in bioreactors



Nutritions required:

- Carbon source
- Nitrogen source
- Oxygen source
- Phosphate source
- ….



stationary phase: lack of some nutriotients limits cell growth; many important products (e.g. antibiotics) produced at this phase.

exp growth: dividing at max rate

adjusting pathways to the new conditions

Bioreactors: Rate laws

$$\text{Substrate} \xrightarrow{Cells} \text{More Cells} + \text{Product}$$

- Monod equation $r_g = \mu C_c$
- Specific growth rate $\mu = \mu_{\max} \dfrac{C_s}{K_s + C_s}$

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

- In many systems the product can inhibit cells growth (e.g. wine making)

$$r_g = k_{obs} \frac{\mu_{\max} C_s C_c}{K_s + C_s} \qquad k_{obs} = \left(1 - \frac{C_p}{C_p^*}\right)^n$$

C_p^*: product concentration where metabolism ceases

- e.g. glucose-to-ethanol: n=0.5, C_p^*=93g/l

Bioreactors: Rate laws

- Other growth equations:
  - Tessier equation $$r_g = \mu_{\max}\left[1-\exp\left(-\frac{C_s}{k}\right)\right]C_c$$
  - Moser equation $$r_g = \frac{\mu_{\max} C_s C_c}{\left(1+kC_s^{-\lambda}\right)}$$

usually give better fit in the beginning and in the end of fermentation

- Cell death rate (due to harsh environment, mixing shear forces, local depletion in nutrients, toxic substances)

$$r_d = \left(k_d + k_t C_t\right)C_c$$

k_d: natural death

$k_t C_t$: death due to toxic environment

- Temperature effect: similar curve with max

Bioreactors: Stoichiometry

$$\text{Substrate} \xrightarrow{Cells} \text{More Cells} + \text{Product}$$

- Yield coefficient for cells and substrate

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

- Yield coefficient for product in the exponential phase

$$Y_{p/c} = \frac{\text{Mass of product formed}}{\text{Mass of new cells formed}} = \frac{\Delta C_P}{\Delta C_C}$$

$$r_p = Y_{p/c} \frac{\mu_{\max} C_s C_c}{K_s + C_s}$$

Bioreactors: Stoichiometry

$$\text{Substrate} \xrightarrow{Cells} \text{More Cells} + \text{Product}$$

- Yield coefficient for product in the stationary phase

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

Usually secondary nutritient

- Maintenance utilization term, typically m=0.05 h^{-1}.

$$r_{sm} = mC_c$$

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

$$-r_s = Y'_{c/s}r_g + Y'_{p/s}r_p + mC_c$$

Bioreactors: Stoichiometry

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

$$-r_s = Y'_{c/s} r_g + Y'_{p/s} r_p + mC_c$$

- In the exponential phase we cannot separate substrate consumption for cell growth and product formation

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

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

- In stationary phase:

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

$$-r_{sn} = Y_{sn/p} r_p + mC_c = \frac{Y_{sn/p} k_p C_{sn}}{K_{sn} + C_{sn}} + mC_c$$

Chemostat (CSTR) operation

- Mass balance:
  - Cell balance $V\frac{dC_c}{dt} = v_0 C_{co} - vC_c + (r_g - r_d)V$
  - Substrate balance $V\frac{dC_s}{dt} = v_0 C_{so} - vC_s + r_s V$

Batch operation

- Mass balance:
  - Cell balance

$$V\frac{dC_c}{dt} = (r_g - r_d)V$$

  - Substrate balance

growth phase

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

stationary phase

$$\frac{dC_s}{dt} = Y_{s/p}(-r_p) - mC_c$$

  - Product balance

growth phase

$$\frac{dC_p}{dt} = r_p = Y_{p/c}r_g$$

stationary phase

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

Example 7-6

- Fermentation of *Saccharomyces cerevisae* in a batch reactor. Plot the concentration of cells, substrate, the product and growth rate as a function of time.
  Initial cell concentration 1g/dm^3, glucose 250g/dm^3

