Chemical Reactor Design

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化學反應裝置設計

第7章

Chemical Reactor Design

Reaction Mechanisms, Pathways, Bioreactions and Bioreactors

Objective

- Discuss the *pseudo-steady-state-hypothesis* and explain how it can be used to solve reaction engineering problems
- Write *reaction pathways* for complex reactions.
- Explain what an *enzyme* is and how it acts as a catalyst.
- Describe *Michealis-Menten enzyme kinetics* and rate law along with its temperature dependence.
- Discss how to distinguish the different types of *enzyme inhibition*.
- Discuss stages of cell growth and rate laws used to describe growth.
- Write material balances on cells, substrates, and products in bioreactors to size chemostats and plot concentration-time trajectoriesin batch reactors.
- Describe how physiologically-based pharmocokinetic models can be used to model alcohol metabolism.

7.1 Active Intermediates and Nonelementary Rate law

- **Elementary rate law**

  - the reaction order of each species is identical with the stoichiometric coefficient of that species for the reaction as written

$$-r_A = kC_A^n$$

- **Non-elementary rate law**

  - no direct correspondence between reaction order and stoichiometry

$CH_3CHO \rightarrow CH_4 + CO$ $\qquad -r_{CH_3CHO} = kC_{CH_3CHO}^{3/2}$

$H_2 + Br_2 \rightarrow 2HBr$ $\qquad -r_{HBr} = \dfrac{k_1 C_{H_2} C_{Br_2}^{3/2}}{C_{HBr} + k_2 C_{Br}}$

Non-elementary Reaction

Non-elementary rate laws involve a number of elementary reactions and at least one 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. ***Active intermediates*** (e.g., A*) can be formed by collision or interaction with other molecules.

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

Here the activation occurs when translational kinetic energy is transferred into energy stored in internal degrees of freedom, particularly vibrational degrees of freedom.

7.1.1 Pseudo-Steady-State Hypothesis (PSSH)

Because a reactive intermediate reacts virtually as fast as it is formed, the net rate of formation of an active intermediate (e.g., A*) is zero, i.e.,

$$r_{A^*} \equiv 0$$

This condition is also referred to as the **Pseudo-Steady-State Hypothesis (PSSH).** If the active intermediate (e.g., A*) appears in n reactions, then

$$r_{A^*} = \sum_{i-1}^{n} r_{iA^*} = 0$$

Pseudo-Steady-State-Hypothesis (PSSH)

Let's consider the gas-phase decomposition of azomethane, AZO, to give ethane and nitrogen.

$$\underset{\text{azomethane (AZO)}}{(CH_3)_2N_2} \longrightarrow \underset{\text{ethane}}{C_2H_6} + \underset{\text{nitrogen}}{N_2}$$

Experimental observation shows that:

High concentration (> 1atm)	$r_{C_2H_6} \sim C_{AZO}$
Low concentration (< 50 mmHg)	$r_{C_2H_6} \sim C_{AZO}^2$

How to explain this first and second order depending on the concentration of AZO?

New proposed mechanism that consisting of three elementary reactions.

Proposed mechanism

rxn 1: $(CH_3)_2N_2 + (CH_3)_2N_2 \xrightarrow{k_1} (CH_3)_2N_2 + [(CH_3)_2N_2]^*$

rxn 2: $[(CH_3)_2N_2]^* + (CH_3)_2N_2 \xrightarrow{k_2} (CH_3)_2N_2 + (CH_3)_2N_2$

rxn 3: $[(CH_3)_2N_2]^* \xrightarrow{k_3} C_2H_6 + N_2$

$$r_{1,AZO^*} = k_1 C_{AZO}^2$$

$$r_{2,AZO^*} = -k_2 C_{AZO^*} C_{AZO}$$

$$r_{3,AZO^*} = -k_3 C_{AZO^*}$$

$$-r_{AZO^*} = \sum_{i=1}^{3} r_{i,AZO^*} = k_1 C_{AZO}^2 - k_2 C_{AZO} C_{AZO^*} - k_3 C_{AZO^*}$$

The concentration of the active intermediate, AZO*, is very difficult to measure, because it is highly reactive and very short-lived (~10^{-9} second).
We need to express C_{AZO}* in terms of C_{AZO}.

Pseudo-Steady-State Hypothesis (PSSH)

1. Life time of active intermediate ~0
2. Active intermediate is present only in low concentrations

rate of formation of the active intermediate= rate of disappearance

Net rate of formation of the active intermediate = 0

$$-r_{AZO*} = 0 = k_1 C_{AZO}^2 - k_2 C_{AZO} C_{AZO*} - k_3 C_{AZO*} \quad \textbf{(7-7)}$$

$$C_{AZO*} = \frac{k_1 C_{AZO}^2}{k_2 C_{AZO} + k_3} \quad \textbf{(7-8)}$$

$$r_{C_2H_6} = k_3 C_{AZO*} = \frac{k_1 k_3 C_{AZO}^2}{k_2 C_{AZO} + k_3} \quad \textbf{(7-9)}$$

$$r_{N_2} = \frac{k_1 k_3 C_{AZO}^2}{k_3 + k_2 C_{AZO}}$$

Low AZO Conc.
$k_2 C_{AZO} \ll k_3$

High AZO Conc.
$k_2 C_{AZO} \gg k_3$

$$r_{N_2} = k_1 C_{AZO}^2$$

$$r_{N_2} = \frac{k_1 k_3}{k_2} C_{AZO} = k C_{AZO}$$

@ low concentration
apparent second order

@ high concentration
apparent first order

It is normal procedure to reduce the additive constant in the denominator to 1

$$r_{N_2} = \frac{k_1 k_3 C_{AZO}^2}{k_3 + k_2 C_{AZO}} \Rightarrow r_{N_2} = \frac{k_1 C_{AZO}^2}{1 + k' C_{AZO}}$$

additive constant

7.1.2 Searching for a Mechanism

In many instances the rate data are correlated before a mechanism is found.

Rules of Thumb for Development of a Mechanism

1. Species having the concentration appearing in the *denominator* of the rate law probably collide with the active intermediate, e.g.,

$$A + A^* \longrightarrow [\text{collision products}]$$

2. If a constant appears in the *denominator*, one of the reaction steps is probably the spontaneous decomposition of the active intermediate, e.g.,

$$A^* \longrightarrow [\text{decomposition products}]$$

3. Species having the concentration appearing in the *numerator* of the rate law probably produce the active intermediate in one of the reaction step, e.g.,

$$[\text{reactant}] \longrightarrow A^* + [\text{other products}]$$

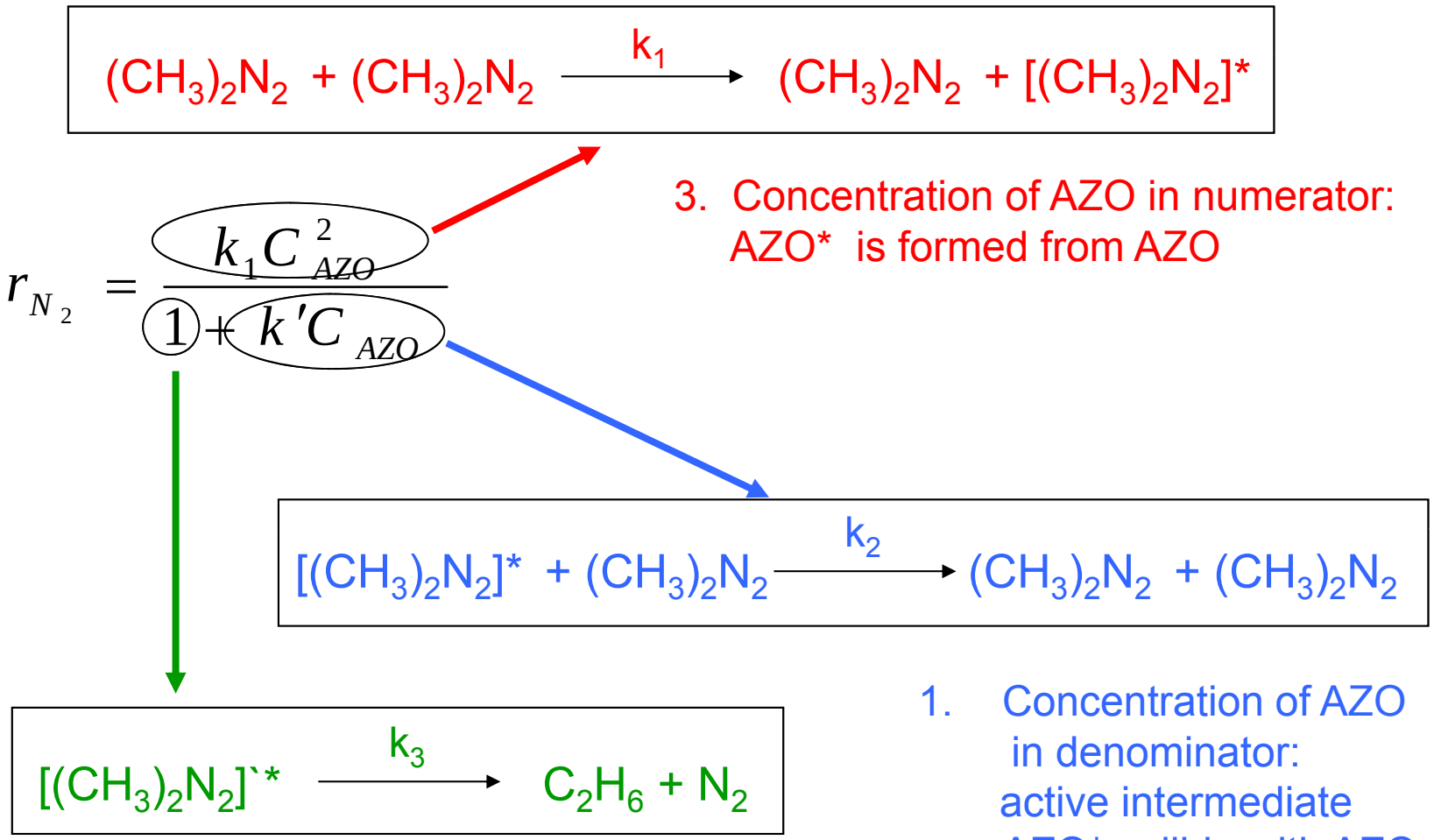
Rules of Thumb for Development of a Mechanism

Upon application of Table 7-1 to azomethane example just discussed, we see the following from rate equation (7-12)

$$r_{C_2H_2} = \frac{k_1 C_{AZO}^{\ 2}}{1 + k' C_{AZO}}$$

1. The active intermediate, AZO*, collides with azomethane, AZO [Reaction 2], resulting in the concentration of AZO in the denominator.
2. AZO* decomposes spontaneously [Reaction 3], resulting in a constant in the denominator of the rate expression.
3. The appearance of AZO in the numerator suggests that the active intermediate AZO* is formed from AZO. Referring to [Reaction 1], we see that this case is indeed true.

Rules of Thumb for Development of a Mechanism



Steps to deduce a rate law

1. Assume an activated intermediate(s)
2. Postulate a mechanism, utilizing the rate law obtained from experimental data, if possible.
3. Model each reaction in the mechanism sequence as an elementary reaction.
4. After writing rate laws for the rate of formation of desired product, write the rate laws for each of the active intermediates.
5. Use the PSSH
6. Eliminate the concentration of the intermediate species in the rate laws by solving the simultaneous equations developed in step 4 and 5.
7. If the derived rate law does not agree with experimental observation, assume a new mechanism and/or intermediates and go to step 3. A strong background in organic and organic chemistry is helpful in predicting the activated intermediates for the reaction under consideration.

7.1.3 Steps in a Chain Reaction

A chain reaction consists of following sequence:

1. ***Initiation*** : formation of an active intermediate

2. ***Propagation or chain transfer*** : interaction of an active intermediate with the reactant or product to produce another active intermediate

3. ***Termination*** : deactivation of the active intermediate

Example 7-2 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

(1) $C_2H_6 \xrightarrow{k_1} 2CH_3\bullet$ $\quad -r_{1,\,C2H6}=k_1[C_2H_6]$

Propagation

(2) $CH_3\bullet + C_2H_6 \xrightarrow{k_2} CH_4 + C_2H_5\bullet$ $\quad -r_{2,\,C2H6}=k_2[CH_3\bullet][C_2H_6]$

(3) $C_2H_5\bullet \xrightarrow{k_3} C_2H_4 + H\bullet$ $\quad -r_{3,\,C2H4}=k_3[C_2H_5\bullet]$

(4) $H\bullet + C_2H_6 \xrightarrow{k_4} C_2H_5\bullet + H_2$ $\quad -r_{4,\,C2H6}=k_4[H\bullet][C_2H_6]$

Termination

(5) $2C_2H_5\bullet \xrightarrow{k_5} C_4H_{10}$ $\quad -r_{5,\,C2H5\bullet}=k_5[C_2H_5\bullet]^2$

(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 ODE mole balances.

Solution

(a) Developing the Rate Law

rate of formation of ethylene: $\boxed{r_{3,C_2H_4} = k_3[C_2H_5\bullet]}$ (1)

for the active intermediates: $CH_3\bullet$, $C_2H_5\bullet$, $H\bullet$ the net rates of rxn are

PSSH

$$r_{C_2H_5\bullet} = r_{2,C_2H_5\bullet} + r_{3,C_2H_5\bullet} + r_{4,C_2H_5\bullet} + r_{5,C_2H_5\bullet} = 0$$

$$= -r_{2,C_2H_6} - \underbrace{r_{3,C_2H_4} - r_{4,C_2H_6}}_{0} + r_{5,C_2H_5\bullet} = 0 \quad (2)$$

$$r_{H\bullet} = r_{3,C_2H_4} + r_{4,C_2H_6} = 0 \quad (3)$$

$$r_{CH_3\bullet} = -2r_{1,C_2H_6} + r_{2,C_2H_6} = 0 \quad (4)$$

Substituting the rate laws into (4)

$$2k_1[C_2H_6] - k_2[CH_3\bullet][C_2H_6] = 0 \quad (5)$$

$$[CH_3\bullet] = \frac{2k_1}{k_2} \quad (6)$$

(2)+(3) yields $-r_{2,C_2H_6} + r_{5,C_2H_5\bullet} = 0$

$$k_2[CH_3\bullet][C_2H_6] - k_5[C_2H_5\bullet]^2 = 0 \quad (7)$$

Solving for $[C_2H_5\bullet]$ gives us

$$[C_2H_5\bullet] = \left\{\frac{k_2}{k_5}[CH_3\bullet][C_2H_6]\right\}^{1/2} = \left\{\frac{2k_1k_2}{k_2k_5}[C_2H_6]\right\}^{1/2}$$

$$= \left\{\frac{2k_1}{k_5}[C_2H_6]\right\}^{1/2} \qquad (8)$$

(8) → (1)

$$\boxed{r_{C_2H_4} = k_3[C_2H_5\bullet] = k_3\left(\frac{2k_1}{k_5}\right)^{1/2}[C_2H_6]^{1/2}} \qquad (9)$$

Rate of disappearance of ethane

$$r_{C_2H_6} = -k_1[C_2H_6] - k_2[CH_3\bullet][C_2H_6] - k_4[H\bullet][C_2H_6] \qquad (10)$$

$$k_3[C_2H_5\bullet] - k_4[H\bullet][C_2H_6] = 0 \qquad (3)$$

$$[H\bullet] = \frac{k_3}{k_4}\left(\frac{2k_1}{k_5}\right)^{1/2}[C_2H_6]^{-1/2} \qquad (11)$$

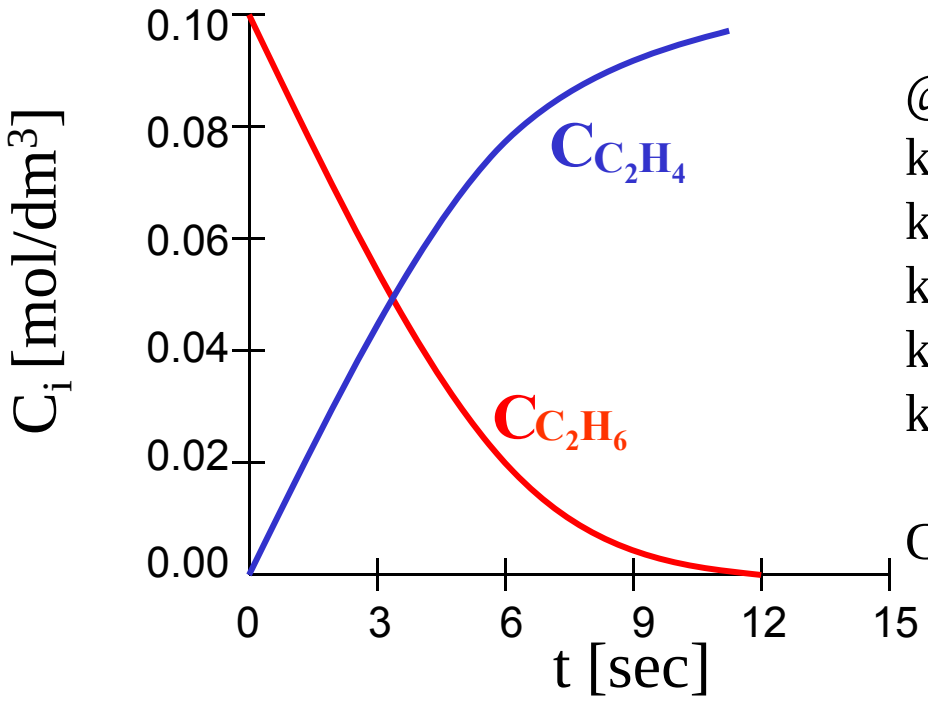
$$\boxed{-r_{C_2H_6} = 3k_1[C_2H_6] + k_3\left(\frac{2k_1}{k_5}\right)^{1/2}[C_2H_6]^{1/2}} \qquad (12)$$

For a constant-volume batch reactor,

Combined Mole balances & rate laws

$$\frac{d[C_2H_6]}{dt} = r_{C_2H_6} = -3k_1[C_2H_6] - k_3\left(\frac{2k_1}{k_5}\right)^{1/2}[C_2H_6]^{1/2}$$

$$\frac{d[C_2H_4]}{dt} = r_{C_2H_4} = k_3\left(\frac{2k_1}{k_5}\right)^{1/2}[C_2H_6]^{1/2}$$



@1000K
k_1=1.5x10^{-3} s^{-1}
k_2=2.3x10^6 dm^3/mol•s
k_3=5.71x10^4 s^{-1}
k_4=9.53x10^8 dm^3/mol•s
k_5=3.98x10^9 dm^3/mol•s

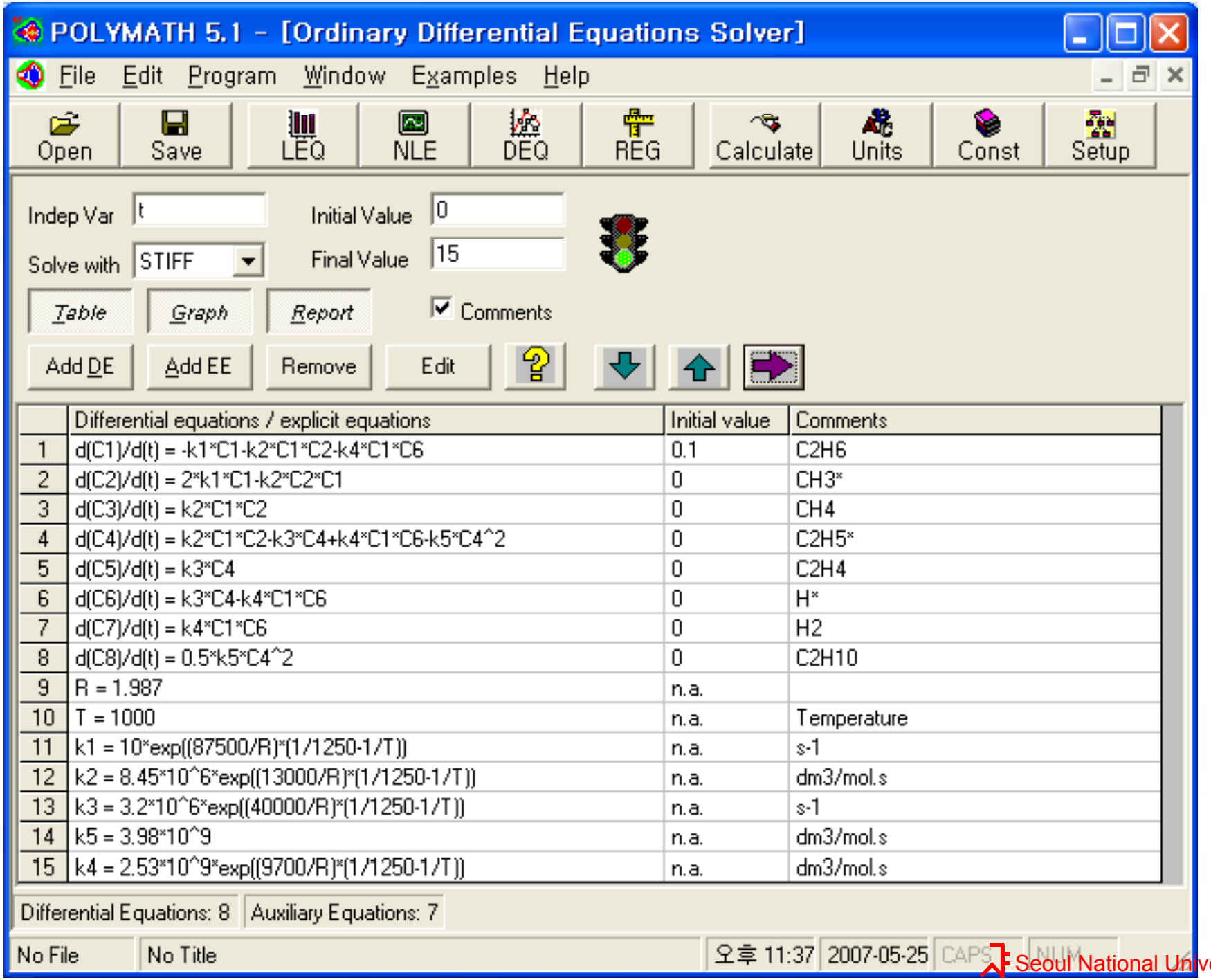
$C_{0,\ C2H6}$=0.1mol/dm^3

Concentration of Intermediates

$$[CH_3\bullet] = \frac{2k_1}{k_2} = \frac{2\times 1.5\times 10^{-3}\,s^{-1}}{2.3\times 10^{6}\,dm^3/mol\cdot s} = 1.3\times 10^{-9}\,\frac{mol}{dm^3}$$

$$[C_2H_5\bullet] = \sqrt{\frac{2k_1}{k_5}[C_2H_6]} = \sqrt{\frac{2\times 1.5\times 10^{-3}\,s^{-1}}{3.98\times 10^{9}\,dm^3/mol\cdot s}[C_2H_6]} = 8.68\times 10^{-7}\sqrt{[C_2H_6]}\,\frac{mol}{dm^3}$$

$$[H\bullet] = \sqrt{\frac{2k_1k_3^2}{k_4^2k_5[C_2H_6]}} = \sqrt{\frac{2\times 1.5\times 10^{-3}\times (5.71\times 10^{4})^2}{(9.53\times 10^{8})^2\times 3.98\times 10^{9}}\frac{1}{[C_2H_6]}} = 5.2\times 10^{-11}\sqrt{\frac{1}{[C_2H_6]}}\,\frac{mol}{dm^3}$$



