Lecture: Non-elementary Reaction Mechanisms & Bioreactors

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Active Intermediates and Nonelementary Rate Laws

Some examples of nonelementary rate laws

rate laws with non-integer powers

$CH_3CHO \rightarrow CH_4 + CO$

$rate = k[CH_3CHO]^{3/2}$

rate laws with a quotient

$H_2 + Br_2 \rightarrow 2HBr$

$$rate = \frac{k_1 C_{H_2} C_{Br_2}^{3/2}}{C_{HBr} + k_2 C_{Br_2}}$$

Rate laws of this form usually involve an active intermediate.

An active intermediate is a high-energy molecule that reacts virtually as fast as it is formed. As a result, it is present in very small concentrations. Frequently, active intermediates are formed during a high energy collision in which translational kinetic energy is converted into kinetic energy associated with an internal degree of freedom, such as a vibrational mode.

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

Pseudo-Steady-State Hypothesis

The active intermediate reacts as quickly as it is formed.

$$r_{A^*} = \sum_{i=1}^{n_r} r_{i,A^*} = 0$$

example: decomposition of Azomethane (AZO)

$(CH_3)_2N_2 \rightarrow C_2H_6 + N_2$

mechanism

creation of an active intermediate

reaction 1: $(CH_3)_2N_2 + (CH_3)_2N_2 \rightarrow (CH_3)_2N_2 + (CH_3)_2N_2^*$

annihilation of an active intermediate (reverse of reaction 1)

reaction 2: $(CH_3)_2N_2^* + (CH_3)_2N_2 \rightarrow (CH_3)_2N_2 + (CH_3)_2N_2$

decomposition of an active intermediate

reaction 3: $(CH_3)_2N_2^* \rightarrow C_2H_6 + N_2$

Each step in the mechanism is an elementary reaction.

$$r_1 = k_1 C_{AZO}^{\ 2}$$

$$r_2 = k_2 C_{AZO} C_{AZO*}$$

$$r_3 = k_3 C_{AZO*}$$

Write a mole balance on the active intermediate.

accumulation = in – out + generation

There are no in or out terms. The PSSH dictates that the accumulation is zero, so we have

$$0 = r_1 - r_2 - r_3 = k_1 C_{AZO}^{\ 2} - k_2 C_{AZO} C_{AZO*} - k_3 C_{AZO*}$$

Solve for the concentration of the active intermediate.

$$C_{AZO*} = \frac{k_1 C_{AZO}^{\ 2}}{k_2 C_{AZO} + k_3}$$

The rate of production of ethane is thus

$$r_3 = k_3 C_{AZO*} = \frac{k_3 k_1 C_{AZO}^{\ 2}}{k_2 C_{AZO} + k_3}$$

At very low concentrations of AZO, $k_2 C_{AZO} << k_3$, so the observed rate is

$$r_3 = k_1 C_{AZO}^{\ 2}$$

At high concentrations of AZO, $k_2 C_{AZO} >> k_3$, so the observed rate is

$$r_3 = \frac{k_3 k_1}{k_2} C_{AZO}$$

In general

mechanism

creation of an active intermediate (M is any molecule)

reaction 1: $A + M \rightarrow M + A^*$

annihilation of an active intermediate (reverse of reaction 1)

reaction 2: $A^* + M \rightarrow A + M$

decomposition of an active intermediate (P is product(s))

reaction 3: $A^* \rightarrow P$

Each step in the mechanism is an elementary reaction.

$$r_1 = k_1 C_A C_M$$

$$r_2 = k_2 C_M C_{A^*}$$

$$r_3 = k_3 C_{A^*}$$

active intermediate mole balance

$$0 = r_1 - r_2 - r_3 = k_1 C_A C_M - k_2 C_M C_{A^*} - k_3 C_{A^*}$$

Solve for the concentration of the active intermediate.

$$C_{A^*} = \frac{k_1 C_A C_M}{k_2 C_M + k_3}$$

Because the concentration of all molecules (M) inert or otherwise is constant, we observe the rate of production as

$$r_3 = k_3 C_{A^*} = \frac{k_3 k_1 C_A C_M}{k_2 C_M + k_3} = k C_A$$

This appears to be first order but it is not an elementary reaction.

Chain Reactions

Chain reactions has a sequence of steps

1. initiation: formation of the active intermediate
2. propagation or chain transfer: interaction of an active intermediate with reactant or product to form another active intermediate
3. termination: deactivation of the active intermediate to form products

example: thermal cracking of ethane

step 1: initiation

$$C_2H_6 \xrightarrow{k_1} 2CH_3^{\bullet} \qquad r_1 = k_1 C_{C_2H_6}$$

step 2: propagation:

$$C_2H_6 + CH_3^{\bullet} \xrightarrow{k_2} CH_4 + C_2H_5^{\bullet} \qquad r_2 = k_2 C_{C_2H_6} C_{CH_3^{\bullet}}$$

$$C_2H_5^{\bullet} \xrightarrow{k_3} C_2H_4 + H^{\bullet} \qquad r_3 = k_3 C_{C_2H_5^{\bullet}}$$

$$C_2H_6 + H^{\bullet} \xrightarrow{k_4} C_2H_5^{\bullet} + H_2 \qquad r_4 = k_4 C_{C_2H_6} C_{H^{\bullet}}$$

step 3: termination:

$$2C_2H_5^{\bullet} \xrightarrow{k_5} C_4H_{10} \qquad r_5 = k_5 C_{C_2H_5^{\bullet}}^{\ 2}$$

mole balances on the active intermediates

$$0 = \sum_{i=1}^{n_r} \nu_{i,\alpha} r_i$$

$H^{\bullet}$: $$0 = \sum_{i=1}^{n_r} \nu_{i,H^{\bullet}} r_i = 0 \cdot r_1 + 0 \cdot r_2 + 1 \cdot r_3 - 1 \cdot r_4 + 0 \cdot r_5 = r_3 - r_4$$