$$C^*_p = 93 g / cm^3 \qquad Y_{c/s} = 0.08\, g / g$$

$$n = 0.52 \qquad Y_{p/s} = 0.45\, g / g$$

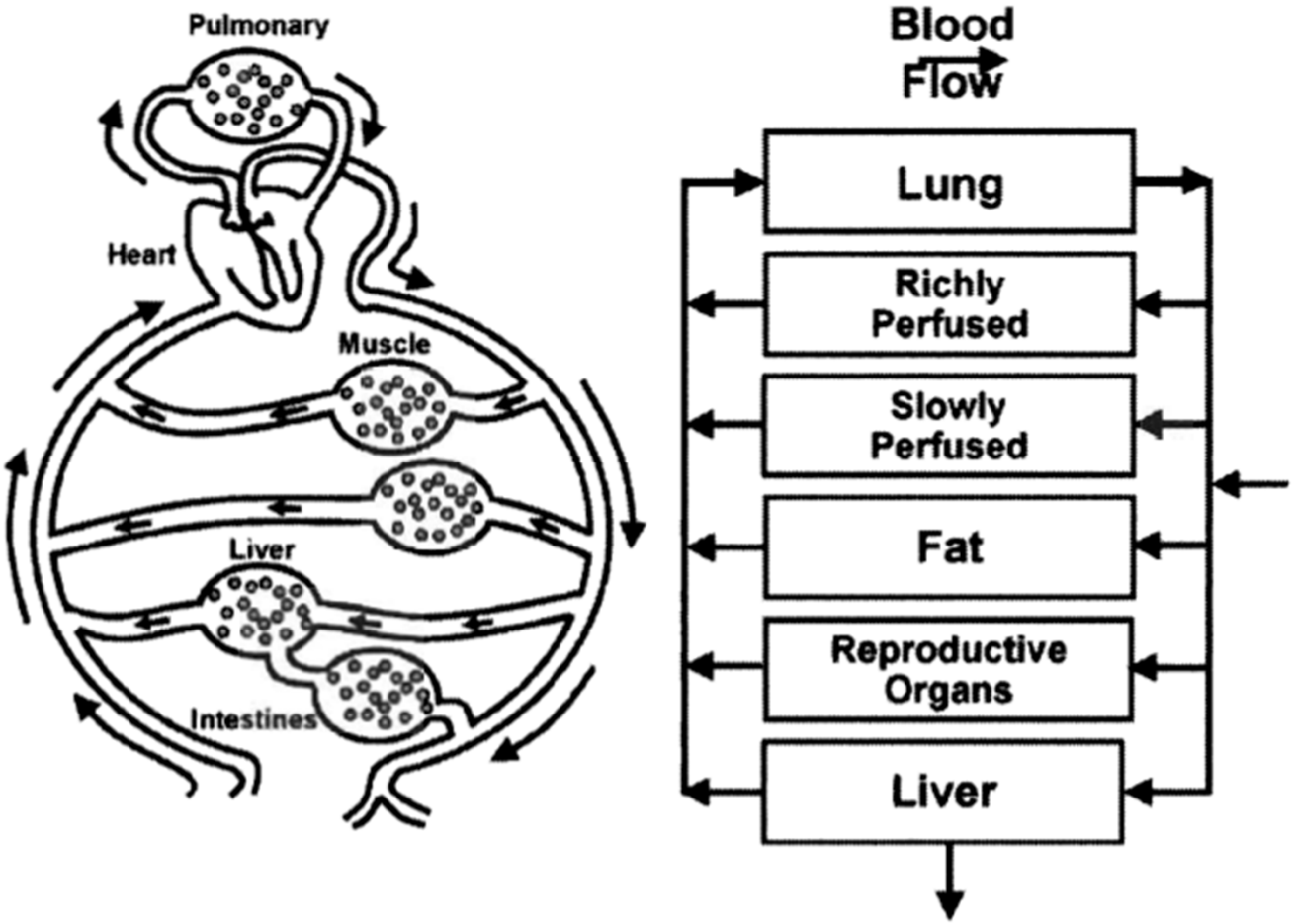
$$\mu_{\max} = 0.33\, h^{-1} \qquad Y_{p/c} = 5.6\, g / g$$

$$K_s = 1.7\, g / dm^3 \qquad k_d = 0.01\, h^{-1}$$

$$m = 0.03\, (g\ substrate) / (g\ cell \cdot h)$$

Physiologically based pharmacokinetics (PBPK)