POLYMATH 5.1 - [Differential Equations Solution #3]

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POLYMATH Results

05-25-2007, Rev5.1.233

Calculated values of the DEQ variables

Variable	initial value	minimal value	maximal value	final value
t	0	0	15	15
C1	0.1	4.445E-23	0.1	4.445E-23
C2	0	0	1.311E-09	1.311E-09
C3	0	0	0.0012476	0.0012476
C4	0	0	2.665E-07	1.858E-21
C5	0	0	0.0981286	0.0981286
C6	0	0	1.62E-08	1.62E-08
C7	0	0	0.0981286	0.0981286
C8	0	0	6.238E-04	6.238E-04
R	1.987	1.987	1.987	1.987
T	1000	1000	1000	1000
k1	0.0014964	0.0014964	0.0014964	0.0014964
k2	2.283E+06	2.283E+06	2.283E+06	2.283E+06
k3	5.71E+04	5.71E+04	5.71E+04	5.71E+04
k5	3.98E+09	3.98E+09	3.98E+09	3.98E+09
k4	9.53E+08	9.53E+08	9.53E+08	9.53E+08

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POLYMATH 5.1 - [Differential Equations Solution #3]

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ODE Report (STIFF)

Differential equations as entered by the user

[1] d(C1)/d(t) = -k1*C1-k2*C1*C2-k4*C1*C6
[2] d(C2)/d(t) = 2*k1*C1-k2*C2*C1
[3] d(C3)/d(t) = k2*C1*C2
[4] d(C4)/d(t) = k2*C1*C2-k3*C4+k4*C1*C6-k5*C4^2
[5] d(C5)/d(t) = k3*C4
[6] d(C6)/d(t) = k3*C4-k4*C1*C6
[7] d(C7)/d(t) = k4*C1*C6
[8] d(C8)/d(t) = 0.5*k5*C4^2

Explicit equations as entered by the user

[1] R = 1.987
[2] T = 1000
[3] k1 = 10*exp((87500/R)*(1/1250-1/T))
[4] k2 = 8.45*10^6*exp((13000/R)*(1/1250-1/T))
[5] k3 = 3.2*10^6*exp((40000/R)*(1/1250-1/T))
[6] k5 = 3.98*10^9
[7] k4 = 2.53*10^9*exp((9700/R)*(1/1250-1/T))

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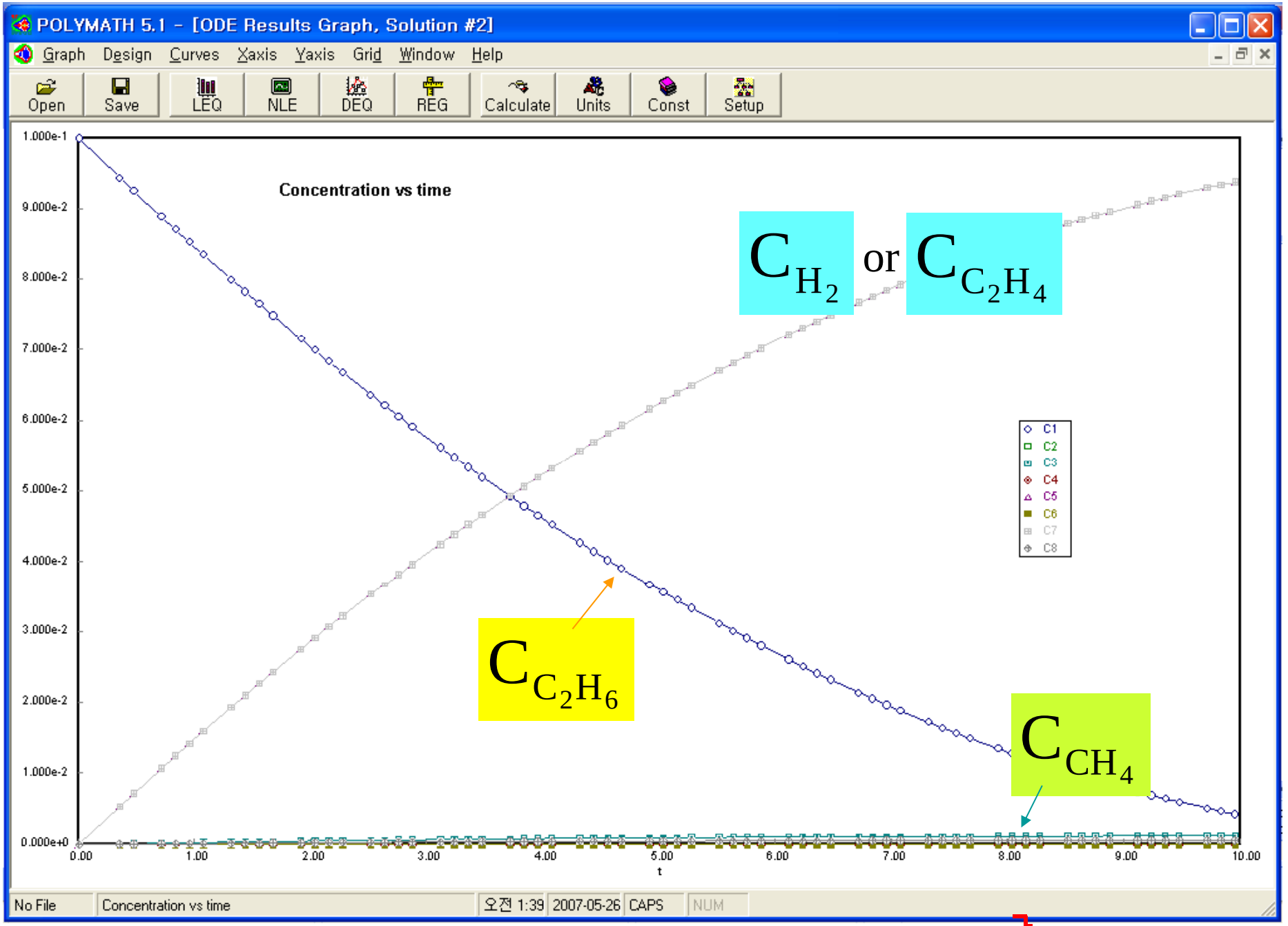
POLYMATH 5.1 - [ODE Results Graph, Solution #2]
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Open Save LEQ NLE DEQ REG Calculate Units Const Setup
Concentration vs time
C_{H_2} or $C_{C_2H_4}$
$C_{C_2H_6}$
C_{CH_4}
C1 C2 C3 C4 C5 C6 C7 C8
1.000e-1
9.000e-2
8.000e-2
7.000e-2
6.000e-2
5.000e-2
4.000e-2
3.000e-2
2.000e-2
1.000e-2
0.000e+0
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
t
No File
Concentration vs time
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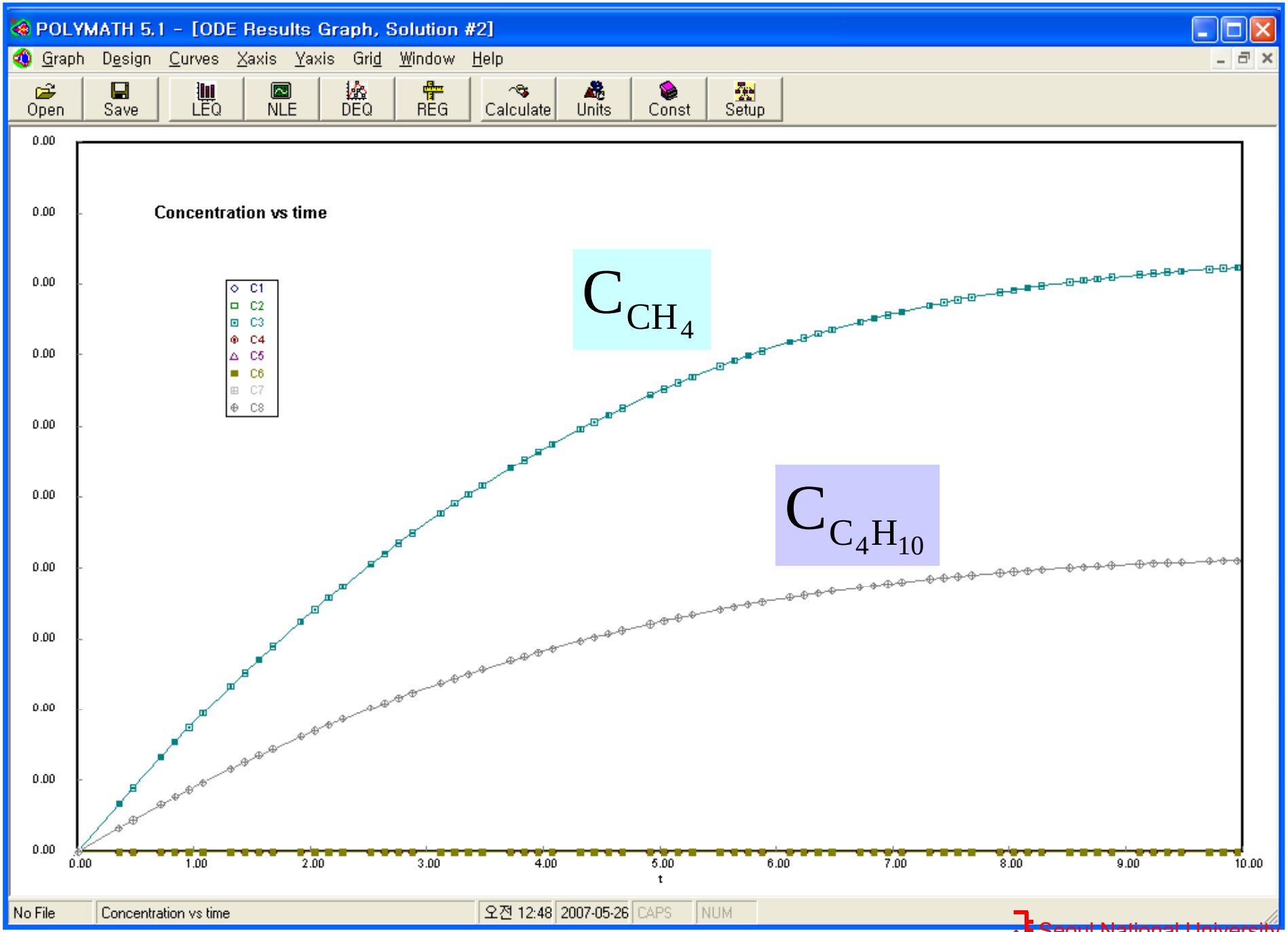

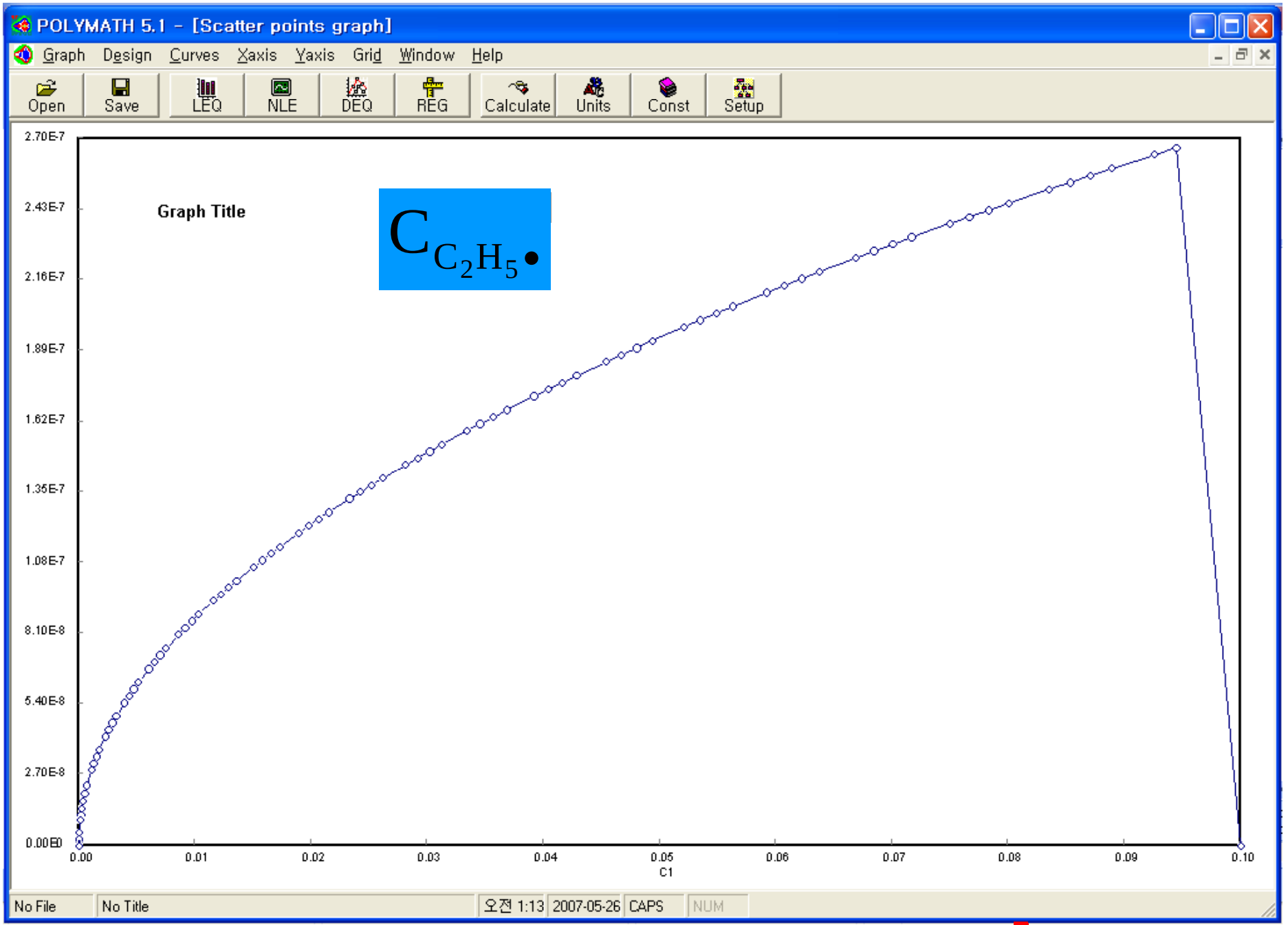

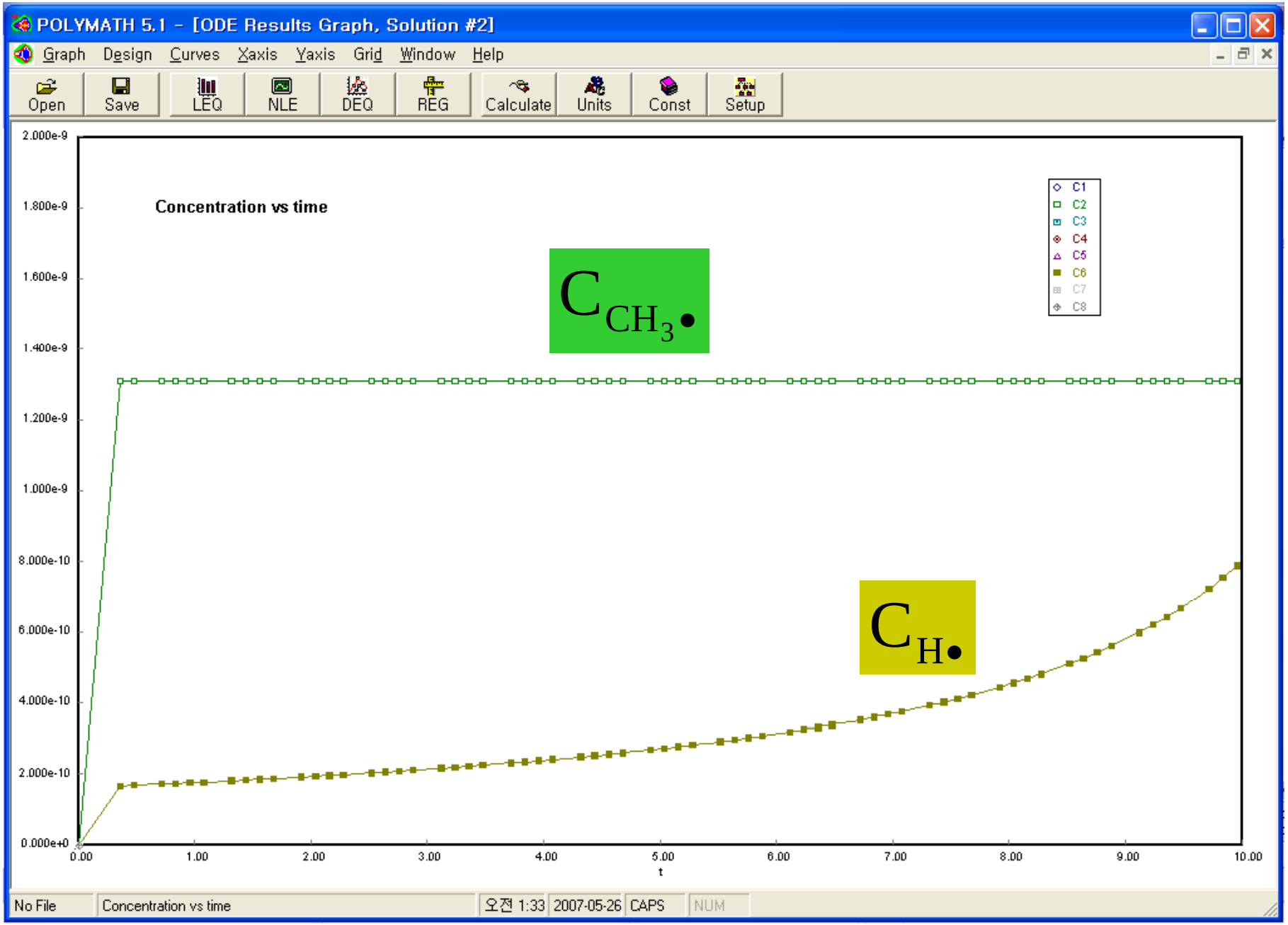
POLYMATH 5.1 - [ODE Results Graph, Solution #2]
Graph Design Curves Xaxis Yaxis Grid Window Help
Open Save LEQ NLE DEQ REG Calculate Units Const Setup
Concentration vs time
$C_{CH_3\bullet}$
$C_{H\bullet}$
C1 C2 C3 C4 C5 C6 C7 C8
2.000e-9
1.800e-9
1.600e-9
1.400e-9
1.200e-9
1.000e-9
8.000e-10
6.000e-10
4.000e-10
2.000e-10
0.000e+0
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
t
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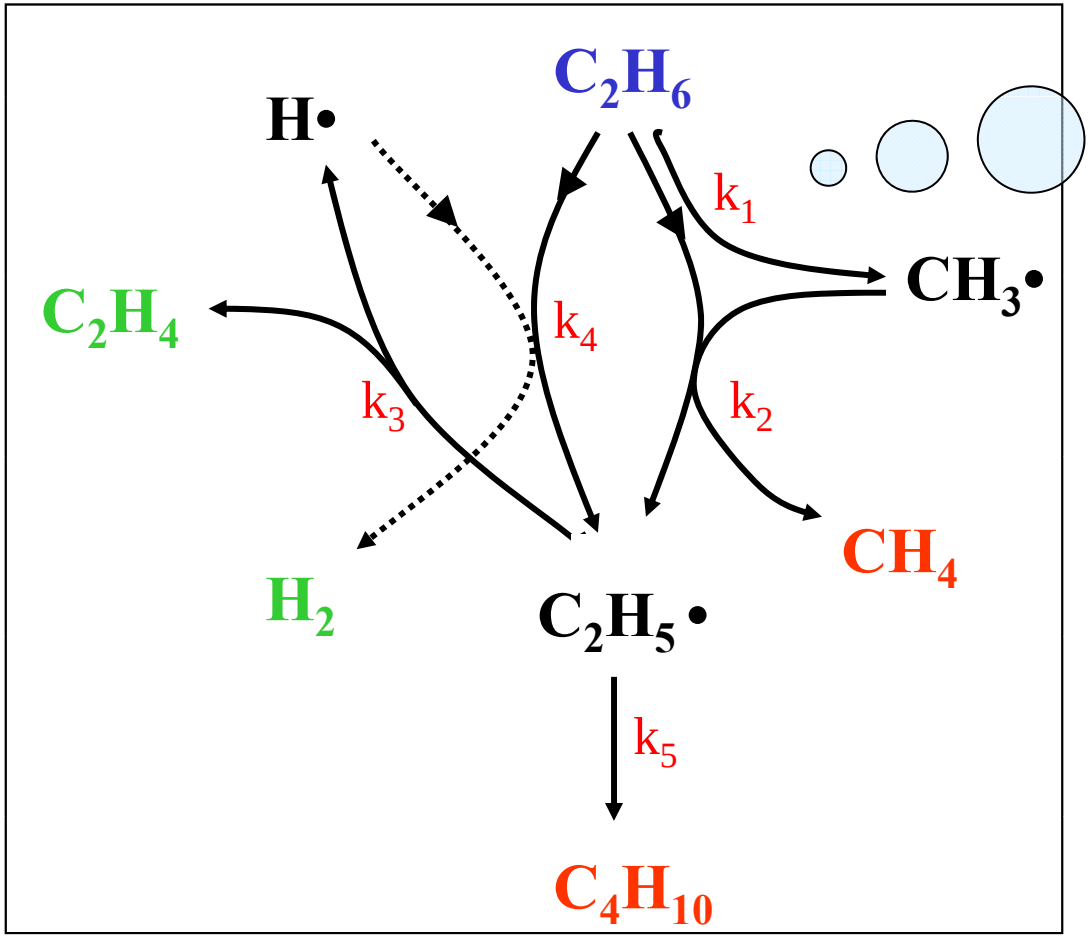
Reaction Pathways of Ethane Cracking

Reaction pathways help see the connection of all interesting species for multiple reaction.