$CH_3^{\bullet}$: $$0 = \sum_{i=1}^{n_r} \nu_{i,CH_3^{\bullet}} r_i = 1 \cdot r_1 - 1 \cdot r_2 + 0 \cdot r_3 + 0 \cdot r_4 + 0 \cdot r_5 = 2r_1 - r_2$$

$C_2H_5^{\bullet}$: $$0 = \sum_{i=1}^{n_r} \nu_{i,C_2H_5^{\bullet}} r_i = 0 \cdot r_1 + 1 \cdot r_2 - 1 \cdot r_3 + 1 \cdot r_4 - 2 \cdot r_5 = r_2 - r_3 + r_4 - r_5$$

Substitute in the values for the rates

$H^{\bullet}$: $$0 = k_3 C_{C_2H_5^{\bullet}} - k_4 C_{C_2H_6} C_{H^{\bullet}}$$

$CH_3^{\bullet}$: $$0 = 2k_1 C_{C_2H_6} - k_2 C_{C_2H_6} C_{CH_3^{\bullet}}$$

$C_2H_5^{\bullet}$: $$0 = k_2 C_{C_2H_6} C_{CH_3^{\bullet}} - k_3 C_{C_2H_5^{\bullet}} + k_4 C_{C_2H_6} C_{H^{\bullet}} - k_5 C_{C_2H_5^{\bullet}}^{\ 2}$$

Solve:

$CH_3^{\bullet}$: $$C_{CH_3^{\bullet}} = 2\frac{k_1}{k_2}$$

$H^{\bullet}$: $$C_{H^{\bullet}} = \frac{k_3}{k_4} \frac{C_{C_2H_5^{\bullet}}}{C_{C_2H_6}}$$

Substitute in for $CH_3^{\bullet}$: and $H^{\bullet}$ in the balance for $C_2H_5^{\bullet}$:

$C_2H_5^{\bullet}$: $$0 = 2k_1 C_{C_2H_6} - k_3 C_{C_2H_5^{\bullet}} + k_3 C_{C_2H_5^{\bullet}} - k_5 C_{C_2H_5^{\bullet}}^{\ 2}$$

Solve

$C_2H_5^{\bullet}$: $$C_{C_2H_5^{\bullet}} = \sqrt{2\frac{k_1}{k_5} C_{C_2H_6}}$$

$H^{\bullet}$: $$C_{H^{\bullet}} = \frac{k_3}{k_4} \sqrt{2\frac{k_1}{k_5}} \frac{1}{\sqrt{C_{C_2H_6}}}$$

The rate of disappearance of ethane is

$$r_1 + r_2 + r_4 = k_1 C_{C_2H_6} + k_2 C_{C_2H_6} C_{CH_3^\bullet} + k_4 C_{C_2H_6} C_{H^\bullet}$$

$$r_1 + r_2 + r_4 = k_1 C_{C_2H_6} + 2k_1 C_{C_2H_6} + k_3 \sqrt{2\frac{k_1}{k_5}} \frac{C_{C_2H_6}}{\sqrt{C_{C_2H_6}}}$$

$$r_1 + r_2 + r_4 = 3k_1 C_{C_2H_6} + k_3 \sqrt{2\frac{k_1}{k_5}} \sqrt{C_{C_2H_6}}$$

The rate of production of C_2H_4 is

$$r_3 = k_3 C_{C_2H_5^\bullet} = k_3 \sqrt{2\frac{k_1}{k_5} C_{C_2H_6}}$$

The rate of production of C_4H_{10} is

$$r_5 = k_5 C_{C_2H_5^\bullet}^{\ 2} = 2k_1 C_{C_2H_6}$$

Reaction Pathways

See Figures 7-2, 7-3 and 7-4 on pp. 391-393

Numerical Example

Provided below are two files that look at the same basic reaction using an active intermediate. The first version of the file does not invoke the PSSH. The second version of the file does invoke the PSSH. The purpose of including the files is several fold.

1. The first file, without using PSSH, shows that the same approach we have been using can be used to model systems of reactions that result in non-elementary rate laws for the composite reaction.

2. How one changes the calculations (or equivalently the code) to account for the PSSH.

3. The goodness of the PSSH is a function of the rate parameters, k1, k2 and k3. For the values given, the results between the two versions of the code are similar. As one narrows the gap between the slow reaction (k1) and the fast reactions (k2 and k3), one can see the goodness of the PSSH approximation deteriorate.

Active Intermediate Example Input File without PSSH

```
function dydt = sysodeinput(x,y,nvec);
%
% active intermediates
% A + M --> A* + M
% A* + M --> A + M
% A* --> P
%
% A = A
% B = M
% C = A*
% D = P
%
% sample usage:
% [y,x]=sysode(2,2000,0,400,[10,40,0,0]);
%
CA = y(1); % mol/liter
CB = y(2);
CC = y(3);
CD = y(4);
%
% stoichiometry
%
nuA1 = -1;
nuB1 = 0;
nuC1 = 1;
nuD1 = 0;
%
nuA2 = 1;
nuB2 = 0;
nuC2 = -1;
nuD2 = 0;
%
nuA3 = 0;
nuB3 = 0;
nuC3 = -1;
nuD3 = 1;

%
% rate laws
%
k1 = 1.0e-3; % liters/mole/sec
r1 = k1*CA*CB; % mole/liter/sec
%
k2 = 1.0e-1; % liters/mole/sec
r2 = k2*CB*CC; % mole/liter/sec
%
k3 = 1.0e-0; % liters/mole/sec
r3 = k3*CC; % mole/liter/sec
%
% mole balances
%
dydt(1) = nuA1*r1 + nuA2*r2 + nuA3*r3;
dydt(2) = nuB1*r1 + nuB2*r2 + nuB3*r3;
dydt(3) = nuC1*r1 + nuC2*r2 + nuC3*r3;
dydt(4) = nuD1*r1 + nuD2*r2 + nuD3*r3;
```