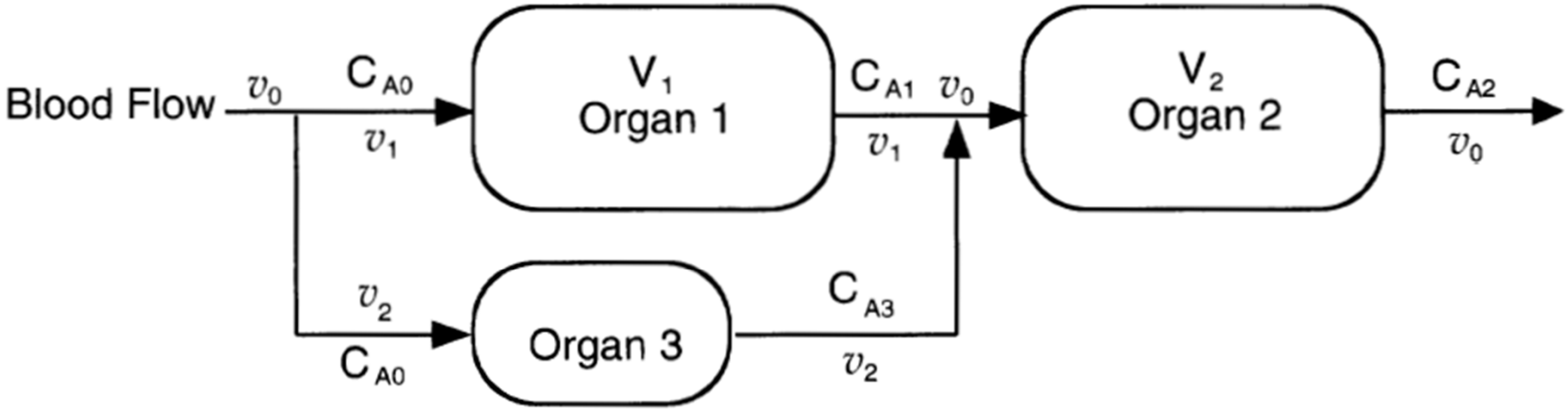
- Chemical reaction engineering approach can be applied to pharmacokinetics



- With every organ we can associate a certain tissue water volume (TWV) and flow rates.

Physiologically based pharmacokinetics (PBPK)

- Chemical reaction engineering approach can be applied to pharmacokinetics



$$V_1\frac{dC_{A1}}{dt} = v_1(C_{A0} - C_{A1}) + r_{A1}V_1$$

$$V_2\frac{dC_{A2}}{dt} = v_1(C_{A1} - C_{A2}) + v_2(C_{A3} - C_{A2}) + r_{A2}V_2$$

$$V_3\frac{dC_{A3}}{dt} = v_2(C_{A0} - C_{A3}) + r_{A3}V_3$$

Problems (for the class)

- Derive the Briggs-Haldane equation
- **P7-25** (http://www.engin.umich.edu/~cre/07chap/frames.htm). Methanol has been ingested, and after pumping the stomach methanol has an initial concentration of C_{Mi} = 0.25 g/dm^3 in the body:
  1. First prove the equations on the left hand side.
  2. How many grams of ethanol are necessary to retard the formation of formaldehyde so that it will not reach the level to cause blindness if the ethanol is to be injected immediately?
  3. What feed rate of ethanol should be used to prevent formaldehyde from reaching a concentration of 0.16 g/dm^3?

$$r_{p1} = \frac{V_{max1}(C_E)}{C_E + K_{M1}\left(1 + \frac{C_M}{K_{M2}}\right)}$$

$$r_{p2} = \frac{V_{Mmx2}(C_M)}{C_M + K_{M2}\left(1 + \frac{C_E}{K_{M1}}\right)}$$

$$\frac{dC_{p2}}{dt} = r_{p2} - k_7 C_{p2}$$

- Use the following values for V_{max1} and K_{M1} for ethanol neglecting the reverse reaction of acetaldehyde to ethanol. As a first approximation, use the same values for methanol. Next, vary V_{max2} the initial methanol concentration (0.1 g/dm^3 < C_M < 2 g/dm^3), (0.1 V_{max1} < V_{max2} < 2V_{max1}), k_7, and the intravenous injection rate, r_{IV}.
- There are 10 mg of methanol per 12 ounce can of diet pop. How many cans and how fast must you need to drink then to cause blindness. Just estimate, no need to modify and run the Polymath program.
- K_{M1} (ethanol) = 1.53 mg/dm^3; K_{M2} (methanol) = 1.07 mg/dm^3; V_{max1} (ethanol) = 3.1 mg/(dm^3 • min); V_{max2} (methanol) 2.16 mg/(dm^3 • min)