Key is to identify which intermediate reactions are important in the overall sequence in predicting the end products.

Reaction Pathways in smog formation

Nitrogen and oxygen react to form nitric oxide in the cylinder of automobile engines. The NO from automobile exhaust is oxidized to NO2 in the presence of peroxide radicals.

$$R\dot{O}O + NO \xrightarrow{k_1} R\dot{O} + NO_2$$

Nitrogen dioxide is then decomposed photochemically to give nascent oxygen

$$NO_2 + h\nu \xrightarrow{k_2} NO + O$$

which reacts to form ozone

$$O + O_2 \xrightarrow{k_3} O_3$$

The ozone then becomes involved in a whole series of reactions with hydrocarbons in the atmosphere to form aldehydes, various free radicals, and other intermediates, which react further to produce undesirable products in air pollution:

$$O_3 + RCH = CHR \xrightarrow{k_4} RCHO + \dot{R}O + H\dot{C}O$$

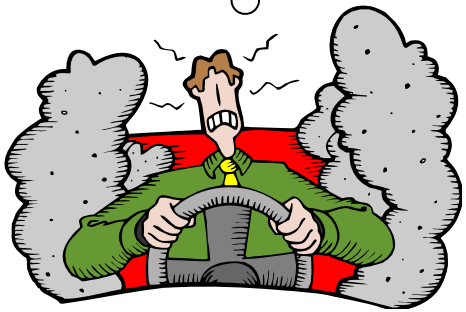
$$RCHO \xrightarrow{k_5} \dot{R} + H\dot{C}O$$

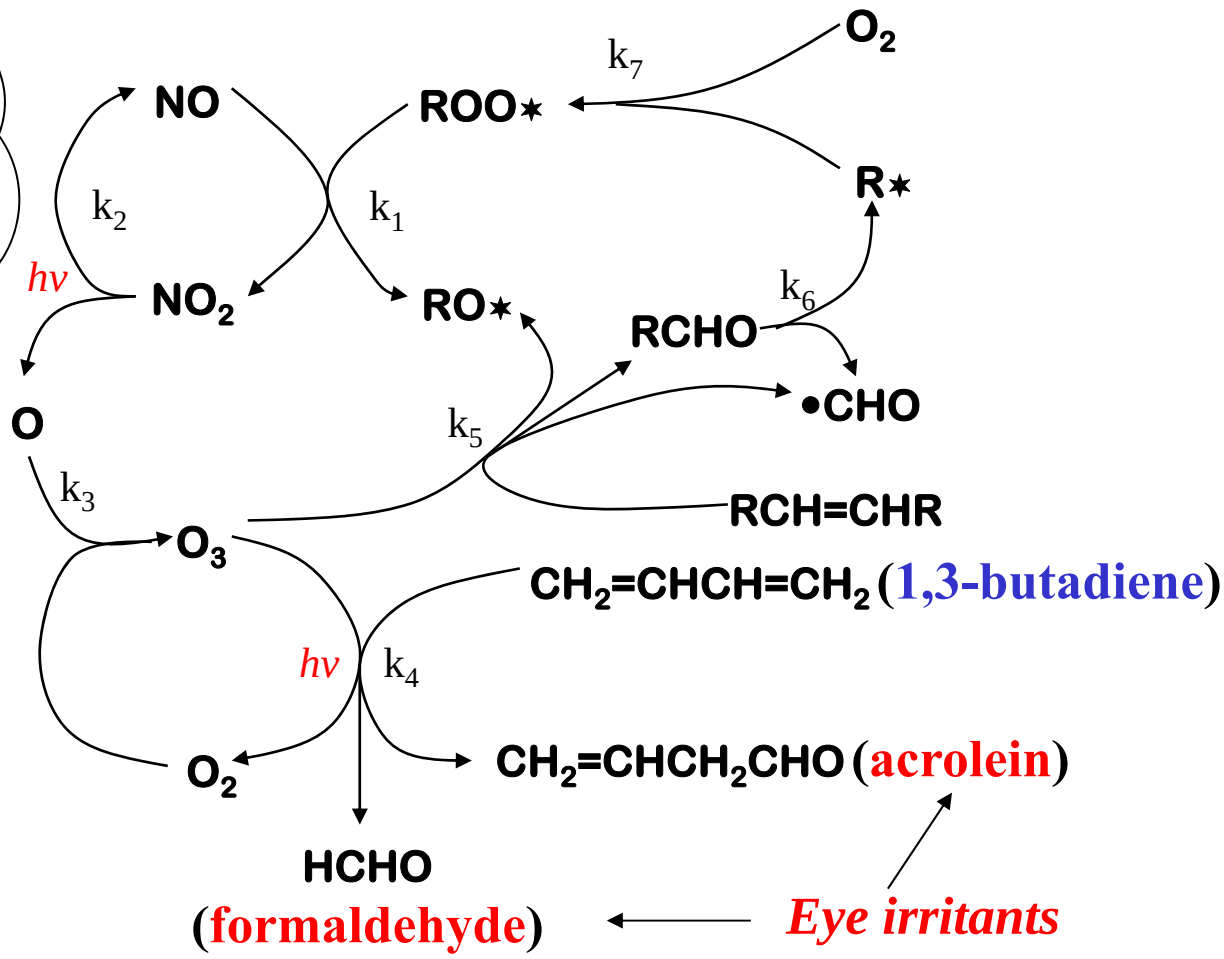
$$\tfrac{2}{3}O_3 + CH_2 = CHCH = CH_2 \xrightarrow{k_6} CH_2 = CHCHO + HCHO$$

$$\dot{R} + O_2 \xrightarrow{k_7} R\dot{O}O$$

Reaction Pathways in smog formation

The cycle has been completed and that with a relative small amount of NO, a large amount of pollutants can be produced.





Smog

Smog

Claude Monet made several trips to London between 1899 and 1901, during which he painted views of the Thames and Houses of Parliament which show the sun struggling to shine through London's smog-laden atmosphere.

7.2 Enzymatic Reaction Fundamentals

- Another class of reactions in which the PSSH is used is the enzymatically catalyzed reaction, which is characteristic of most biological reactions.

$$\mathbf{E+S \leftrightarrow E \bullet S \rightarrow E+P}$$

- **E**: Enzyme is a protein or proteinlike substances with catalytic properties.

- **S**: Substance that is chemically transformed at an accelerated rate because of the action of the enzyme on it.

- **E•S**: active intermediate, enzyme-substrate complex

7.2 Enzymatic Reaction Fundamentals

- It provides a pathway for the substrate to proceed at a faster rate, usually 10^3 to 10^{17} times faster than the uncatalyzed rate.
- Enzymes are usually present in small quantities and are not consumed during the course of the reaction nor do they affect the chemical reaction equilibrium.
- Enzymes provide an alternate pathway for the reaction to occur thereby requiring a lower activation energy.
- Degradation of urea by urease where the degradation rate is on the order of 10^{14} higher than without urease.

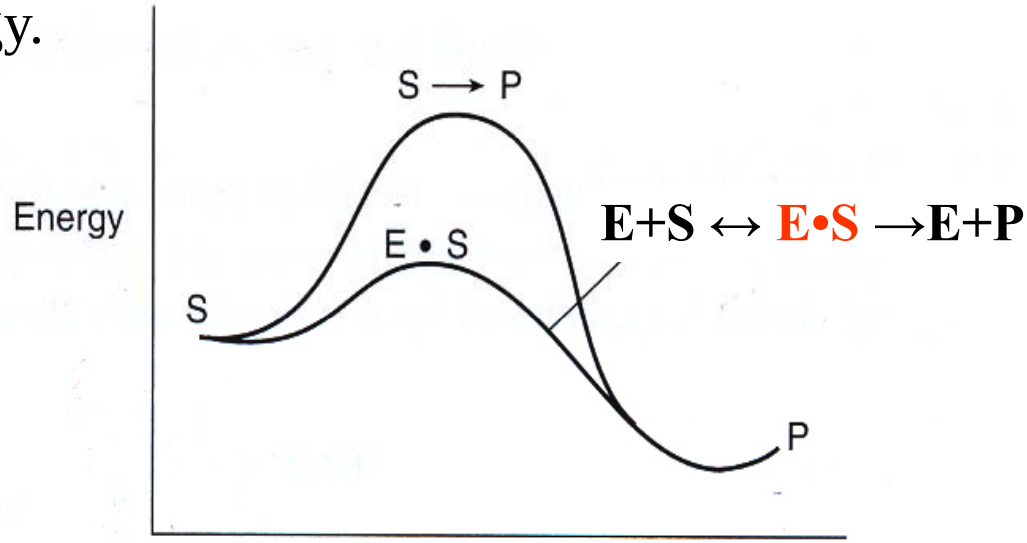


Figure 7-4 Reaction coordinate for enzyme catalysis.

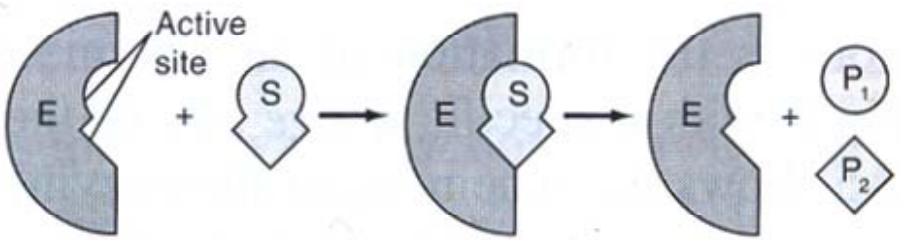
7.2 Enzymatic Reaction Fundamentals

- Enzyme is specific in that one enzyme can usually catalyze only one type of reaction. Consequently, unwanted products are easily controlled.
  - Protease hydrolyze only bonds specific between specific amino acids in proteins
  - An amylase works on bonds between glucose molecules in starch
  - Lipase attacks fats, degrading them to fatty acids and glycerol.

- Enzymes usually work under mild conditions:
  pH 4~9 & temp 24~70^{o}C.

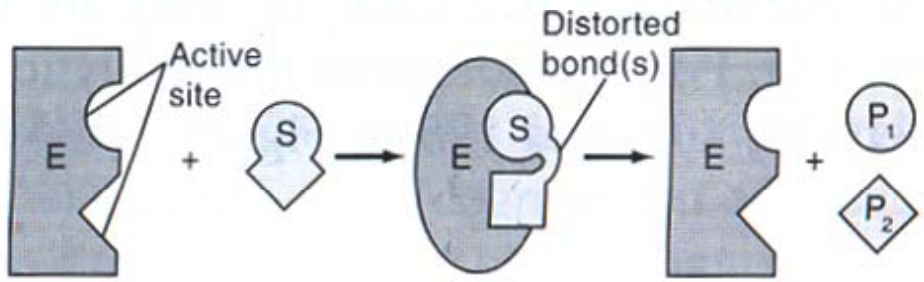
- Enzymes are produced only by living organisms, and commercial enzymes are generally produced by bacteria.

Two models for substrate-enzyme interactions



Lock and Key Model

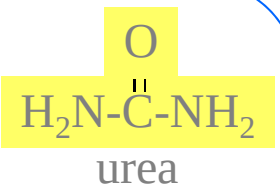
For many years, the **lock and key model** was preferred because of sterospecific effects of one enzyme acting on one substrate.



Induced Fit Model

However, the **induced fit model** is more useful model. In the induced fit model both the enzyme molecule and the substrate molecules are distorted. These changes in conformation distort one or more of the substrate bonds, thereby stressing and weakening the bond to make the molecule more susceptible to rearrangement or attachment.

7.2.2 Mechanisms



1. The enzyme urease (E) reacts with the substrate urea (S) to form an enzyme-substrate complex (E•S)

$$NH_2CONH_2 + urease \xrightarrow{k_1} [NH_2CONH_2 \cdot urease]^* \quad \textbf{(7-13)}$$

2. This complex can decompose back to urea(S) and urease (E)

$$[NH_2CONH_2 \cdot urease]^* \xrightarrow{k_2} urease + NH_2CONH_2 \quad \textbf{(7-14)}$$

3. Or it can react with water to give ammonia, carbon dioxide, and urease (E)

$$[NH_2CONH_2 \cdot urease]^* + H_2O \xrightarrow{k_3} 2NH_3 + CO_2 + urease \quad \textbf{(7-15)}$$

Letting

E : enzyme
S : substrate
W : water
E•S : enzyme-substrate complex
P : reaction product

(1) $E + S \xrightarrow{k_1} E \cdot S$ **(7-16)**

(2) $E \cdot S \xrightarrow{k_2} E + S$ **(7-17)**

(3) $E \cdot S + W \xrightarrow{k_3} P + E$ **(7-18)**

$Here\ P = 2NH_3 + CO_2$

$$r_{1S} = -k_1(E)(S) \quad \textbf{(7-16A)}$$

$$r_{2S} = k_2(E \cdot S) \quad \textbf{(7-17A)}$$

$$r_{3P} = k_3(E \cdot S)(W) \quad \textbf{(7-18A)}$$

The net rate of disappearance of the substrate

$$-r_S = k_1(E)(S) - k_2(E \cdot S) \qquad (7\text{-}19)$$

The net rate of formation of the enzyme-substrate complex

$$r_{E \cdot S} = k_1(E)(S) - k_2(E \cdot S) - k_3(W)(E \cdot S) \qquad (7\text{-}20)$$

Total enzyme concentration

$$(E_t) = (E) + (E \cdot S) \qquad (7\text{-}23)$$

Enzyme concentration

$$(E) = (E_t) - (E \cdot S) \qquad (7\text{-}23A)$$

using the PSSH for the enzyme complex

$$-r_{E\cdot S} = \mathbf{0} = k_1(E)(S) - k_2(E\cdot S) - k_3(W)(E\cdot S)$$

Solving for (E•S)

$$(E\cdot S) = \frac{k_1(E)(S)}{k_2 + k_3(W)} \qquad (7\text{-}21)$$

Substituting (7-21) into (7-19), then we get

$$-r_S = k_1(E)(S) - k_2(E\cdot S) = k_1(E)(S) - k_2\frac{k_1(E)(S)}{k_2 + k_3(W)}$$

$$\boxed{-r_s = \frac{k_1 k_3(E)(S)(W)}{k_2 + k_3(W)}} \qquad (7\text{-}22)$$

We *still* cannot use this rate law because we cannot measure the unbound enzyme concentration (E); however, we can measure the total enzyme concentration, E_t.

Total enzyme concentration

$$(E \cdot S) = \frac{k_1(E)(S)}{k_2 + k_3(W)}$$

$$(E_t) = (E) + (E \cdot S) \qquad (7\text{-}23)$$

Enzyme concentration

$$(E) = \frac{(E_t)(k_2 + k_3(W))}{k_2 + k_3(W) + k_1(S)} \qquad (7\text{-}23A)$$

The rate law for substrate consumption is

$$-r_s = \frac{k_1 k_3(E)(S)(W)}{k_2 + k_3(W)}$$

$$\boxed{-r_s = \frac{k_1 k_3(W)(E_t)(S)}{k_1(S) + k_2 + k_3(W)}} \qquad (7\text{-}24)$$

Throughout, E_t=(E_t)=total concentration of enzyme with typical units (kmol/m^3 or g/dm^3).

7.2.3 Michaelis-Menten Equation

$$-r_s = \frac{k_1 k_3 (W)(E_t)(S)}{k_1 (S) + k_2 + k_3 (W)}$$

Because the reaction of urea and urease is carried out in aqueous solution, water is, of course, in excess, and concentration of water is therefore considered constant.

Let $k_{cat}=k_3(W)$ and $K_M=(k_{cat}+k_2)/k_1$

Michaelis-Menten equation

$$-r_s = \frac{k_{cat}(E_t)(S)}{(S) + K_M} \qquad (7\text{-}25)$$

7.2.3 Michaelis-Menten Equation

Turnover number

$$-r_s = \frac{k_{cat}(E_t)(S)}{(S) + K_M} \qquad (7\text{-}25)$$

Turnover number (k_{cat}) :

It is the number of substrate molecules converted to produce in a given time on a single-enzyme molecule when the enzyme is saturated with substrate (i.e., all the active sites on the enzyme are occupied, $S >> K_M$).

For example, turnover number for the decomposition H_2O_2 by the enzyme catalase is 40×10^6 s^{-1}. That is, 40 million molecules of H_2O_2 are decomposed every second on a single-enzyme molecule saturated with H_2O_2.

7.2.3 Michaelis-Menten Equation

$$-r_s = \frac{k_{cat}(E_t)(S)}{(S) + K_M} \qquad (7\text{-}25)$$

Michaelis constant

Michaelis constant (K_M) :

For simple system, the Michaelis constant is a measure of the attraction of the enzyme for its substrate, so it's also called the ***affinity constant***.

The Michaelis constant, K_M, for the decomposition of H_2O_2 discussed earlier is 1.1 M while that for chymotrypsin is 0.1 M.

7.2.3 Michaelis-Menten Equation

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

Let $V_{max} = k_{cat}(E_t)$:

maximum rate of reaction for a given total enzyme concentration,

Michaelis-Menten equation

$$-r_s = \frac{V_{\max}(S)}{K_M + (S)} \qquad (7\text{-}26)$$

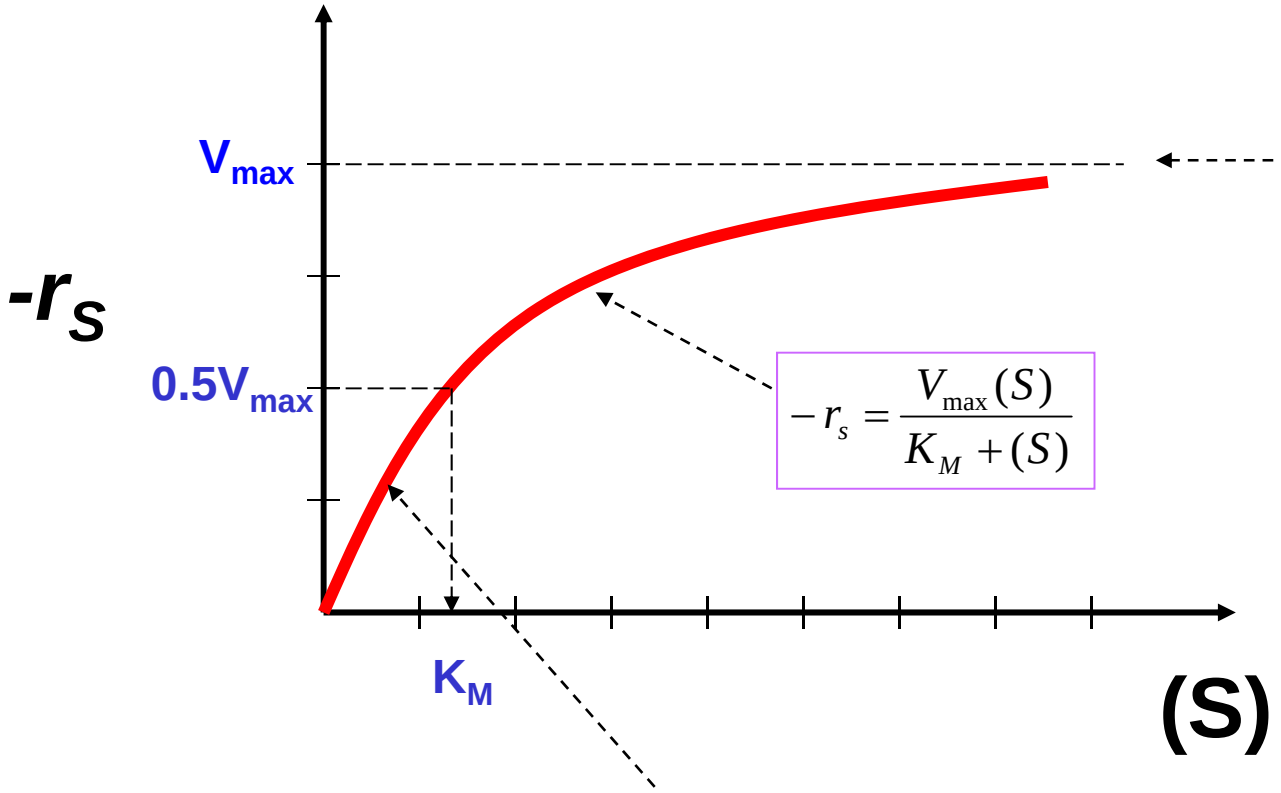
7.2.3 Michaelis-Menten Equation

Leonor Michaelis

Born in Berlin (Germany), he studied medicine in Freiburg, where he graduated in 1897. He then moved to Berlin, where he received his doctorate the same year. Michaelis worked as assistant to Paul Ehrlich (1898–1899), Moritz Litten (1899–1902) and Ernst Viktor von Leyden (1902–1906). In 1906 he started as director of the bacteriology lab in Berlins Charité hospital, becoming Professor extraordinary at Berlin University in 1908. In 1922 he moved to the Medical School of the University of Nagoya (Japan) as Professor of biochemistry, 1926 to Johns Hopkins University in Baltimore, Maryland as resident lecturer in medical research and 1929 to the Rockefeller Institute of Medical Research in New York City, where he retired 1941.