Active Intermediate Example Input File with PSSH

```
function dydt = sysodeinput(x,y,nvec);
%
% active intermediates
% A + M --> A* + M
% A* + M --> A + M
% A* --> P
%
% A = A
% B = M
% C = P
% A* is determined from PSSH
%
% sample usage:
% [y,x]=sysode(2,2000,0,400,[10,40,0]);
%
CA = y(1); % mol/liter
CB = y(2);
CC = y(3);
%
% stoichiometry
%
nuA1 = -1;
nuB1 = 0;
nuC1 = 0;
%
nuA2 = 1;
nuB2 = 0;
nuC2 = 0;
%
nuA3 = 0;
nuB3 = 0;
nuC3 = 1;
%
% rate constants
%
k1 = 1.0e-3; % liters/mole/sec
k2 = 1.0e-1; % liters/mole/sec
k3 = 1.0e-0; % liters/mole/sec
%
%  PSSH expression for concentration of active intermediate
%
CAstar = k1*CA*CB/(k2*CB+k3);
%
% rate laws
%
r1 = k1*CA*CB; % mole/liter/sec
r2 = k2*CB*CAstar; % mole/liter/sec
r3 = k3*CAstar; % mole/liter/sec
%
% mole balances
%
dydt(1) = nuA1*r1 + nuA2*r2 + nuA3*r3;
dydt(2) = nuB1*r1 + nuB2*r2 + nuB3*r3;
dydt(3) = nuC1*r1 + nuC2*r2 + nuC3*r3;
```

Enzymatic Reaction Fundamentals

overall reaction: S = substrate, P = product

$S \rightarrow P$

reaction pathway E = enzyme

$$E + S \leftrightarrow E \bullet S \rightarrow E + P$$

enzymes are specific. Typically, one enzyme catalyze only one type of reaction.
Therefore, unwanted by-products of side-reactions are usually avoided in enzymatic reactions.

Enzyme-Substrate Complex

enzyme have a specific active site which binds the substrate to form the enzyme-substrate complex.

If the enzyme is exposed to extreme temperatures or extreme pH, it may unfold, losing its active sites, becoming denatured.

two popular models
- lock-and-key (older but less preferred today)
- induced fit

See Figure on page 396.

Six and only six classes of enzymes

1. oxidoreductases	$AH_2 + B + E \rightarrow A + BH_2 + E$	reducing A
2. transferases	$AB + C + E \rightarrow AC + B + E$	replacing B with C on A
3. hydrolases	$AB + H_2O + E \rightarrow AH + BOH + E$	splitting water in H and OH
4. isomerases	$A + E \rightarrow isoA + E$	forming an isomer
5. lyases	$AB + E \rightarrow A + B + E$	splitting a molecule
6. ligases	$A + B + E \rightarrow AB + E$	joining two molecules

Enzymatic Reaction Mechanisms

example
- substrate: S = urea
- enzyme: E = urease
- product : P = ammonia and carbon dioxide

reaction 1: formation of the enzymatic complex

$$S + E \xrightarrow{k_1} S \bullet E^* \qquad r_1 = k_1 C_S C_E$$

reaction 2: reverse of reaction 1

$$S \bullet E^* \xrightarrow{k_2} S + E \qquad r_2 = k_2 C_{S \bullet E^*}$$

reaction 3: product formation

$$S \bullet E^* + H_2O \xrightarrow{k_3} 2NH_3 + CO_2 + E \qquad r_3 = k_3 C_{S \bullet E^*} C_{H_2O}$$

The challenge is that we can only measure the total E concentration.

$$E_t = E + S \bullet E^*$$

A mole balance on the enzymatic species yields

$$S \bullet E^*: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,S \bullet E^*} r_i = 1 \cdot r_1 - 1 \cdot r_2 - 1 \cdot r_3 = r_1 - r_2 - r_3$$

$$S \bullet E^*: \qquad 0 = k_1 C_S C_E - k_2 C_{S \bullet E^*} - k_3 C_{S \bullet E^*} C_{H_2O}$$

Solve for the concentration of the enzymatic complex

$$S \bullet E^*: \qquad C_{S \bullet E^*} = \frac{k_1 C_S C_E}{k_2 + k_3 C_{H_2O}}$$

the rate of disappearance of substrate is given by a mole balance on S

$$S: \qquad \frac{dC_S}{dt} = \sum_{i=1}^{n_r} \nu_{i,S} r_i = -1 \cdot r_1 + 1 \cdot r_2 = -r_1 + r_2$$

$$S: \qquad \frac{dC_S}{dt} = -k_1 C_S C_E + k_2 C_{S \bullet E^*}$$

$$S: \qquad \frac{dC_S}{dt} = -k_1 C_S C_E + k_2 \frac{k_1 C_S C_E}{k_2 + k_3 C_{H_2O}} = k_1 \left(\frac{k_2}{k_2 + k_3 C_{H_2O}} - 1 \right) C_S C_E$$

This expression is no good in a practical sense, since we can only measure the total enzyme concentration.

$$C_{E_t} = C_E + C_{S \bullet E^*} = C_E + \frac{k_1 C_S C_E}{k_2 + k_3 C_{H_2O}}$$

$$C_E = C_{E_t} \frac{1}{1 + \dfrac{k_1 C_S}{k_2 + k_3 C_{H_2O}}} = C_{E_t} \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S + k_2 + k_3 C_{H_2O}}$$

Substituting this into the balance for the substrate yields

$$\frac{dC_S}{dt} = k_1 \left(\frac{k_2}{k_2 + k_3 C_{H_2O}} - 1 \right) \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S + k_2 + k_3 C_{H_2O}} C_S C_{E_t}$$

$$= -\frac{k_1 k_3}{k_1 C_S + k_2 + k_3 C_{H_2O}} C_S C_{E_t} C_{H_2O}$$

Michaelis-Menton Equation

The reaction is carried out in aqueous solution, so water is in excess and can be considered constant.

$$k_{cat} \equiv k_3 C_{H_2O}$$

$$K_M \equiv \frac{k_{cat} + k_2}{k_1}$$

Putting these new definitions into the balance for the substrate yields

$$\frac{dC_S}{dt} = -\frac{k_{cat}}{C_S + K_M} C_S C_{E_t}$$

The turnover number is given by k_{cat}. This is the number of substrates converted to product per unit time. For example, for the enzyme catalase with the substrate H_2O_2, the turnover number is 40×10^6 s^{-1}. Forty million hydrogen peroxide molecules are decomposed by this enzyme every second.

The constant K_M is the Michaelis constant. It is a measure of the attraction of the enzyme for its substrate. It is also called the affinity constant. For the example above, the Michaelis constant is 1.1 M.

If we define another variable, V_{max}, which represent the maximum rate of reaction for a given total enzyme concentration,

$$V_{\max} \equiv k_{cat} C_{E_t}$$

We have

$$\frac{dC_S}{dt} = -\frac{V_{\max} C_S}{C_S + K_M}$$

This is called the Michaelis-Menten equation.

See Figure 7-6 on page 400.

At low concentrations of substrate, $C_S << K_M$,

$$\frac{dC_S}{dt} = -\frac{V_{\max} C_S}{K_M}$$

The reaction is apparently first order in the substrate.

At high concentrations of substrate, $C_S >> K_M$,

$$\frac{dC_S}{dt} = -\frac{V_{\max} C_S}{C_S} = -V_{\max}$$

The reaction is apparently zeroth order in the substrate.

Furthermore, K_M is the concentration that yields $\frac{dC_S}{dt} = -\frac{V_{\max}}{2}$.

Michaelis-Menten plot is –rate vs concentration of substrate. (See Figure 7.6)

$$\frac{dC_S}{dt} = -\frac{V_{\max} C_S}{C_S + K_M}$$

Lineweaver-Burk plot is -1/rate vs $1/C_S$. Invert equation above. (See Figure E7-3.1) Straight line.

$$-\frac{1}{\frac{dC_S}{dt}} = \frac{C_S + K_M}{V_{\max} C_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{C_S}$$

So you can get V_{max} from the intercept and K_m from the slope.

If the generation of product is reversible:

reaction 1: formation of the enzymatic complex

$$S + E \xrightarrow{k_1} S \bullet E^* \qquad r_1 = k_1 C_S C_E$$

reaction 2: reverse of reaction 1

$$S \bullet E^* \xrightarrow{k_2} S + E \qquad r_2 = k_2 C_{S \bullet E^*}$$

reaction 3: product formation

$$S \bullet E^* + H_2O \xrightarrow{k_3} P + E \qquad r_3 = k_3 C_{S \bullet E^*} C_{H_2O}$$

reaction 4: product returns to enzyme

$$P + E \xrightarrow{k_4} S \bullet E^* + H_2O \qquad r_4 = k_4 C_P C_E$$

The challenge is that we can only measure the total E concentration.

$$E_t = E + S \bullet E^*$$

A mole balance on the enzymatic species yields

$$S \bullet E^*: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,S \bullet E^*} r_i = 1 \cdot r_1 - 1 \cdot r_2 - 1 \cdot r_3 + 1 \cdot r_4 = r_1 - r_2 - r_3 + r_4$$

$$S \bullet E^*: \qquad 0 = k_1 C_S C_E - k_2 C_{S \bullet E^*} - k_3 C_{S \bullet E^*} C_{H_2O} + k_4 C_P C_E$$

Solve for the concentration of the enzymatic complex

$$S \bullet E^*: \qquad C_{S \bullet E^*} = \frac{k_1 C_S C_E + k_4 C_P C_E}{k_2 + k_3 C_{H_2O}}$$

the rate of disappearance of substrate is given by a mole balance on S

$$S: \qquad \frac{dC_S}{dt} = \sum_{i=1}^{n_r} \nu_{i,S} r_i = -1 \cdot r_1 + 1 \cdot r_2 = -r_1 + r_2$$

$$S: \qquad \frac{dC_S}{dt} = -k_1 C_S C_E + k_2 C_{S \bullet E^*}$$

$$S: \qquad \frac{dC_S}{dt} = -k_1 C_S C_E + k_2 \frac{k_1 C_S C_E + k_4 C_P C_E}{k_2 + k_3 C_{H_2O}} = \left(\frac{-k_1 k_3 C_{H_2O} C_S + k_2 k_4 C_P}{k_2 + k_3 C_{H_2O}} \right) C_E$$

This expression is no good in a practical sense, since we can only measure the total enzyme concentration.

$$C_{E_t} = C_E + C_{S \bullet E^*} = C_E + \frac{k_1 C_S C_E + k_4 C_P C_E}{k_2 + k_3 C_{H_2O}}$$

$$C_E = C_{E_t} \frac{1}{1 + \frac{k_1 C_S + k_4 C_P}{k_2 + k_3 C_{H_2O}}} = C_{E_t} \frac{k_2 + k_3 C_{H_2O}}{k_2 + k_3 C_{H_2O} + k_1 C_S + k_4 C_P}$$