Maud Leonora Menten

Michaelis-Menten Plot
for identifying V_{max} and K_M



$$-r_s \sim V_{\max}$$

At high substrate concentration (S) >>K_m

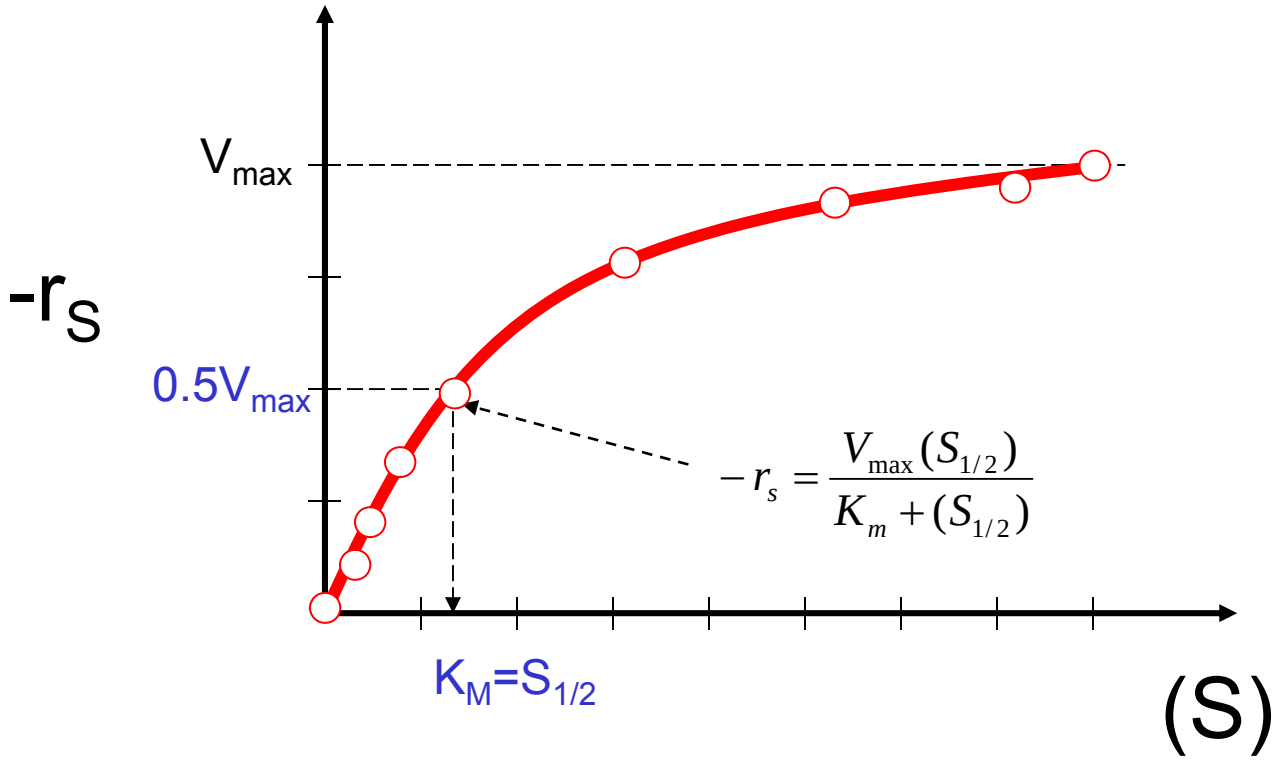
$$-r_s = \frac{V_{\max}(S)}{K_M + (S)}$$

At low substrate concentration (S) << K_m

$$-r_s \sim \frac{V_{\max}(S)}{K_M}$$

Consider the case when the substrate concentration is such that the reaction rate is equal to one-half the maximum rate,

$$-r_s = \frac{V_{\max}}{2} = \frac{V_{\max}(S_{1/2})}{K_M + (S_{1/2})} \Rightarrow K_M = (S_{1/2})$$



The Michaelis constant is equal to the substrate concentration at which the rate of reaction is equal to one-half the maximum rate.

The parameters V_{max} and K_M characterize the enzymatic reactions that are described by Michaelis-Menten kinetics. V_{max} is dependent on total enzyme concentration, whereas K_M is not.

Two enzymes may have the same values for k_{cat} but different reaction rate because of different value of K_M. **One way to compare the catalytic efficiencies of different enzymes is to compare the ratio k_{cat}/K_M.**

When this ratio approaches 10^8 to 10^9 ($dm^3/mol/s$) the reaction rate approaches becoming diffusion-limited. That is, it takes a long time for the enzyme and substrate to find each other, but once they do they react immediately.

Evaluation of Michaelis-Menten Parameter, V_{max} and K_M

1. Lineweaver-Burk plot
2. Eadie-Hofsee plot
3. Hanes-Woolf plot
4. Nonlinear regression

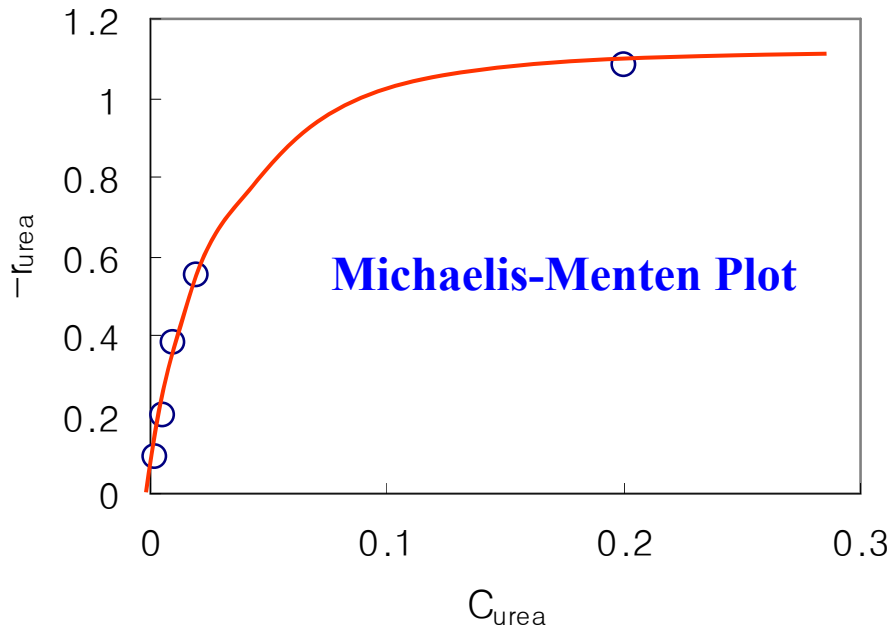
Example 7-3: Evaluation of Michaelis-Menten Parameter, V_{max} and K_M

Determine the Michaelis-Menten parameter V_{max} and K_m for the reaction

$$\text{urea} + \text{urease} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\text{urea}\bullet\text{urease}] \xrightarrow[-H_2O]{k_3} 2NH_3 + CO_2 + \text{urease}$$

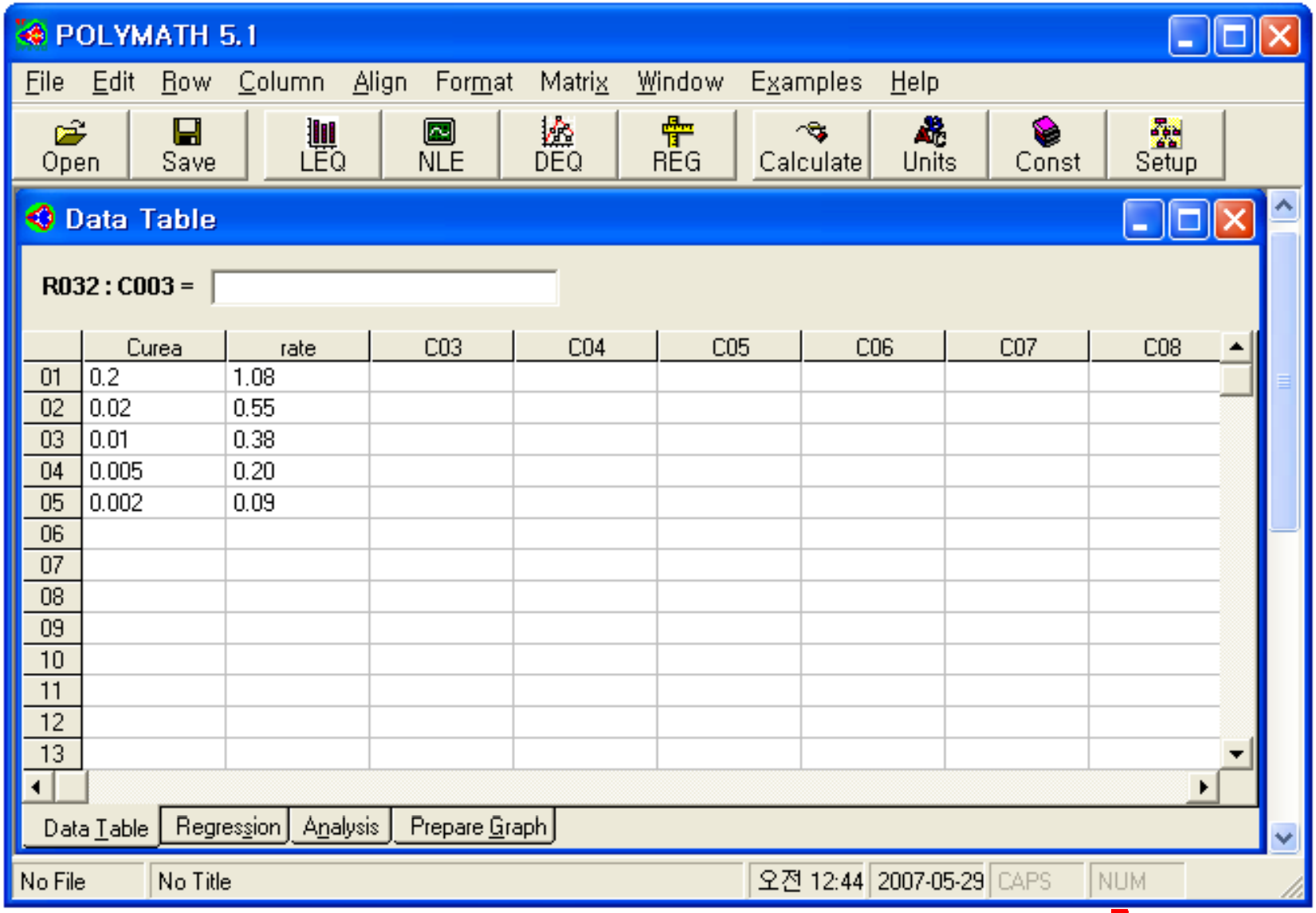
The rate of reaction is given as a function of urea concentration

C_{urea} (kmol/m^3)	0.20	0.02	0.01	0.005	0.002
$-r_{urea}$ (kmol/m^3•s)	1.08	0.55	0.38	0.20	0.09