Substituting this into the balance for the substrate yields

$$\frac{dC_S}{dt} = \left(\frac{-k_1 k_3 C_{H_2O} C_S + k_2 k_4 C_P}{k_2 + k_3 C_{H_2O}} \right) C_E = \left(\frac{-k_1 k_3 C_{H_2O} C_S + k_2 k_4 C_P}{k_2 + k_3 C_{H_2O}} \right) \frac{k_2 + k_3 C_{H_2O}}{k_2 + k_3 C_{H_2O} + k_1 C_S + k_4 C_P} C_{E_t}$$

$$= \frac{-k_1 k_3 C_{H_2O} C_S + k_2 k_4 C_P}{k_2 + k_3 C_{H_2O} + k_1 C_S + k_4 C_P} C_{E_t}$$

$$k_{cat} \equiv k_3 C_{H_2O}$$

$$\frac{dC_S}{dt} = \frac{-k_1 k_{cat} C_S + k_2 k_4 C_P}{k_2 + k_{cat} + k_1 C_S + k_4 C_P} C_{E_t}$$

$$K_M \equiv \frac{k_{cat} + k_2}{k_1}$$

$$\frac{dC_S}{dt} = \frac{-k_{cat} C_S + k_2 \frac{k_4}{k_1} C_P}{K_M + C_S + \frac{k_4}{k_1} C_P} C_{E_t}$$

$$V_{\max} \equiv k_{cat} C_{E_t}$$

$$\frac{dC_S}{dt} = \frac{-V_{\max} C_S + k_2 \frac{k_4}{k_1} C_P \frac{V_{\max}}{k_{cat}}}{K_M + C_S + \frac{k_4}{k_1} C_P}$$

$K_P \equiv \frac{k_4}{k_1}$ ratio of formation of enzymatic complex from substrate to that from product

$$\frac{dC_S}{dt} = \frac{-V_{\max} C_S + k_2 K_P C_P \frac{V_{\max}}{k_{cat}}}{K_M + C_S + K_P C_P}$$

$K_C \equiv \frac{k_{cat}}{k_2 K_P} = \frac{k_{cat}}{k_2} \frac{k_1}{k_4} = \frac{k_3}{k_2} \frac{k_1}{k_4} C_{H_2O}$ ratio of many rates…

$$\frac{dC_S}{dt} = -\frac{V_{\max}\left(C_S - C_P \middle/ K_C\right)}{K_M + C_S + K_P C_P}$$ (equation 7-29) of Fogler

This is the Briggs-Haldane Equation.

Section 7.2 Batch Reactors for Enzyme Reactions

In the simplest case, we have in a batch reactor a mole balance on the substrate

accumulation = generation

$$\frac{dC_S}{dt} = \nu_S r$$

$$\frac{dC_S}{dt} = -\frac{V_{\max} C_S}{C_S + K_M}$$

Reminder: This is the Michaelis-Menten equation. It can be analytically integrated.

$$\frac{C_S + K_M}{C_S} dC_S = -V_{\max} dt$$

$$\left(\frac{1}{K_M}+\frac{1}{C_S}\right)dC_S=-\frac{V_{\max}}{K_M}dt$$

$$\int_{C_{S,o}}^{C_S}\left(\frac{1}{K_M}+\frac{1}{C_S}\right)dC_S=-\frac{V_{\max}}{K_M}\int_{t_o}^{t}dt$$

$$\frac{1}{K_M}\left(C_S-C_{S,o}\right)+\ln\left(\frac{C_S}{C_{S,o}}\right)=-\frac{V_{\max}}{K_M}\left(t-t_o\right)$$

This expression relates reactor time to substrate concentration. It can not be analytically solved for the substrate concentration.

Effect of Temperature on Enyzmatic Reactions

Enzymatic reactions are very sensitive to temperature and have an optimum temperature.
See Figure 7-8. page 407.

The same can be said for pH.

Inhibition of Enzyme Reactions

Inhibitors are a molecule or species that renders the enzyme unable to catalyze a specific reaction. In biological systems, some inhibitors have negative effects (e.g. cyanide acts an inhibitor to a single enzyme in the aerobic oxidation process that will lead to death) and positive effects (e.g. in the treatement of leukemia). Aspirin inhibits enzyme that catalyzes the synthesis of a compound involved in the pain-producing process.

Three most common types of reversible inhibition are

competitive - inhibitor and substrate compete for the same active site

uncompetitive – inhibitor deactivates the enzyme-substrate complex

noncompetitive – enzyme has two types of sites and the presence of the inhibitor in one of them deactivates the enzyme

Competitive Inhibition

reaction 1: formation of the enzymatic complex

$$S + E \xrightarrow{k_1} S \bullet E \qquad r_1 = k_1 C_S C_E$$

reaction 2: reverse of reaction 1

$$S \bullet E \xrightarrow{k_2} S + E \qquad r_2 = k_2 C_{S \bullet E}$$

reaction 3: product formation

$$S \bullet E + W \xrightarrow{k_3} P + E \qquad r_3 = k_3 C_{S \bullet E} C_W$$

reaction 4: formation of the inhibited enzymatic complex

$$I + E \xrightarrow{k_4} I \bullet E \qquad r_4 = k_4 C_I C_E$$

reaction 5: reverse of reaction 4

$$I \bullet E \xrightarrow{k_5} I + E \qquad r_5 = k_5 C_{I \bullet E}$$

The rate of the formation of the product is still the rate of reaction 3.
We use the PSSH to determine the concentrations of both the substrate-enzyme and inhibitor-enzyme complex.

Actually, the concentrations of the substrate-enzyme complex is unaffected by the inhibitor.

$$S \bullet E: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,S \bullet E} r_i = 1 \cdot r_1 - 1 \cdot r_2 - 1 \cdot r_3 = r_1 - r_2 - r_3$$

$$S \bullet E: \qquad 0 = k_1 C_S C_E - k_2 C_{S \bullet E} - k_3 C_{S \bullet E} C_{H_2O}$$

Solve for the concentration of the enzymatic complex

$$S \bullet E: \qquad C_{S\bullet E} = \frac{k_1 C_S C_E}{k_2 + k_3 C_{H_2O}} \quad \text{or} \quad C_E = \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S} C_{S\bullet E}$$

The concentrations of the inhibitor-enzyme complex is unaffected by the substrate.

$$I \bullet E: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,I\bullet E} r_i = 1 \cdot r_4 - 1 \cdot r_5 = r_4 - r_5$$

$$I \bullet E: \qquad 0 = k_4 C_I C_E - k_5 C_{I\bullet E}$$

Solve for the concentration of the enzymatic complex

$$I \bullet E: \qquad C_{I\bullet E} = \frac{k_4 C_I C_E}{k_5}$$

The rate will be expressed in terms of the total enzyme concentration

$$E_t = E + S \bullet E + I \bullet E$$

$$C_{E_t} = C_E + C_{S\bullet E} + \frac{k_4 C_I C_E}{k_5} = C_{S\bullet E} + \left(1 + \frac{k_4 C_I}{k_5}\right) C_E$$

$$= C_{S\bullet E} + \left(1 + \frac{k_4 C_I}{k_5}\right) \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S} C_{S\bullet E} = \left[1 + \left(1 + \frac{k_4 C_I}{k_5}\right) \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S}\right] C_{S\bullet E}$$

The rate of production therefore becomes

$$r_3 = k_3 C_{S\bullet E} C_{H_2O} = k_3 C_{H_2O} C_{E_t} \frac{1}{1 + \left(1 + \frac{k_4 C_I}{k_5}\right) \frac{k_2 + k_3 C_{H_2O}}{k_1 C_S}}$$

$$k_{cat} \equiv k_3 C_{H_2O}$$

$$r_3 = k_{cat} C_{E_t} \frac{1}{1 + \left(1 + \frac{k_4 C_I}{k_5}\right) \frac{k_2 + k_{cat}}{k_1 C_S}}$$

$$K_M \equiv \frac{k_{cat} + k_2}{k_1} \quad \text{and} \quad V_{\max} \equiv k_{cat} C_{E_t}$$

$$r_3 = V_{\max} \frac{1}{1 + \left(1 + \frac{k_4 C_I}{k_5}\right) \frac{K_M}{C_S}}$$

$$K_I \equiv \frac{k_5}{k_4}$$

$$r_3 = \frac{V_{\max} C_S}{C_S + \left(1 + \frac{C_I}{K_I}\right) K_M}$$

an effective Michaelis constant is observed

$$K_M^{'} = \left(1 + \frac{C_I}{K_I}\right) K_M$$

Uncompetitive Inhibition

reaction 1: formation of the enzymatic complex

$$S + E \xrightarrow{k_1} S \bullet E \qquad r_1 = k_1 C_S C_E$$

reaction 2: reverse of reaction 1

$$S \bullet E \xrightarrow{k_2} S + E \qquad r_2 = k_2 C_{S \bullet E}$$

reaction 3: product formation

$$S \bullet E \xrightarrow{k_3} P + E \qquad r_3 = k_3 C_{S \bullet E}$$

reaction 4: formation of the inhibited substrate-enzymatic complex

$$I + S \bullet E \xrightarrow{k_4} I \bullet E \bullet S \qquad r_4 = k_4 C_I C_{S \bullet E}$$

reaction 5: reverse of reaction 4

$$I \bullet E \bullet S \xrightarrow{k_5} I + S \bullet E \qquad r_5 = k_5 C_{I \bullet E \bullet S}$$

The rate of the formation of the product is still the rate of reaction 3.

We use the PSSH to determine the concentrations of both the substrate-enzyme and inhibitor-substrate-enzyme complex.

$$S \bullet E: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,S\bullet E} r_i = r_1 - r_2 - r_3 - r_4 + r_5$$

$$S \bullet E: \qquad 0 = k_1 C_S C_E - k_2 C_{S\bullet E} - k_3 C_{S\bullet E} - k_4 C_I C_{S\bullet E} + k_5 C_{I\bullet E\bullet S}$$

$$I \bullet E \bullet S: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,I\bullet E\bullet S} r_i = r_4 - r_5 = k_4 C_I C_{S\bullet E} - k_5 C_{I\bullet E\bullet S}$$

Solve for the concentration of the inhibited enzymatic complex

$$I \bullet E \bullet S: \qquad C_{I\bullet E\bullet S} = \frac{k_4}{k_5} C_I C_{S\bullet E}$$

Substitute into balance for substrate complex

$$S \bullet E: \qquad 0 = k_1 C_S C_E - k_2 C_{S\bullet E} - k_3 C_{S\bullet E} - k_4 C_I C_{S\bullet E} + k_5 \frac{k_4}{k_5} C_I C_{S\bullet E}$$

Simplify

$$S \bullet E: \qquad C_E = \frac{k_2 + k_3}{k_1 C_S} C_{S\bullet E}$$

The rate will be expressed in terms of the total enzyme concentration

$$C_{E_t} = C_E + C_{S\bullet E} + C_{I\bullet E\bullet S}$$

$$C_{E_t} = \frac{k_2 + k_3}{k_1 C_S} C_{S\bullet E} + C_{S\bullet E} + \frac{k_4}{k_5} C_I C_{S\bullet E} = \left[1 + \frac{k_2 + k_3}{k_1 C_S} + \frac{k_4}{k_5} C_I \right] C_{S\bullet E}$$

The rate of production therefore becomes

$$r_3 = k_3 C_{S\bullet E} = k_3 \frac{1}{1 + \frac{k_2 + k_3}{k_1 C_S} + \frac{k_4}{k_5} C_I} C_{E_t}$$

$$K_M \equiv \frac{k_3 + k_2}{k_1} \quad \text{and} \quad V_{\max} \equiv k_3 C_{E_t} \quad \text{and} \quad K_I \equiv \frac{k_5}{k_4}$$

$$r_3 = \frac{V_{\max}}{1 + \frac{K_M}{C_S} + \frac{C_I}{K_I}} = \frac{V_{\max} C_S}{K_M + \left(1 + \frac{C_I}{K_I}\right) C_S}$$