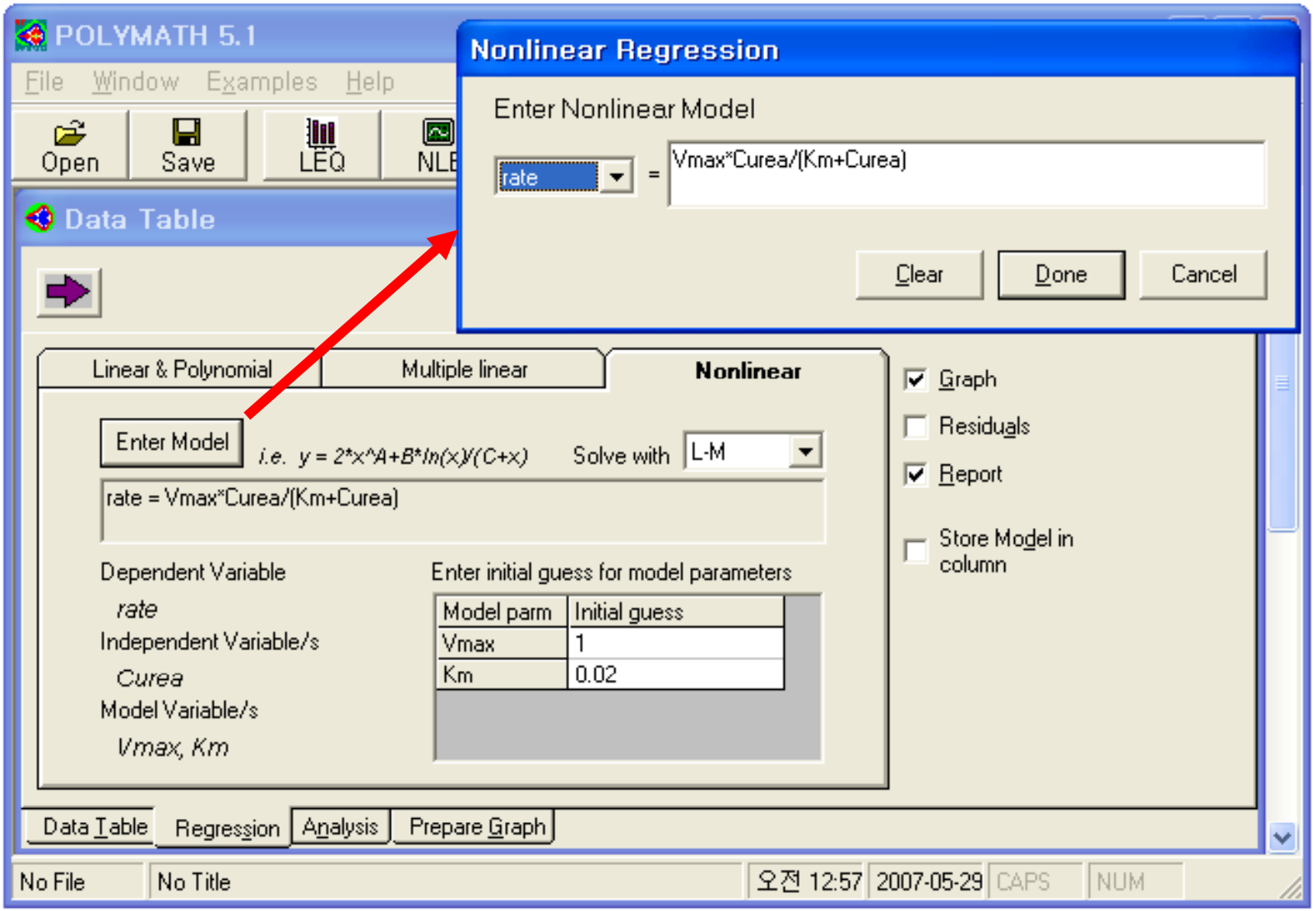
POLYMATH 5.1

Michaelis-Menten Plot



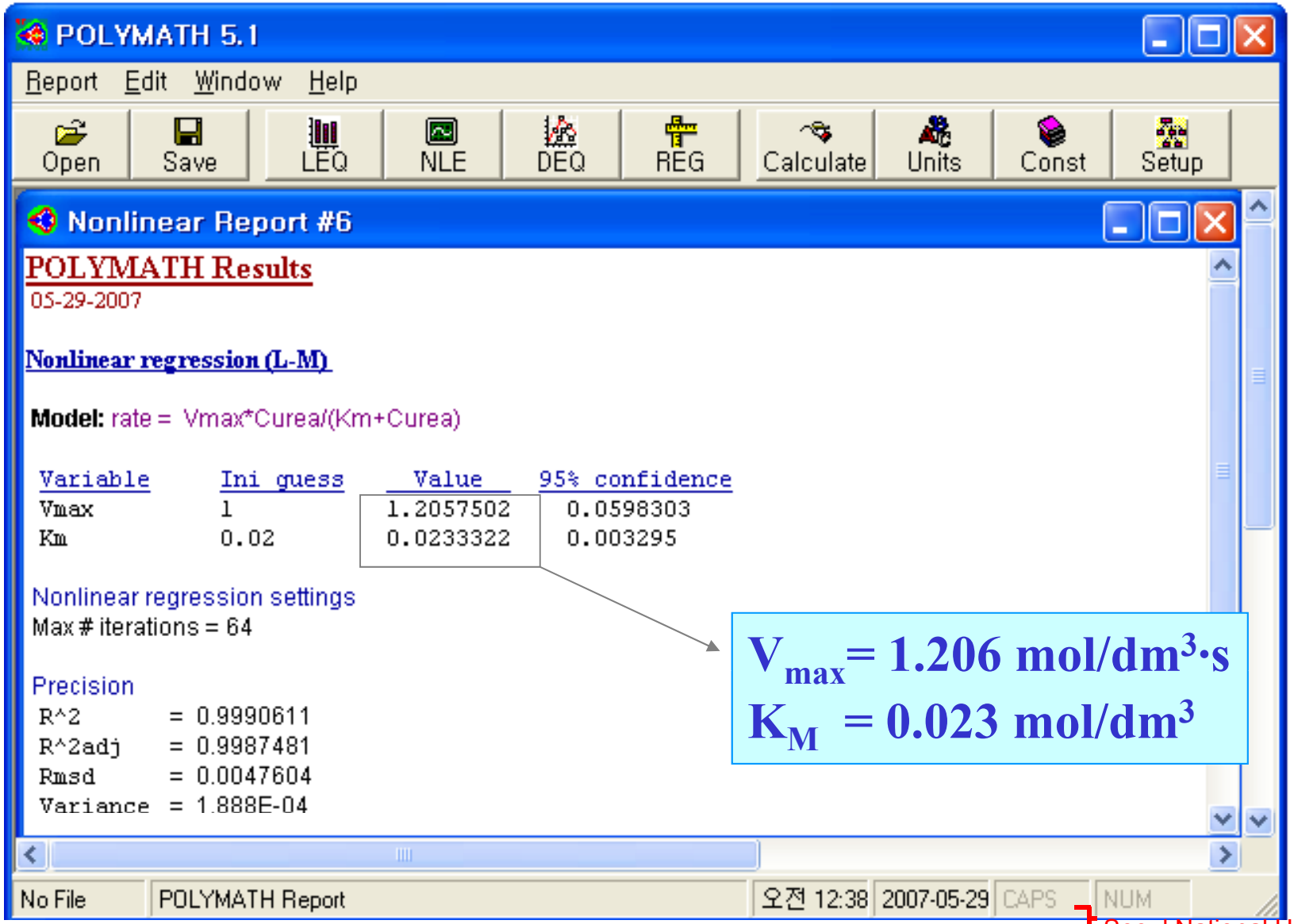
POLYMATH 5.1

Michaelis-Menten Plot



POLYMATH 5.1

Michaelis-Menten Plot



$V_{max} = 1.206$ mol/dm^3·s

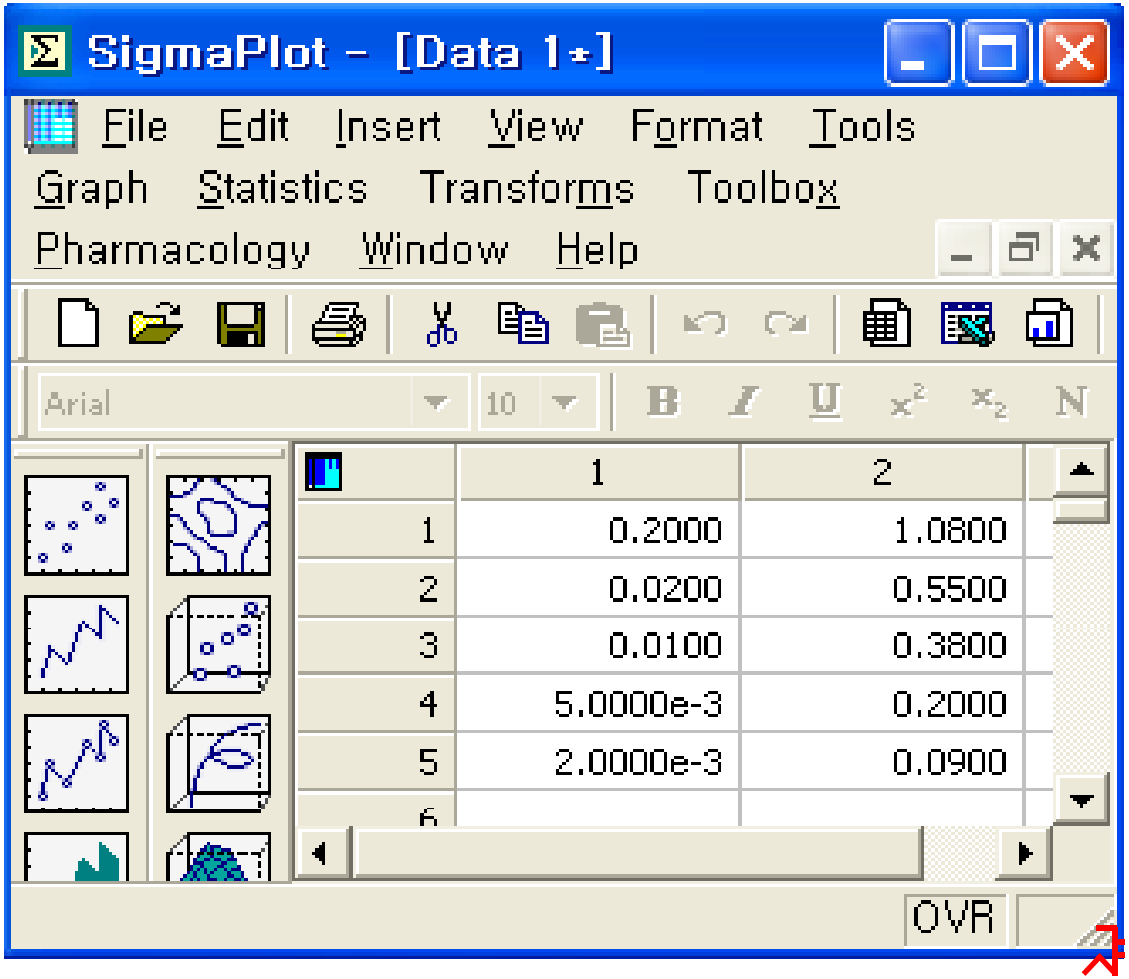
$K_M = 0.023$ mol/dm^3

SigmaPlot

Michaelis-Menten Plot

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

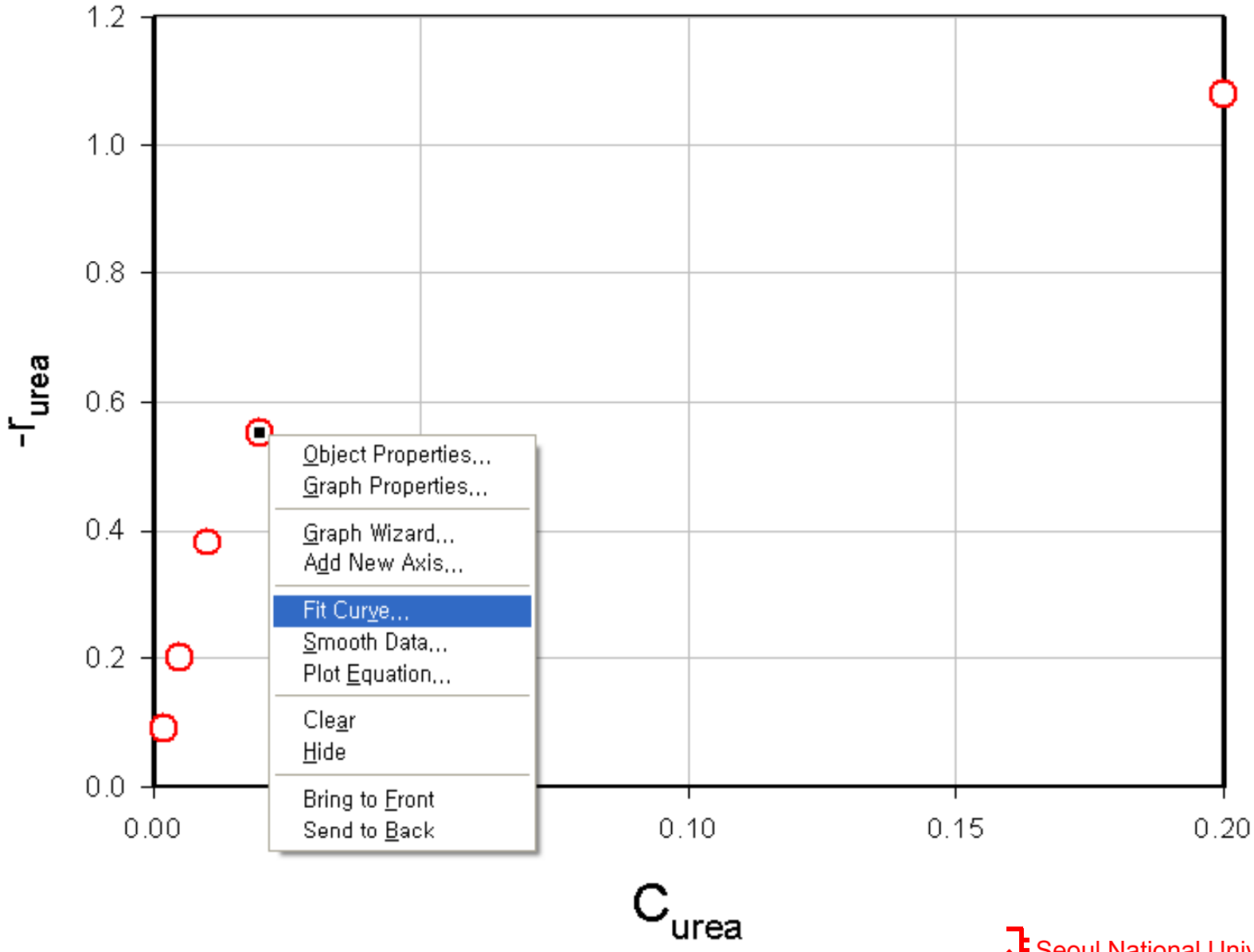
C_{urea} (kmol/m^3)	0.20	0.02	0.01	0.005	0.002
$-r_{urea}$ (kmol/m^3•s)	1.08	0.55	0.38	0.20	0.09



SigmaPlot

Michaelis-Menten Plot

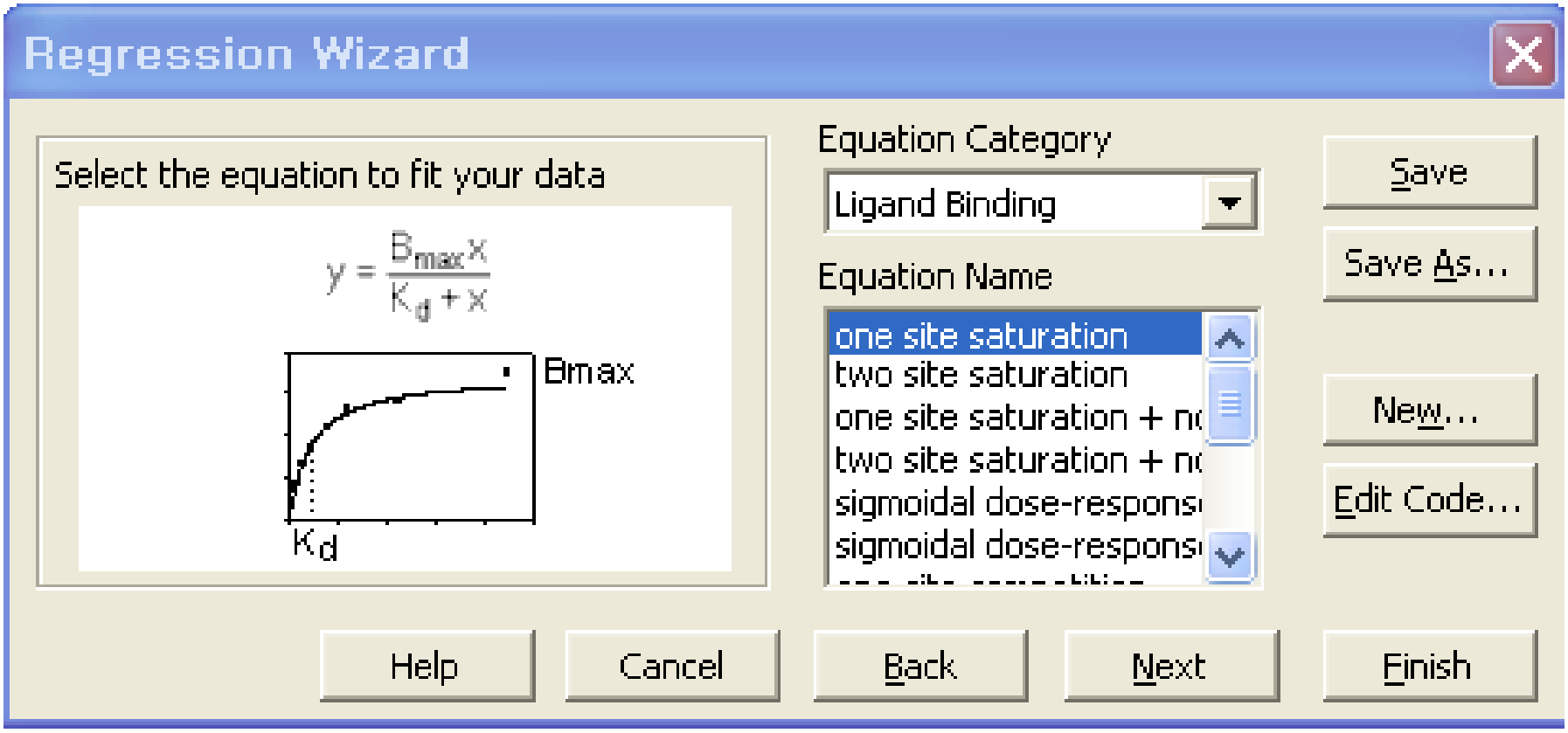
$$-r_s = \frac{V_{max}(S)}{K_M + (S)}$$



SigmaPlot

Michaelis-Menten Plot

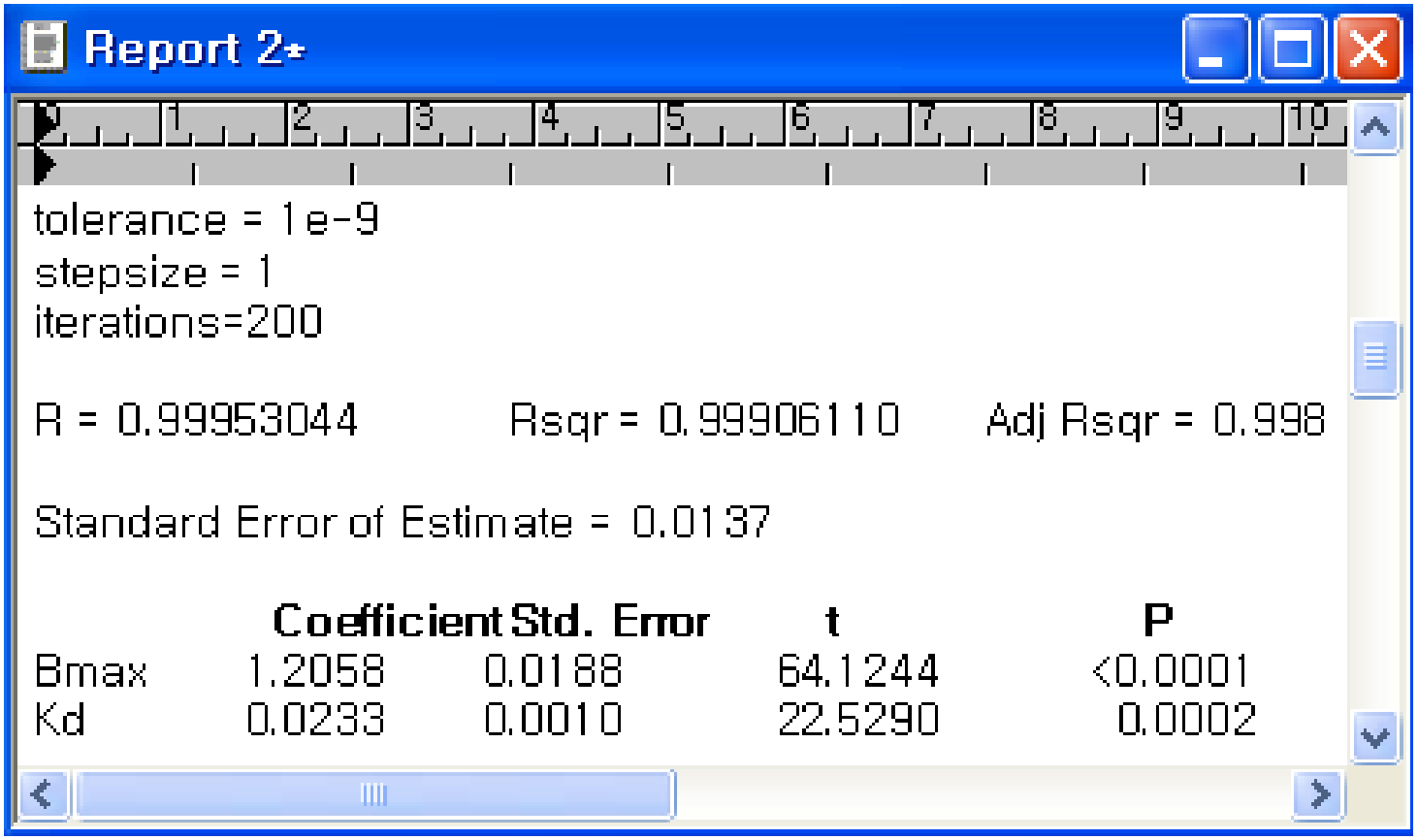
$$-r_s = \frac{V_{\max}(S)}{K_M + (S)}$$



SigmaPlot

Michaelis-Menten Plot

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



V_{max} = 1.206 mol/dm^3·s

K_M = 0.023 mol/dm^3

Example 7-3: Evaluation of Michaelis-Menten Parameter, V_{max} and K_M

Solution:

$$-r_s = \frac{V_{\max}(S)}{K_M + (S)} \quad \xrightarrow{\text{inverting}} \quad \frac{1}{-r_s} = \frac{(S)+K_M}{V_{\max}(S)} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\frac{1}{(S)}$$

$$\frac{1}{-r_{urea}} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\left(\frac{1}{C_{urea}}\right)$$

Lineweaver-Burk plot

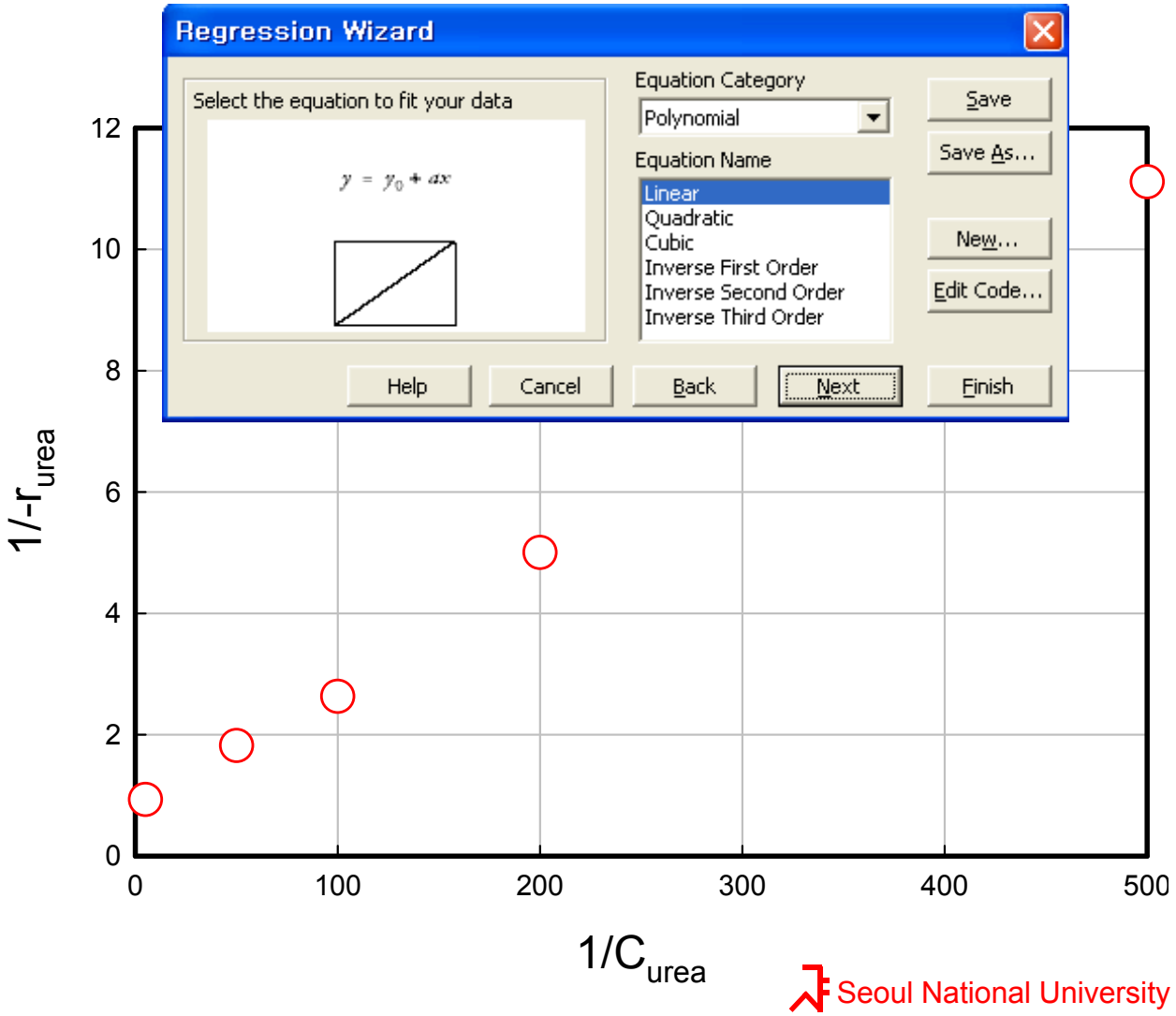
A plot of the reciprocal reaction rate versus the reciprocal urea concentration should be a straight line with an intercept $1/V_{max}$ and slope K_m/V_{max}. This type of plot is called a ***Lineweaver-Burk plot***.