Noncompetitive Inhibition

reaction 1: formation of the enzymatic complex

$$S + E \xrightarrow{k_1} S \bullet E \qquad r_1 = k_1 C_S C_E$$

reaction 2: reverse of reaction 1

$$S \bullet E \xrightarrow{k_2} S + E \qquad r_2 = k_2 C_{S \bullet E}$$

reaction 3: product formation

$$S \bullet E \xrightarrow{k_3} P + E \qquad r_3 = k_3 C_{S \bullet E}$$

reaction 4: formation of the inhibited enzymatic complex

$$I + E \xrightarrow{k_4} I \bullet E \qquad r_4 = k_4 C_I C_E$$

reaction 5: reverse of reaction 4

$$I \bullet E \xrightarrow{k_5} I + E \qquad r_5 = k_5 C_{I \bullet E}$$

reaction 6: formation of the inhibited substrate-enzymatic complex

$$I + S \bullet E \xrightarrow{k_6} I \bullet E \bullet S \qquad r_6 = k_6 C_I C_{S \bullet E}$$

reaction 7: reverse of reaction 6

$$I \bullet E \bullet S \xrightarrow{k_7} I + S \bullet E \qquad r_7 = k_7 C_{I \bullet E \bullet S}$$

reaction 8: formation of the inhibited substrate-enzymatic complex

$$S + I \bullet E \xrightarrow{k_8} I \bullet E \bullet S \qquad r_8 = k_8 C_S C_{I \bullet E}$$

reaction 9: reverse of reaction 8

$$I \bullet E \bullet S \xrightarrow{k_9} S + I \bullet E \qquad r_9 = k_9 C_{I \bullet E \bullet S}$$

The rate of the formation of the product is still the rate of reaction 3.

We use the PSSH to determine the concentrations of both the substrate-enzyme and inhibitor-enzyme and inhibitor-substrate enzyme complexes.

$$S \bullet E: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,S\bullet E} r_i = r_1 - r_2 - r_3 - r_6 + r_7$$

$$S \bullet E: \qquad 0 = k_1 C_S C_E - k_2 C_{S\bullet E} - k_3 C_{S\bullet E} - k_6 C_I C_{S\bullet E} + k_7 C_{I\bullet E\bullet S}$$

$$I \bullet E: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,I\bullet E} r_i = r_4 - r_5 - r_8 + r_9 = k_4 C_I C_E - k_5 C_{I\bullet E} - k_8 C_S C_{I\bullet E} + k_9 C_{I\bullet E\bullet S}$$

$$I \bullet E \bullet S: \qquad 0 = \sum_{i=1}^{n_r} \nu_{i,I\bullet E\bullet S} r_i = r_6 - r_7 + r_8 - r_9 = k_6 C_I C_{S\bullet E} - k_7 C_{I\bullet E\bullet S} + k_8 C_S C_{I\bullet E} - k_9 C_{I\bullet E\bullet S}$$

Solve for the concentrations of $I \bullet E$ and $I \bullet E \bullet S$ first.

$$I \bullet E: \qquad C_{I\bullet E} = \frac{k_4 C_I C_E + k_9 C_{I\bullet E\bullet S}}{k_5 + k_8 C_S}$$

$$I \bullet E \bullet S: \qquad 0 = k_6 C_I C_{S\bullet E} - k_7 C_{I\bullet E\bullet S} + k_8 C_S \frac{k_4 C_I C_E + k_9 C_{I\bullet E\bullet S}}{k_5 + k_8 C_S} - k_9 C_{I\bullet E\bullet S}$$

$$C_{I\bullet E\bullet S} = \frac{k_6 C_I C_{S\bullet E} + \dfrac{k_4 k_8 C_S C_I C_E}{k_5 + k_8 C_S}}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9}$$

Substitute into balance for substrate complex

$$S \bullet E: \qquad 0 = k_1 C_S C_E - k_2 C_{S\bullet E} - k_3 C_{S\bullet E} - k_6 C_I C_{S\bullet E} + k_7 \frac{k_6 C_I C_{S\bullet E} + \dfrac{k_4 k_8 C_S C_I C_E}{k_5 + k_8 C_S}}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9}$$

Simplify

$$S \bullet E: \qquad \left(k_2 + k_3 + k_6 C_I - \frac{k_7 k_6 C_I}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9} \right) C_{S\bullet E} = \left(k_1 C_S + \frac{\dfrac{k_7 k_4 k_8 C_S C_I}{k_5 + k_8 C_S}}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9} \right) C_E$$

$$S \bullet E: \qquad C_E = \left(\frac{k_2 + k_3 + k_6 C_I - \dfrac{k_7 k_6 C_I}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9}}{k_1 C_S + \dfrac{\dfrac{k_7 k_4 k_8 C_S C_I}{k_5 + k_8 C_S}}{k_7 - \dfrac{k_9 k_8 C_S}{k_5 + k_8 C_S} + k_9}} \right) C_{S \bullet E}$$

Simplify

$$C_E = \left(\frac{\left((k_7 + k_9)(k_5 + k_8 C_S) - k_9 k_8 C_S\right)(k_2 + k_3 + k_6 C_I) - (k_5 + k_8 C_S) k_7 k_6 C_I}{\left((k_7 + k_9)(k_5 + k_8 C_S) - k_9 k_8 C_S\right) k_1 C_S + (k_5 + k_8 C_S) k_7 k_4 k_8 C_S C_I} \right) C_{S \bullet E}$$

The rate will be expressed in terms of the total enzyme concentration

$$C_{E_t} = C_E + C_{S \bullet E} + C_{I \bullet E} + C_{I \bullet E \bullet S}$$

Substitute in for E, $I \bullet E$ and $I \bullet E \bullet S$, and solve for $C_{S \bullet E}$ in terms of C_{E_t}. Substitute this expression in the rate of production. (I skipped all these algebraic steps, as did Fogler.)

The rate of production therefore becomes

$$r_3 = k_3 C_{S \bullet E}$$

$$r_3 = \frac{V_{\max} C_S}{(K_M + C_S)\left(1 + \dfrac{C_I}{K_I}\right)}$$ End Result from Fogler. Noncompetitive

Compare with:

$$r_3 = \frac{V_{\max} C_S}{C_S + \left(1 + \dfrac{C_I}{K_I}\right) K_M}$$ Competitive

$$r_3 = \frac{V_{\max} C_S}{K_M + \left(1 + \dfrac{C_I}{K_I}\right) C_S}$$ Uncompetitive

Substrate Inhibition: In any of these cases, the inhibitor could be the substrate. Substitute S for I in these results for rate law.