SigmaPlot

Lineweaver-Burk plot

$$\frac{1}{-r_{urea}} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\left(\frac{1}{C_{urea}}\right)$$

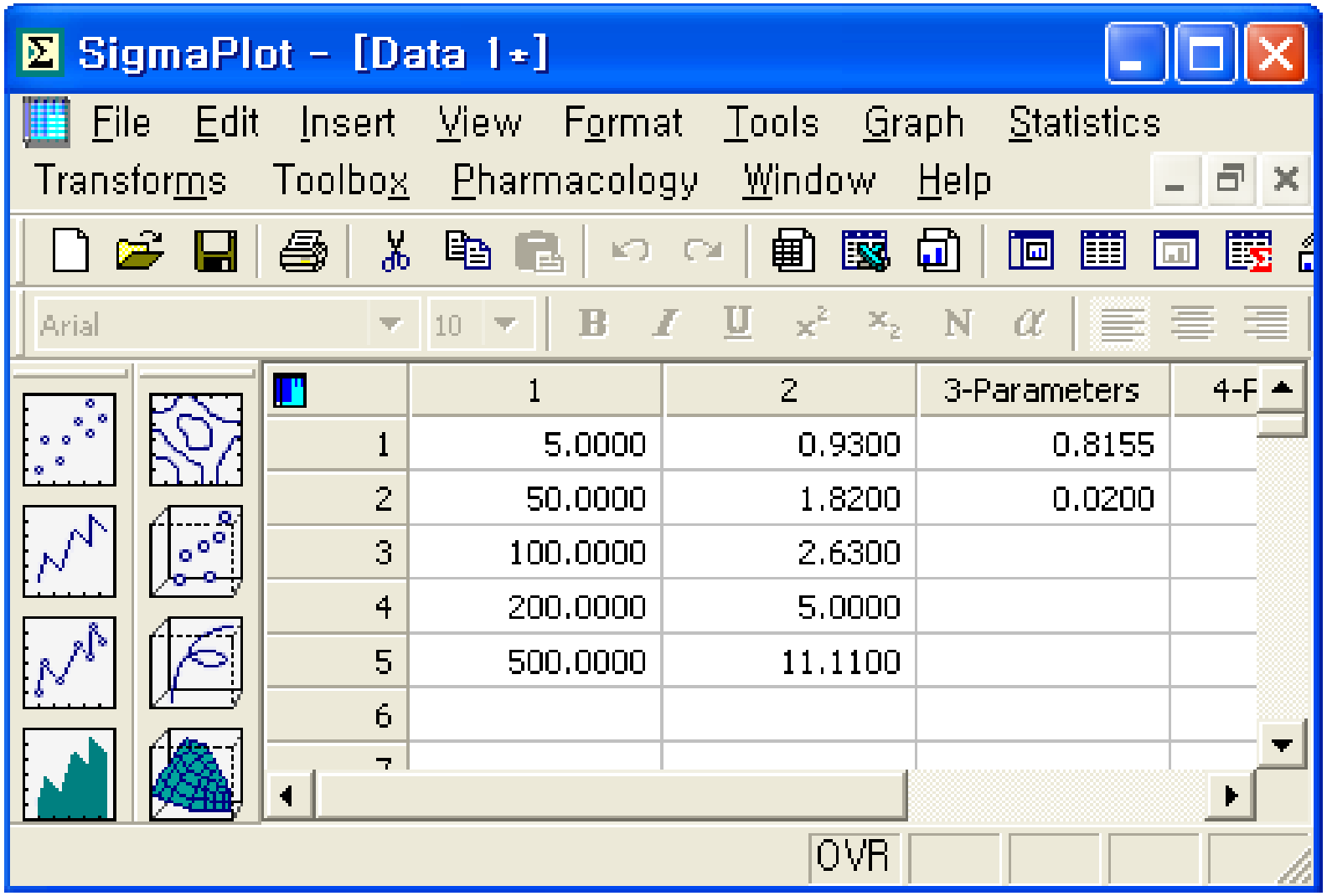
$1/C_{urea}$ ($m^3/kmol$)	$1/-r_{urea}$ ($m^3 \cdot s/kmol$)
5.0	0.93
50.0	1.82
100.0	2.63
200.0	5.00
500.0	11.11



SigmaPlot

Lineweaver-Burk plot

$$\frac{1}{-r_{urea}} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\left(\frac{1}{C_{urea}}\right)$$

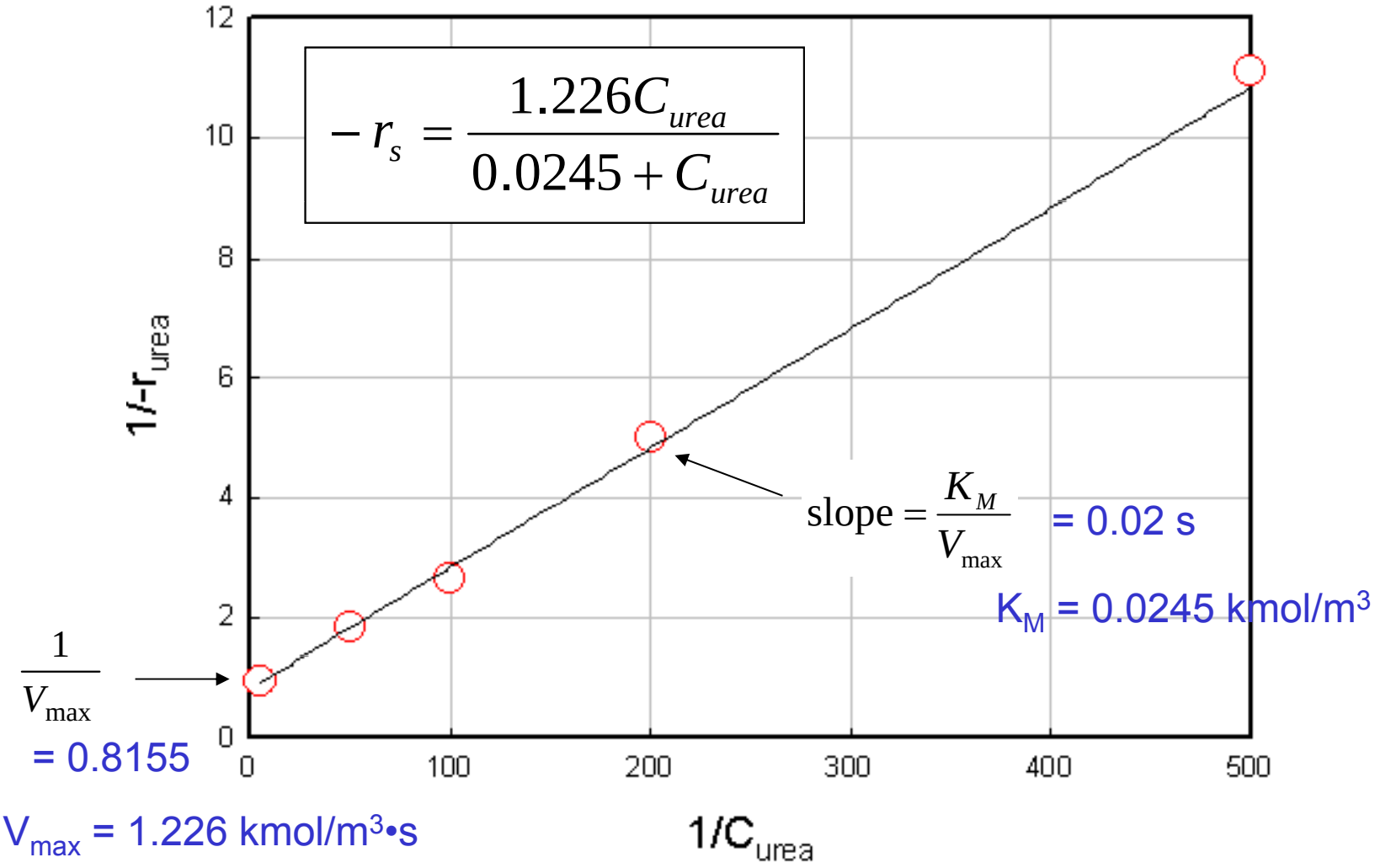


	1	2	3-Parameters	4-P
1	5.0000	0.9300	0.8155	
2	50.0000	1.8200	0.0200	
3	100.0000	2.6300		
4	200.0000	5.0000		
5	500.0000	11.1100		
6				

SigmaPlot

Lineweaver-Burk plot

$$\frac{1}{-r_{urea}} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(\frac{1}{C_{urea}}\right)$$



In addition to the Lineweaver–Burk plot, one can also use a Hanes–Woolf plot or an Eadie–Hofstee plot. Here $S \equiv C_{urea}$, and $-r_S \equiv -r_{urea}$. Equation (7-26)

$$-r_S = \frac{V_{max}(S)}{K_M + (S)} \tag{7-26}$$

can be rearranged in the following forms. For the Eadie–Hofstee form,

$$\boxed{-r_S = V_{max} - K_M\left(\frac{-r_S}{(S)}\right)} \tag{E7-3.4}$$

For the Hanes–Woolf form, we have

$$\boxed{\frac{(S)}{-r_S} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}}(S)} \tag{E7-3.5}$$

For the Eadie–Hofstee model we plot $-r_S$ as a function of $(-r_S/S)$ and for the Hanes–Woolf model, we plot $[(S)/-r_S]$ as a function of (S). The Eadie–Hofstee plot does not bias the points at low substrated concentrations, while the Hanes–Woolf plot gives a more accurate evaluation of V_{max}. In Table E7-3.2, we add two columns to Table E7-3.1 to generate these plots ($C_{urea} \equiv S$).

TABLE E7-3.2. RAW AND PROCESSED DATA

S (kmol/m^3)	$-r_S$ (kmol/m^3 • s)	$1/S$ (m^3/kmol)	$1/-r_S$ (m^3 • s/kmol)	$S/-r_s$ (s)	$-r_S/S$ (1/s)
0.20	1.08	5.0	0.93	0.185	5.4
0.02	0.55	50.0	1.82	0.0364	27.5
0.01	0.38	100.0	2.63	0.0263	38
0.005	0.20	200.0	5.00	0.0250	40
0.002	0.09	500.0	11.11	0.0222	45

Plotting the data in Table E7-3.2, we arrive at Figures E7-3.2 and E7-3.3.

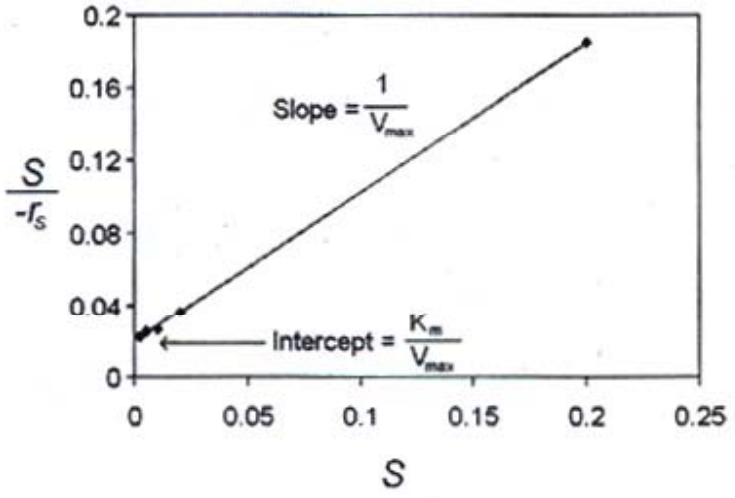


Figure E7-3.2 Hanes–Woolf plot.

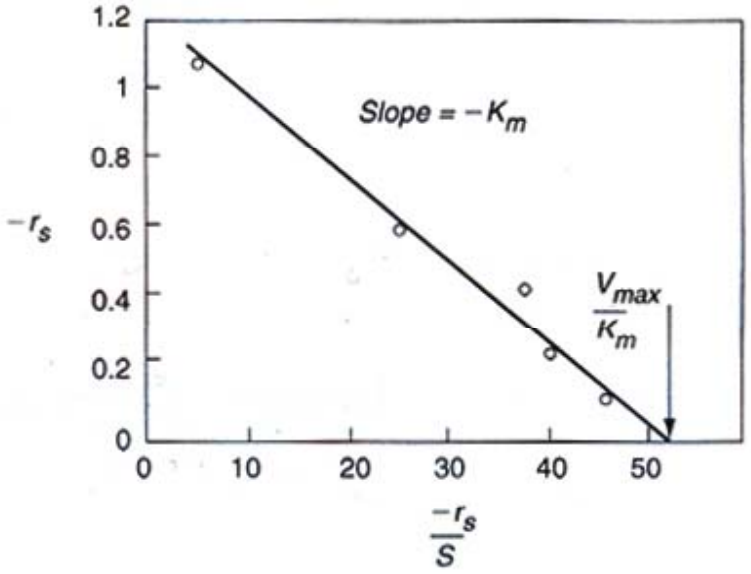
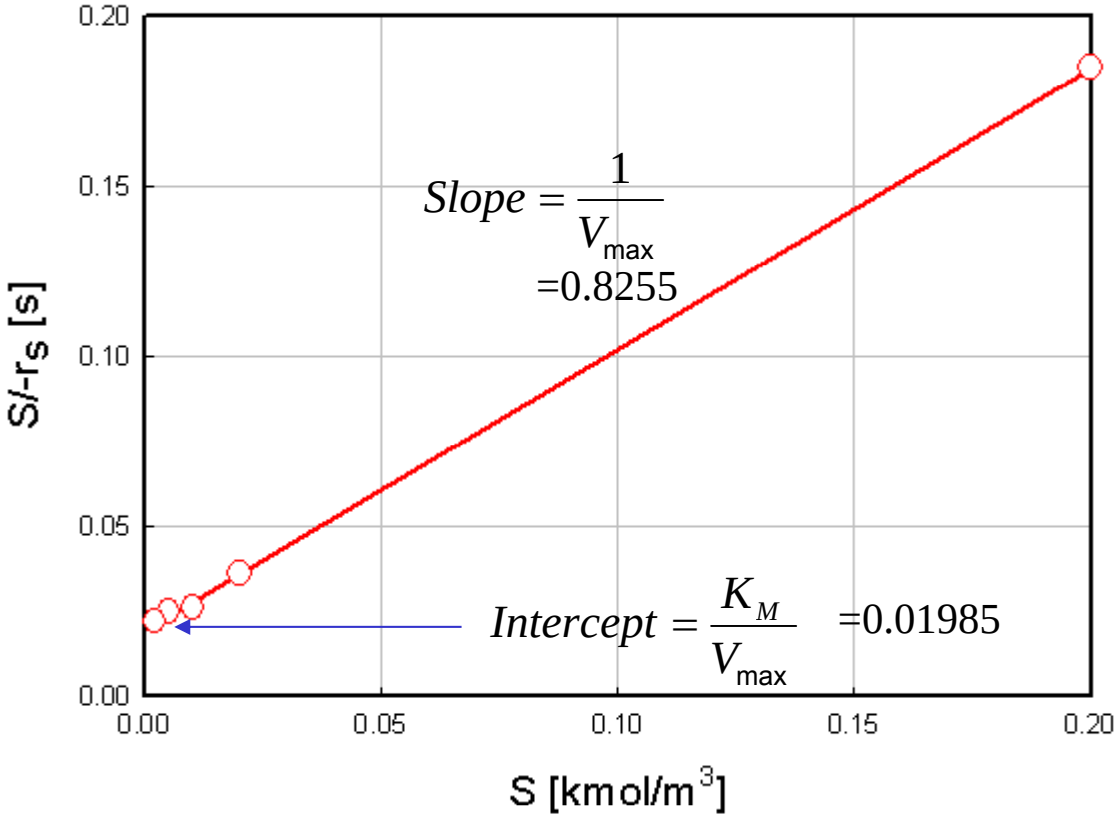


Figure E7-3.3 Eadie–Hofstee plot.

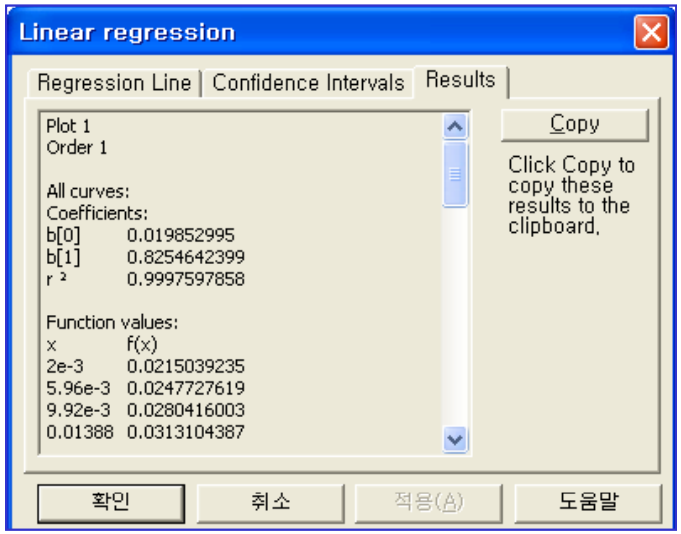
SigmaPlot

Hanes-Woolf plot

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



Linear Regression by SigmaPlot



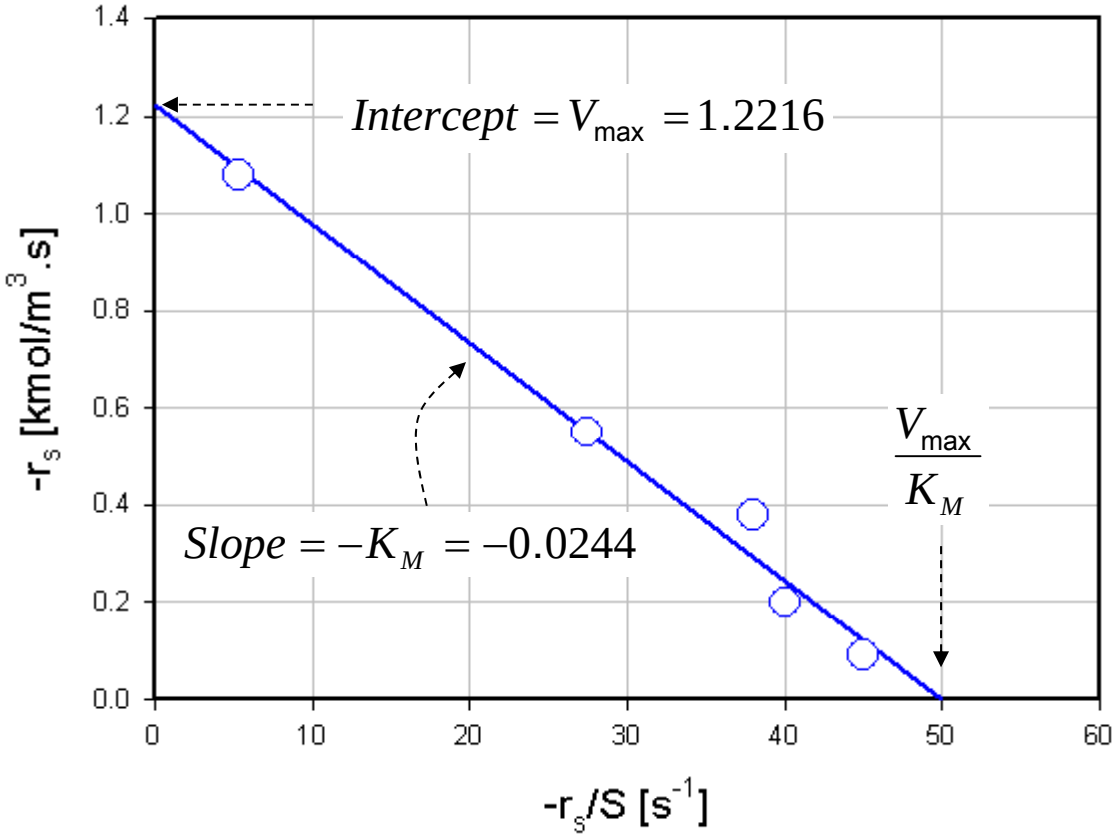
V_{max}= 1.211 mol/dm³·s

K_M = 0.024 mol/dm³

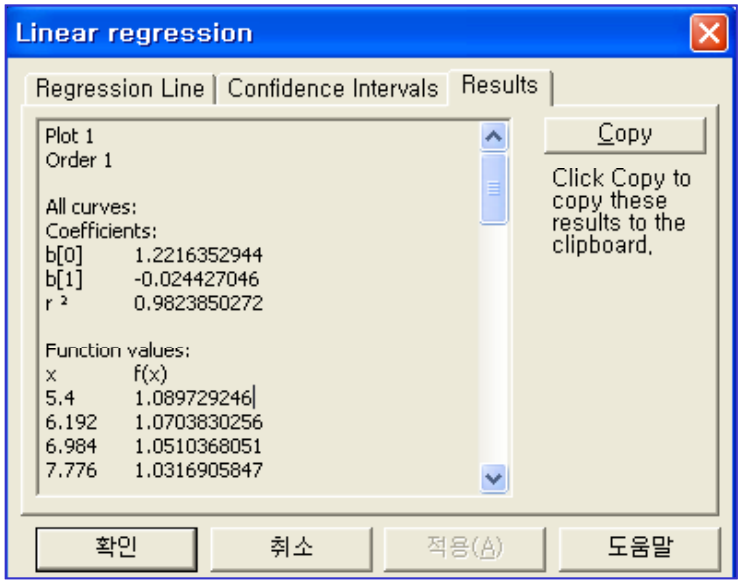
SigmaPlot

Eadie-Hofstee plot

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



Linear Regression by SigmaPlot



V_{max} = 1.221 mol/dm^3·s
K_M = 0.024 mol/dm^3

Evaluation of Michaelis-Menten Parameter, V_{max} and K_M

	V_{max}	K_M
1. Lineweaver-Burk plot	1.226	0.0245
2. Eadie-Hofsee plot	1.221	0.024
3. Hanes-Woolf plot	1.211	0.024
4. Nonlinear regression	1.206	0.023

The Product-Enzyme Complex

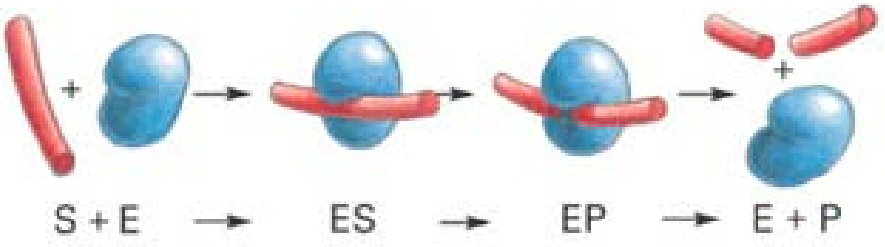
In many reactions the enzyme and product complex (E • P) is formed directly from the enzyme substrate complex (E • S) according to the sequence

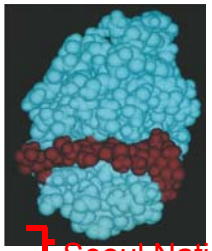
$$E + S \rightleftharpoons E \bullet S \rightleftharpoons E \bullet P \rightleftharpoons P + E$$

Applying the PSSH to both (E • S) and (E • P), we obtain

$$-r_S = \frac{V_{max}(C_S - C_P/K_C)}{C_S + K_{max} + K_P C_P} \qquad (7\text{-}29)$$

which is often referred to as the **Briggs-Haldane Equation** (see Problem P7-10) and the application of the PSSH to enzyme kinetics often called the Briggs–Haldane approximation.





7.2.4 Batch Reactor Calculations for Enzyme Reactions

Mole balance on urea

$$-\frac{dN_{urea}}{dt} = -r_{urea}V$$

For liquid phase

$$-\frac{dC_{urea}}{dt} = -r_{urea} \qquad \textbf{(7-30)}$$

The rate law for urea decomposition

$$-r_{urea} = \frac{V_{\max}C_{urea}}{K_M + C_{urea}} \qquad \textbf{(7-31)}$$

Combine & integrate

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

Time to achieve a conversion X in a batch enzymatic reaction

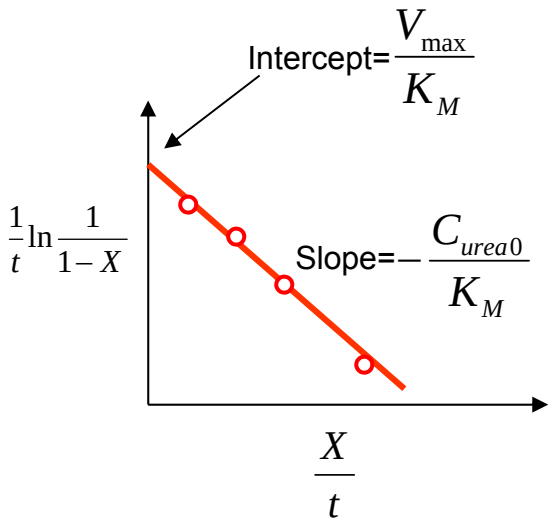
$$\boxed{t = \frac{K_M}{V_{\max}} \ln\frac{1}{1-X} + \frac{C_{urea0}X}{V_{\max}}} \qquad C_{urea} = C_{urea0}(1-X) \qquad \textbf{(7-32)}$$

Determine K_m and V_{max} from batch reactor data

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

Multiple $\frac{V_{\max}}{K_M t}$

$$\frac{1}{t} \ln \frac{1}{1-X} = \frac{V_{\max}}{K_M} - \frac{C_{urea0} X}{K_M t}$$

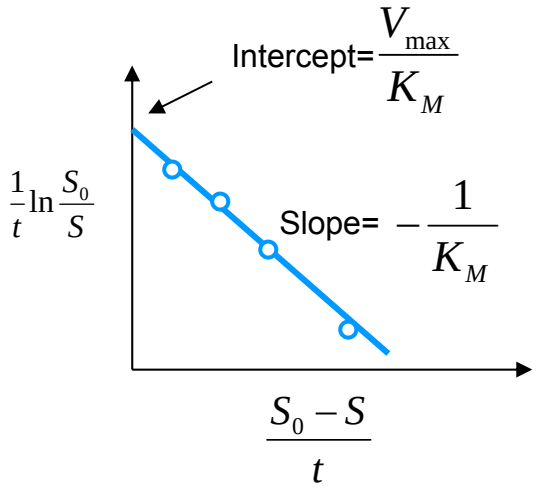


Michaelis-Menten equation

$$\frac{S}{S_0} = 1 - X$$

$$S_0 - S = C_{urea0} X$$

$$\frac{1}{t} \ln \frac{S_0}{S} = \frac{V_{\max}}{K_M} - \frac{S_0 - S}{K_M t}$$



S_0=initial concentration of substrate

C_S=(S)=S

Example 7–4 Batch Enzymatic Reactors

Calculate the time needed to convert 99% of the urea to ammonia and carbon dioxide in a 0.5-dm^3 batch reactor. The initial concentration of urea is 0.1 mol/dm^3, and the urease concentration is 0.001 g/dm^3. The reaction is to be carried out isothermally at the same temperature at which the data in Table E7-3.2 were obtained.

TABLE E7-3.2. RAW AND PROCESSED DATA

S ($kmol/m^3$)	$-r_S$ ($kmol/m^3 \cdot s$)	$1/S$ ($m^3/kmol$)	$1/-r_S$ ($m^3 \cdot s/kmol$)	$S/-r_s$ (s)	$-r_S/S$ (1/s)
0.20	1.08	5.0	0.93	0.185	5.4
0.02	0.55	50.0	1.82	0.0364	27.5
0.01	0.38	100.0	2.63	0.0263	38
0.005	0.20	200.0	5.00	0.0250	40
0.002	0.09	500.0	11.11	0.0222	45

Solution

V_{max} = 1.33kmol/m^3•s

K_M = 0.0266 kmol/m^3

We can use Equation (7-32),

$$t = \frac{K_M}{V_{max}} \ln \frac{1}{1-X} + \frac{C_{urea0} X}{V_{max}} \qquad (7\text{-}32)$$

where $K_M = 0.0266$ mol/dm^3, $X = 0.99$, and $C_{urea0} = 0.1$ mol/dm^3, V_{max} was 1.33 mol/dm$^3 \cdot$s. However, for the conditions in the batch reactor, the enzyme concentration is only 0.001 g/dm^3 compared with 5 g in Example 7-3. Because $V_{max} = E_t \cdot k_3$, V_{max} for the second enzyme concentration is

$$V_{max2} = \frac{E_{t2}}{E_{t1}} V_{max1} = \frac{0.001}{5} \times 1.33 = 2.66 \times 10^{-4} \text{ mol/s} \cdot \text{dm}^3$$

$$K_M = 0.0266 \text{ mol/dm}^3 \quad \text{and} \quad X = 0.99$$

Substituting into Equation (7-32)

$$t = \frac{2.66 \times 10^{-2} \text{ mol/dm}^3}{2.66 \times 10^{-4} \text{ mol/dm}^3/\text{s}} \ln\left(\frac{1}{0.01}\right) + \frac{(0.1 \text{ mol/dm}^3)(0.99)}{2.66 \times 10^{-4} \text{ mol/dm}^3/\text{s}} \text{s}$$

$$= 460 \text{ s} + 380 \text{ s}$$

$$= 840 \text{ s (14 minutes)}$$

Effect of temperature on enzymatic reaction

The effect of temperature on enzymatic reactions is very complex. If the enzyme structure would remain unchanged as the temperature is increased, the rate would probably follow the Arrhenius temperature dependence. However, as the temperature increases, the enzyme can unfold and/or become denatured and lose its catalytic activity. Consequently, as the temperature increases, the reaction rate, $-r_S$, increases up to a maximum with increasing temperature and then decreases as the temperature is increased further. The descending part of this curve is called temperature inactivation or thermal denaturizing.[10] Figure 7-9 shows an example of this optimum in enzyme activity.[11]

Effect of temperature on enzymatic reaction

Thermal Denaturizing

Temperature Inactivation

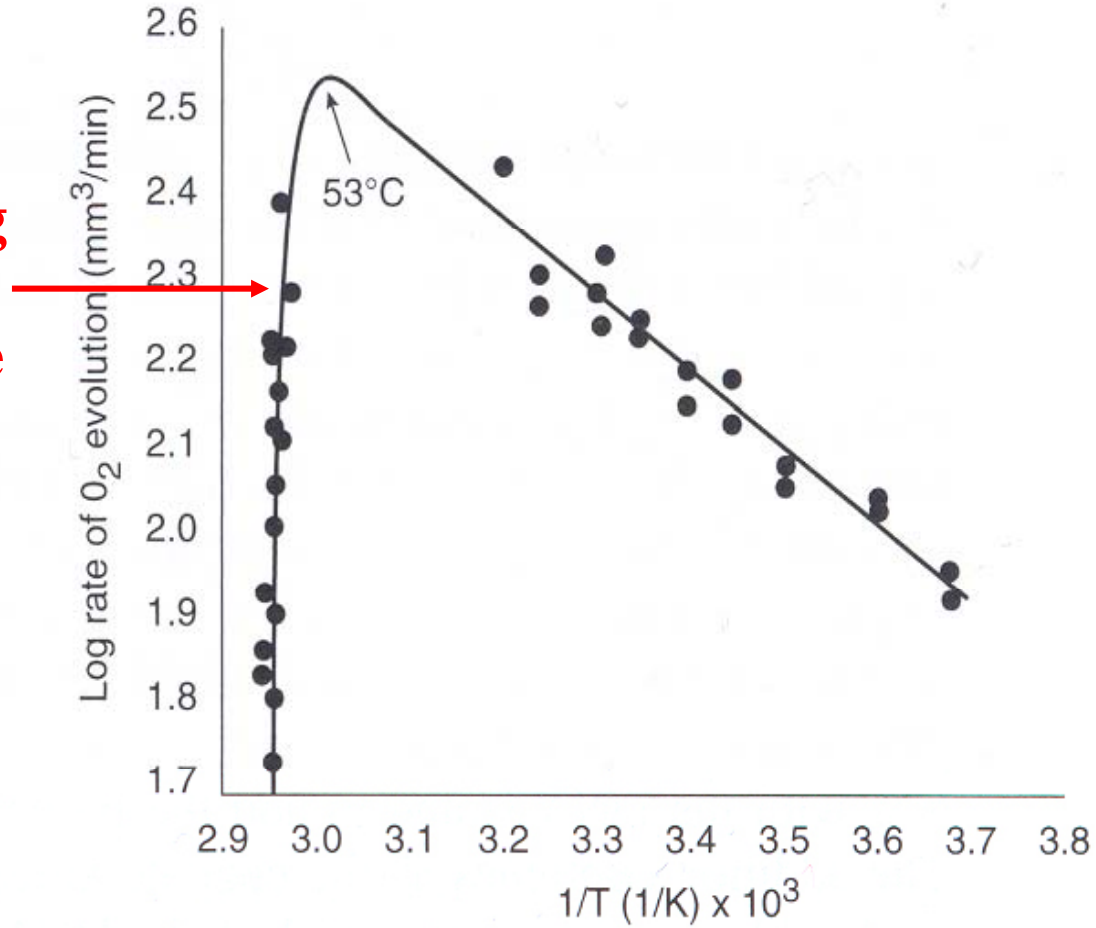


Figure 7-8 Catalytic breakdown rate of H_2O_2 depending on temperature. Courtesy of S. Aiba, A. E. Humphrey, and N. F. Mills, *Biochemical Engineering*, Academic Press (1973).

Inhibition of Enzyme Reaction

In addition to temperature, and solution pH, another factor that greatly influences the rates of enzyme-catalyzed reactions is the presence of an inhibitor. Inhibitors are species that interact with enzymes and render the enzyme ineffective to catalyze its specific reaction. The most dramatic consequences of enzyme inhibition are found in living organism where the inhibition of any particular enzyme involved in a primary metabolic pathway will render the entire pathway inoperative, resulting in either serious damage or death of the organism.

For example, the inhibition of a single enzyme, cytochrome oxidase, by cyanide will cause the aerobic oxidation process to stop; death occurs in a very few minutes.

There are also beneficial inhibitors such as the ones used in the treatment of leukemia and other neoplastic diseases. Aspirin inhibits the enzyme that catalyzes the synthesis of prostaglandin involved in the pain-producing process.

leukemia:=백혈병, neoplastic = 종양의, prostaglandin = 전립선, 정낭(精囊) 따위에서 분비되는 호르몬과 같은 불포화 지방산의 약제

Inhibition of Enzyme Reaction

The three most common types of reversible inhibition occurring in enzymatic reactions are

(1) Competitive inhibition 경쟁하는

(2) Uncompetitive inhibition 경쟁하지 않는

(3) Noncompetitive inhibition 경쟁이 없는

(4) Substrate inhibition

Inhibition of Enzyme Reaction

The enzyme molecule is analogous to a heterogeneous catalytic surface in that it contains active sites.

When ***competitive inhibition*** occurs, the substrate and inhibitor are usually similar molecules that compete for the same site on the enzyme.

Uncompetitive inhibition occurs when the inhibitor deactivates the enzyme-substrate complex, sometimes by attaching itself to both the substrate and enzyme molecules of the complex.

Noncompetitive inhibition occurs with enzymes containing at least two different types of sites. The substrate attaches only to one type of site, and the inhibitor attaches only to the other to render the enzyme inactive. 경쟁이 없는

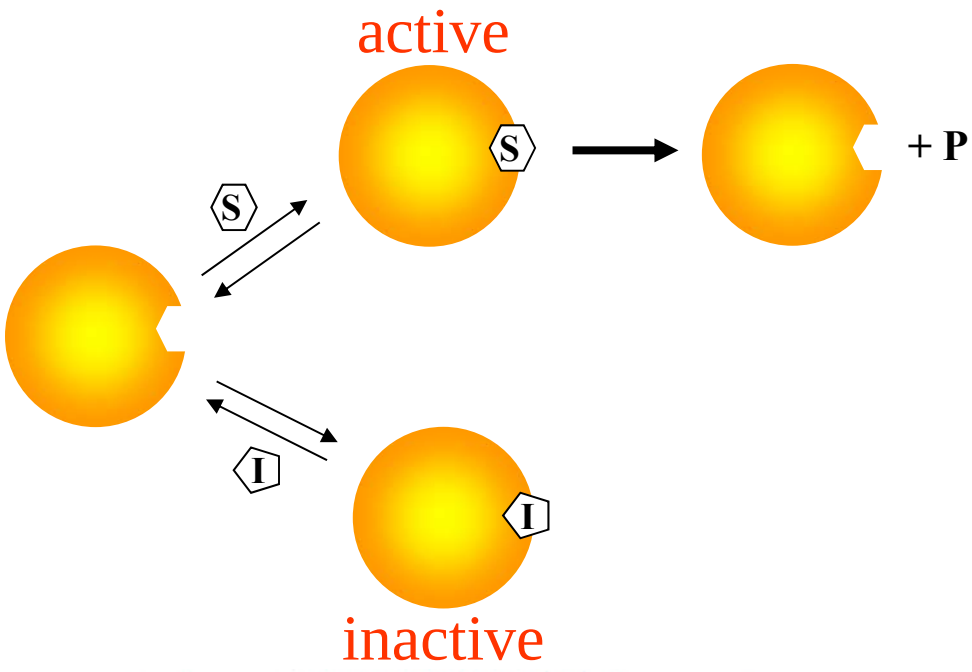
7.3.1 Competitive Inhibition

Competitive inhibition is of particular importance in pharmacokinetics (drug therapy).

If a patient were administered two or more drugs that react simultaneously within the body with a common enzyme, cofactor, or active species, this interaction could lead to competitive inhibition in the formation of the respective metabolites and produce serious consequences.

7.3.1 Competitive Inhibition

In competitive inhibition, another substance, **I**, competes with the substrate for the enzyme molecules to form a inhibitor-enzyme complex



경쟁하는

(a) Competitive inhibition. Courtesy of D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000), p. 266.

7.3.1 Competitive Inhibition

Reaction steps

$$(1)\quad E + S \xrightarrow{k_1} E \bullet S$$
$$(2)\quad E \bullet S \xrightarrow{k_2} E + S$$
$$(3)\quad E \bullet S \xrightarrow{k_3} P + E$$
$$(4)\quad I + E \xrightarrow{k_4} E \bullet I \text{ (inactive)}$$
$$(5)\quad E \bullet I \xrightarrow{k_5} E + I$$

In addition to the three Michaelis–Menten reaction steps, there are two additional steps as the inhibitor reversely ties up the enzyme as shown in reaction steps 4 and 5.

The rate law for the formation of product is the same [cf. Equation (7-18A)] as it was before in the absence of inhibitor

$$r_P = k_3 (E \bullet S) \qquad (7\text{-}34)$$

$$r_P = k_3 (E \bullet S) \tag{7-34}$$

Applying the PSSH, the net rate of reaction of the enzyme–substrate complex is

$$r_{E \bullet S} = 0 = k_1 (E)(S) - k_2(E \bullet S) - k_3 (E \bullet S) \tag{7-35}$$

The net rate of reaction of enzyme-inhibitor complex is also zero

$$r_{E \bullet I} = 0 = k_4 (E)(I) - k_5(E \bullet I) \tag{7-36}$$

The total enzyme concentration is the sum of the bound and unbound enzyme concentrations

$$E_t = E + (E \bullet S) + (E \bullet I) \tag{7-37}$$

Combining Equations (7-35), (7-36), and (7-37) and solving for (E • S) and substituting in Equation (7-34) and simplifying

Rate law for competitive inhibition

$$\boxed{r_P = -r_S = \frac{V_{max}(S)}{S + K_M\left(1 + \frac{I}{K_I}\right)}} \tag{7-38}$$

V_{max} and K_M are the same as before when no inhibitor is present, that is,

$$V_{max} = k_3 E_t \text{ and } K_M = \frac{k_2 + k_3}{k_1}$$

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

and the inhibition constant K_I (mol/dm^3) is

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

$$-r_s = \frac{V_{max}(S)}{S + K'_M}$$

By letting $K'_M = K_M(1 + I/K_I)$, we can see that the effect of a competitive inhibition is to increase the "apparent" Michaelis constant, K'_M. A consequence of the larger "apparent" Michaelis constant K'_M is that a larger substrate concentration is needed for the rate of substrate decomposition, $-r_S$, to reach half its maximum rate.

Rearranging in order to generate a Lineweaver–Burk plot,

$$\frac{1}{-r_{urea}} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(\frac{1}{C_{urea}}\right) \rightarrow \boxed{\frac{1}{-r_s} = \frac{1}{V_{max}} + \frac{1}{(S)}\quad\frac{K_M}{V_{max}}\left(1 + \frac{(I)}{K_I}\right)} \qquad (7\text{-}39)$$

From the Lineweaver–Burk plot (Figure 7-10), we see that as the inhibitor (I) concentration is increased the slope increases (i.e., the rate decreases) while the intercept remains fixed.

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

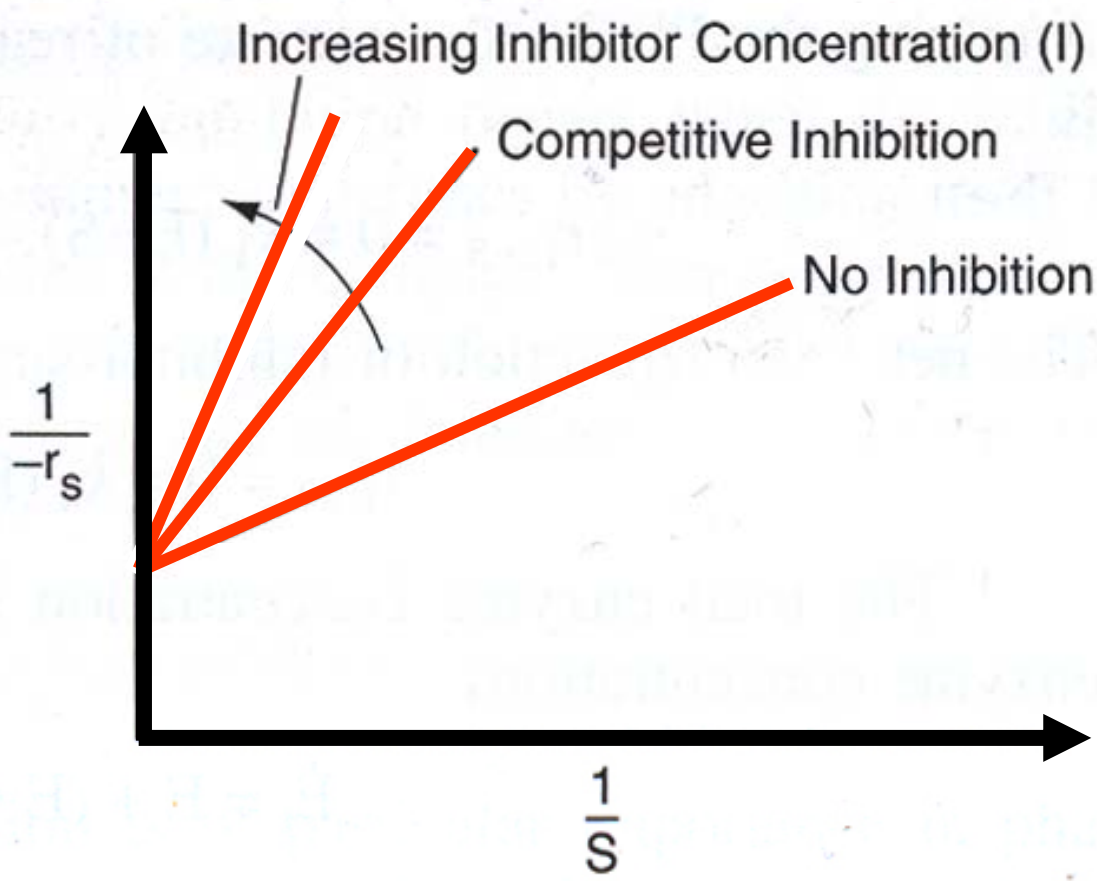


Figure 7-10 Lineweaver–Burk plot for competitive inhibition.

7.3.1 Competitive Inhibition

Methanol Poisoning

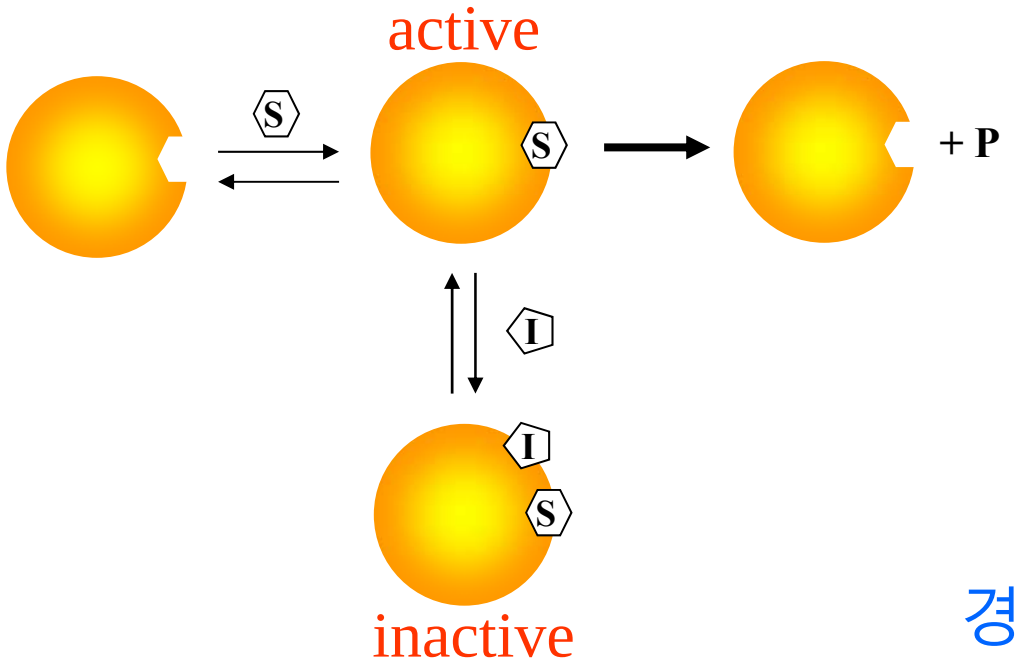
An interesting and important example of competitive substrate inhibition is the enzyme ***alcohol dehydrogenase (ADH)*** in the presence of ethanol and methanol.