Enzyme co-factors: In many enzymatic reactions, you need two substrates. A rate law could be derived following these same procedures.

Bioreactors

A bioreactor is a reactor that sustains and supports life for cells and tissue cultures.

reaction

cells + substrate → more cells + product

Monod equation for cell growth

$$r_g = \mu C_C$$

where r_g, is the rate of growth in grams/liter/second
μ, is specific growth rate in 1/second
C_C, is the cell concentration in grams/liter

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

where C_S, is the substrate concentration in grams/liter
K_S, is the Monod constant in grams/liter

Monod equation for bacterial cell growth rate

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

Compare to the Michaelis-Menten equation for enzymatic reaction rates without inhibition.

$$-\frac{dC_S}{dt} = \frac{V_{\max} C_S}{K_M + C_S}$$

In the case of many substrates, the concentration of the limiting substrate is used.

In the presence of inhibition, an empirical factor is added

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

where $k_{obs} = \left(1 - \frac{C_p}{C_p^*}\right)^n$,

C_p is concentration of product

C_p^* is concentration of product where metabolism ceases

n is an empirical constant.

There are other empirical growth equations as well:

$$r_g = \mu_{\max} C_C \left[1 - \exp\left(-\frac{C_S}{k}\right)\right] \qquad \text{Tessier equation}$$

$$r_g = \mu_{\max} C_C \left[\frac{1}{1 + kC_S^{-\lambda}}\right] \qquad \text{Moser equation}$$

Cell death rate

$$r_d = (k_d + k_t C_t) C_C$$

where k_d is an environmental cell death rate

k_t is a toxicity death rate

C_t is the concentration of the toxin

Growth and death are a strong function of temperature.

Stoichiometry

Cells + Substrate → more cells + Product

We don't have clean stoichiometry.
Instead, we shall use yield coefficients,

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

$$Y_{c/s} = \frac{1}{Y_{s/c}}$$

Product formation can take place during different phases of the cell growth cycle.

Product formation during the exponential growth phase

$$r_p = Y_{p/c} r_g = Y_{p/c} \mu C_C = Y_{p/c} \mu_{\max} C_C \frac{C_S}{K_S + C_S}$$

Product formation during the stationary phase where no cell growth occurs, we relate product growth to substrate consumption

$$r_p = -Y_{p/s} r_s$$

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

Part of the substrate must be used to maintain a cell's daily activities: maintenance utilization term

$$m = \frac{mass\ of\ substrate\ consumed\ for\ maintenance}{mass\ of\ cells \cdot time}$$

typical value m = 0.05 g S per g dry weight per hour

The rate of substrate consumption for maintenance

$$r_{sm} = mC_C$$

If it is possible to sort out the S consumed for cell growth and S consumed for P then

$$S \rightarrow Y'_{c/s}\, C + Y'_{p/s}\, P$$

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

$$Y'_{c/s} = \frac{mass\ of\ new\ cells\ formed}{mass\ of\ substrate\ consumed\ to\ form\ new\ cells}$$

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

$$Y'_{p/s} = \frac{mass\ of\ product\ formed}{mass\ of\ substrate\ consumed\ to\ form\ product}$$

substrate utilization

balance

acculumation = in – out + generation

assume batch reactor – no in and no out
assume steady state – no accumulation

net generation = rate consumed by cells + rate consumed to form product + rate consumed for maintenance

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

During the growth phase, you may not be able to separate purpose of substrate consumption

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

The product rate of formation is then

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

During stationary phase, growth is zero and these equations don't work

$$r_p = k_p C_C \frac{C_{Sn}}{K_{Sn} + C_{Sn}} \text{ (Monod equation)}$$

Sn = secondary nutrient

$$-r_{Sn} = Y_{Sn/p} r_p + mC_C = Y_{Sn/p} k_p C_C \frac{C_{Sn}}{K_{Sn} + C_{Sn}} + mC_C$$

Determine yield coefficients experimentally.
These equations apply for two asymptotic cases. Production during exponential growth phase. Production during the stationary phase. Some reactors don't fall into either of these asymptotic cases.

Mass Balances

chemostat = CSTR with microorganisms

acc = in – out + generation

Cell balance

$$V\frac{dC_C}{dt} = F_{in}C_{C,in} - F_{out}C_C + V\left(r_g - r_d\right)$$

Cell balance has a growth and death term. Usually, $C_{C,in} = 0$.

substrate balance

$$V\frac{dC_S}{dt} = F_{in}C_{S,in} - F_{out}C_S + Vr_s$$

For a batch reactor, no in and out terms.

Cell Balance

$$V\frac{dC_C}{dt} = V\left(r_g - r_d\right)$$

Substrate balance

growth phase

$$V\frac{dC_S}{dt} = Vr_s = -VY_{s/c}r_g - VmC_C$$

stationary phase

$$V\frac{dC_S}{dt} = Vr_s = -VY_{s/p}r_p - VmC_C$$

product

$$V\frac{dC_P}{dt} = Vr_p = -VY_{p/s}r_s$$ (seems obviously incorrect since some substrate is used for maintenance, not production)

This seems better:

$$V\frac{dC_P}{dt} = Vr_p = -VY'_{p/s}r_s$$ (seems obviously incorrect since)

Other notes:

dilution rate

$$D = \frac{F}{V} = \frac{1}{\tau_R}$$

CSTRs at steady state

cells: $$\frac{dC_C}{dt} = 0 = DC_{C,in} - DC_C + r_g - r_d$$

substrate: $$\frac{dC_S}{dt} = 0 = DC_{S,in} - DC_S - r_s$$

substrate: $$\frac{dC_P}{dt} = 0 = DC_{P,in} - DC_P + r_p$$

These are algebraic equations.

The dilution rate can be adjusted to optimize reactor performance.