If a person ingests methanol, ADH will convert it to formaldehyde and then formate, which cause blindness. Consequently, the treatment involves intravenously injecting ethanol (which metabolized at a slower rate than methanol) at a controlled rate to tie up ADH to slow the metabolism of *methanol-to-formaldehyde-to-formate* so that the kidneys have time to filter out the methanol which is then excreted in the urine. With this treatment, blindness is avoided.

Excrete=노폐물을 배설하다.

7.3.2 Uncompetitive Inhibition

Here, the inhibitor has no affinity for the enzyme itself and thus does not compete with the substrate for the enzyme; instead it ties up the enzyme-substrate complex by forming an inhibitor-enzyme-substrate complex, (I•E•S), is inactive. In ***uncompetitive inhibition***, the inhibitor reversibly ties up enzyme-substrate complex after it has been formed.



경쟁하지 않는

7.3.2 Uncompetitive Inhibition

Reaction steps

$$(1)\quad E + S \xrightarrow{k_1} E \bullet S$$

$$(2)\quad E \bullet S \xrightarrow{k_2} E + S$$

$$(3)\quad E \bullet S \xrightarrow{k_3} P + E$$

$$(4)\quad I + E \bullet S \xrightarrow{k_4} I \bullet E \bullet S \ \text{(inactive)}$$

$$(5)\quad I \bullet E \bullet S \xrightarrow{k_5} I + E \bullet S$$

7.3.2 Uncompetitive Inhibition

$$r_P = k_3 (E \bullet S) \tag{7-34}$$

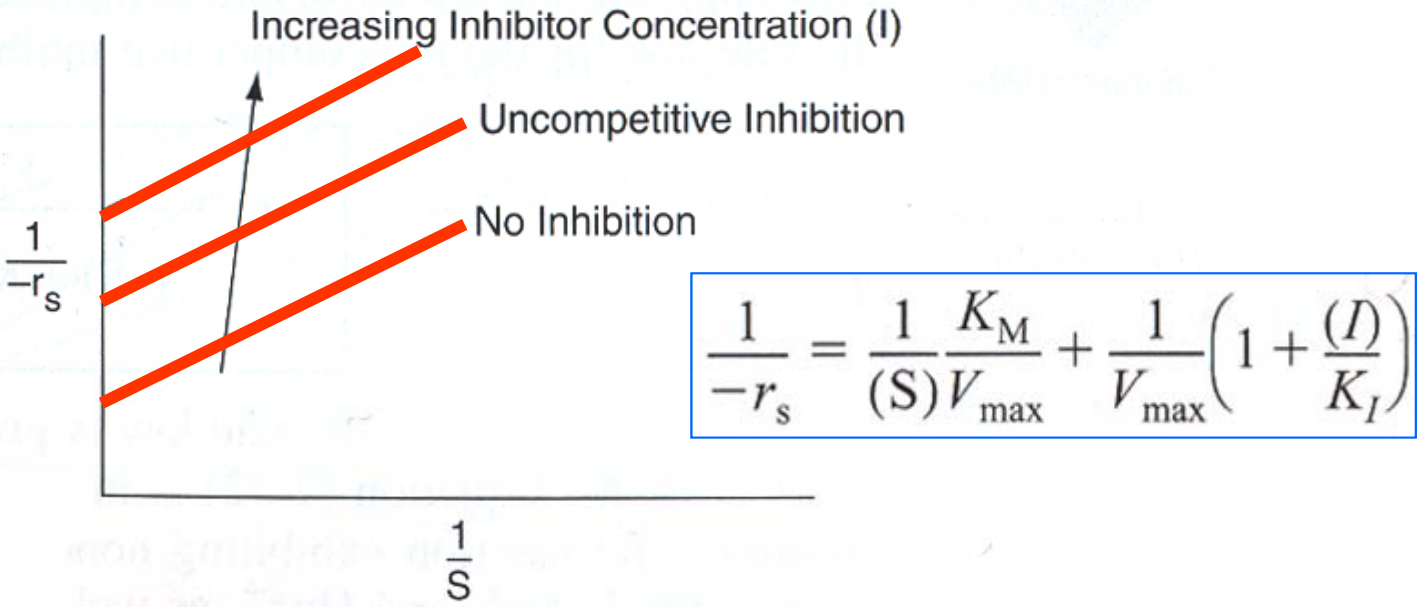
Starting with equation for rate of formation of product, Equation (7-34), and then applying the pseudo-steady-state hypothesis to the intermediate (I • E • S), we arrive at the rate law for uncompetitive inhibition

$$-r_s = r_p = \frac{V_{max}(S)}{K_M + (S)\left(1 + \frac{(I)}{K_I}\right)} \quad \text{where } K_I = \frac{k_5}{k_4} \tag{7-40}$$

Rearranging

$$\frac{1}{-r_s} = \frac{1}{(S)}\frac{K_M}{V_{max}} + \frac{1}{V_{max}}\left(1 + \frac{(I)}{K_I}\right) \tag{7-41}$$

The Lineweaver–Burk plot is shown in Figure 7-11 for different inhibitor concentrations. The slope (K_M/V_{max}) remains the same as the inhibition (I) concentration is increased, while the intercept ($1 + (I)/K_I$) increases.



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

Figure 7-11 Lineweaver–Burk plot for uncompetitive inhibition.

7.3.3 Noncompetitive Inhibition (Mixed Inhibition)

In ***noncompetitive inhibition***, also called *mixed inhibition*, the substrate and inhibitor molecules react with different types of sites on the enzyme molecule. Whenever the inhibitor is attached to the enzyme, it is inactive and can not form products. Consequently, the deactivating complex (I•E•S) can be formed by two reversible reaction paths.

1. After a substrate molecule attaches to the enzyme molecule at the substrate site, the inhibitor molecule attaches to the enzyme at the inhibitor site.
2. After an inhibitor molecule attaches to the enzyme molecule at the inhibitor site, the substrate molecule attaches to the enzyme at the substrate site.

7.3.3 Noncompetitive Inhibition (Mixed Inhibition)

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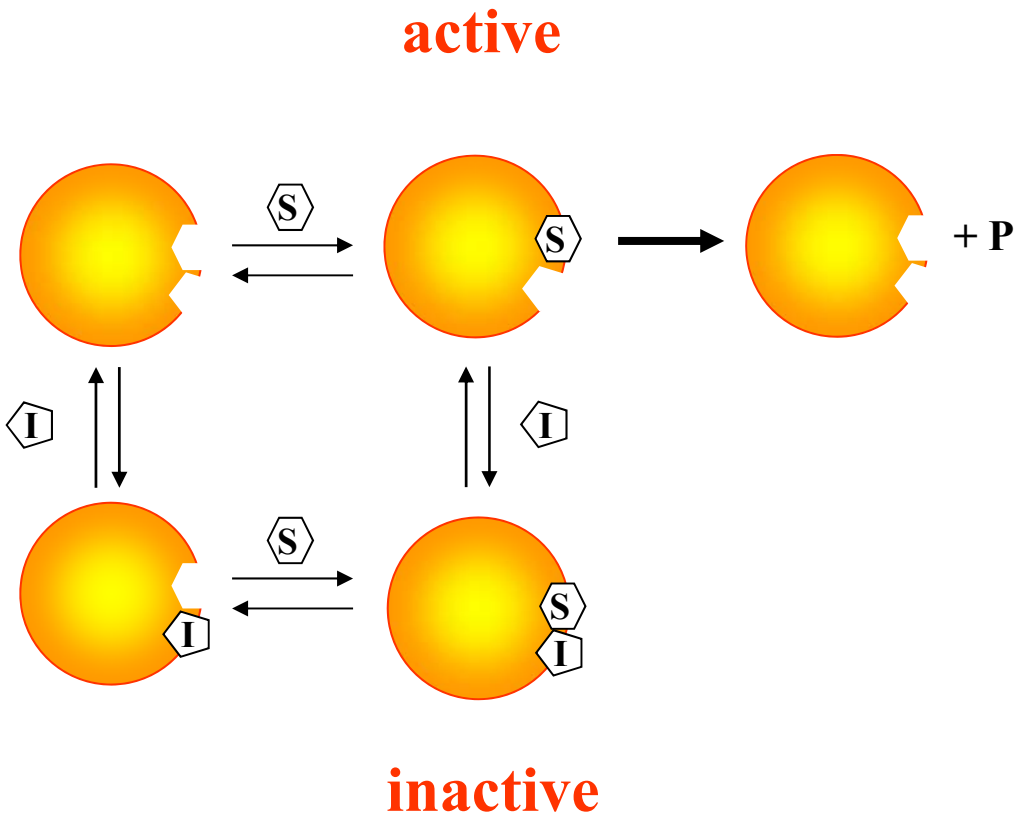


7.3.3 Noncompetitive Inhibition (Mixed Inhibition)

Reaction steps

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

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

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

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

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

Again starting with the rate law for the rate of formation of product and then applying the PSSH to the complexes (I • E) and (I • E • S) we arrive at the rate law for the noncompetitive inhibition

$$-r_s = \frac{V_{max}(S)}{((S) + K_M)\left(1 + \frac{(I)}{K_I}\right)} \tag{7-42}$$

The derivation of the rate law is given in the *Summary Notes* on the web and CD-ROM. Equation (7-42) is in the form of the rate law that is given for an enzymatic reaction exhibiting noncompetitive inhibition. Heavy metal ions such as Pb^{2+}, Ag^{+}, and Hg^{2+}, as well as inhibitors that react with the enzyme to form chemical derivatives, are typical examples of noncompetitive inhibitors.

Rearranging

$$\frac{1}{-r_s} = \frac{1}{V_{max}}\left(1 + \frac{(I)}{K_I}\right) + \frac{1}{(S)}\frac{K_M}{V_{max}}\left(1 + \frac{(I)}{K_I}\right) \qquad (7\text{-}43)$$

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

Intercept = $\frac{1}{V_{max}}\left(1 + \frac{(I)}{K_I}\right)$

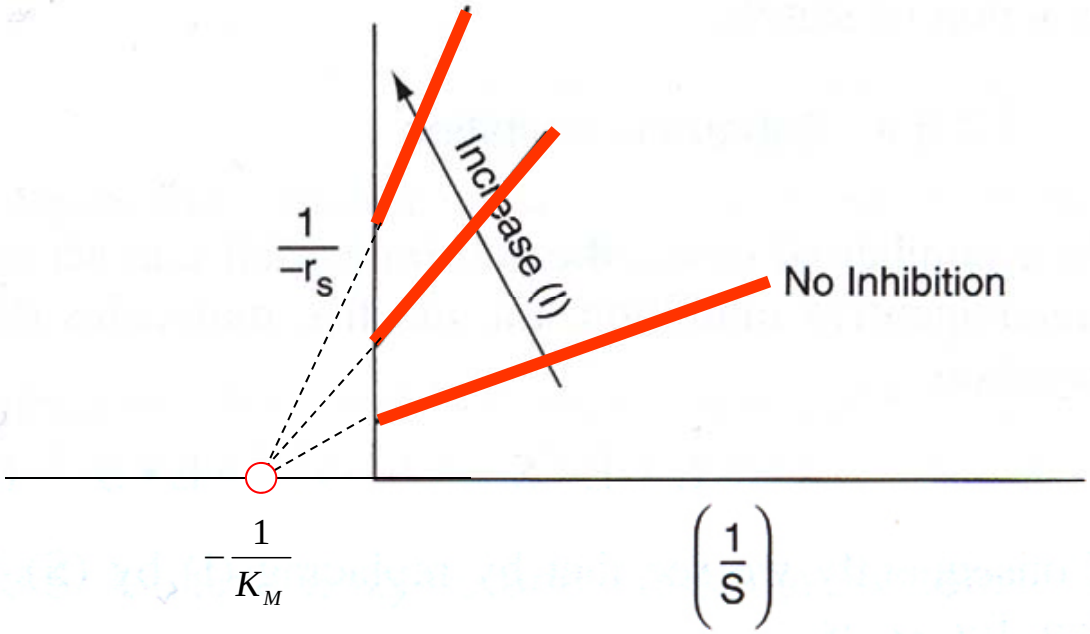


Figure 7-12 Lineweaver–Burk plot for noncompetitive enzyme inhibition.

For noncompetitive inhibition, we see in Figure 7-12 that both the slope $\left(\frac{K_M}{V_{max}}\left[1+\frac{(I)}{K_I}\right]\right)$ and intercept $\left(\frac{1}{V_{max}}\left[1+\frac{(I)}{K_I}\right]\right)$ increase with increasing inhibitor concentration. In practice, *uncompetitive inhibition* and *mixed inhibition* are observed only for enzymes with two or more substrates, S_1 and S_2.

The three types of inhibition are compared with a reaction in which no inhibitors are present on the Lineweaver–Burk plot shown in Figure 7-13.

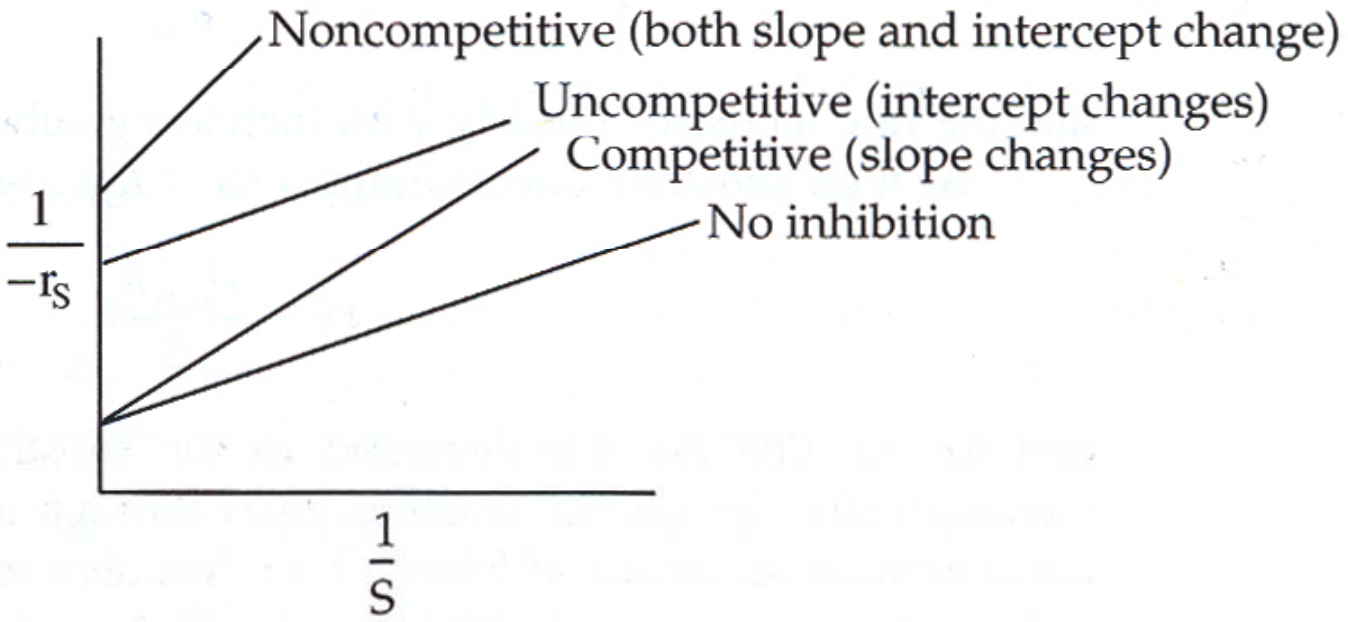


Figure 7-13 Lineweaver–Burk plots for three types of enzyme inhibition.

Summary: Inhibition of Enzyme Reaction

(1) In ***competitive inhibition,*** the slope increases with increasing inhibitor concentration, while intercept remains fixed.

(2) In ***uncompetitive inhibition***, the y-intercept increases with increasing inhibitor concentration, while the slope remains fixed.

(3) In ***noncompetitive inhibition (mixed inhibition)***, both the y-intercept and slope will increase with increasing inhibitor concentration.

7.3.4 Substrate Inhibition

In a number of cases, the substrate itself can act as a inhibitor. In the case of uncompetitive inhibition, the inactive molecules (S • E • S) is formed by the reaction

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

Consequently we see that by replacing (I) by (S) in Equation (7-40) the rate law for $-r_s$ is

$$-r_s = \frac{V_{max} S}{K_M + S + \frac{S^2}{K_I}} \tag{7-44}$$

$$-r_s = r_p = \frac{V_{max}(S)}{K_M + (S)\left(1 + \frac{(I)}{K_I}\right)} \tag{7-40}$$

We see that at low substrate concentrations

$$K_M >> \left(S + \frac{S^2}{K_I}\right) \tag{7-45}$$

$$-r_s \sim \frac{V_{max} S}{K_M} \tag{7-46}$$

and the rate increases linearly with increasing substrate concentration.

At high substrate concentrations $(S^2 / K_I) >> (K_M + S)$, then

$$-r_S = \frac{V_{max} K_I}{S} \tag{7-47}$$

and we see that the rate decreases as the substrate concentration increases.

Consequently, the rate of reaction gives through a maximum in the substrate concentration as shown in Figure 7-14. We also see there is an optimum substrate concentration at which to operate. This maximum is found by taking the derivative of Equation (7-44) wrt S, to obtain

$$S_{max} = \sqrt{K_M K_I} \tag{7-48}$$

When substrate inhibition is possible, a semibatch reactor called a ***fed batch*** is often used as a CSTR to maximize the reaction rate and conversion.

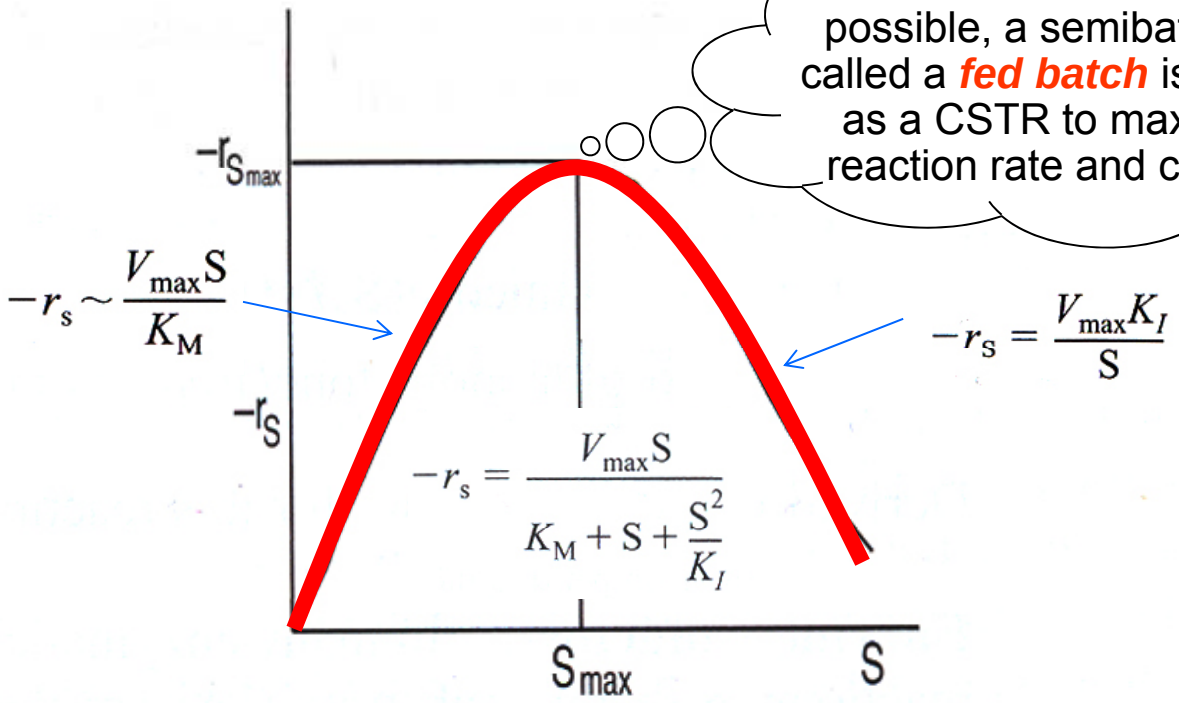


Figure 7-14 Substrate reaction rate as a function of substrate concentration for substrate inhibition.

7.4 Bioreactors

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

- Virtually all cellular reactions necessary to maintain life are mediated by enzymes as they catalyze various aspects of cell metabolism such as the transformation of chemical energy and the construction, breakdown, and digestion of cellular components.

tissue cultures: 조직배양
cellular reaction: 세포반응

7.4 Bioreactors

- Because enzymatic reactions are involved in the growth of microorganisms, we now proceed to study microbial growth and bioreactors.

- Not surprisingly, the Monod equation, which describes the growth law for a number of bacteria, is similar to the Michaelis-Menten equation.

- Consequently, even though bioreactors are not truly homogeneous because of the presence of living cells, we include them in this chapter as a logical progression from enzymatic reactions.

Jacques Monod: 모노 (프랑스의 생화학자; 노벨 생리의학상 수상, 1965)

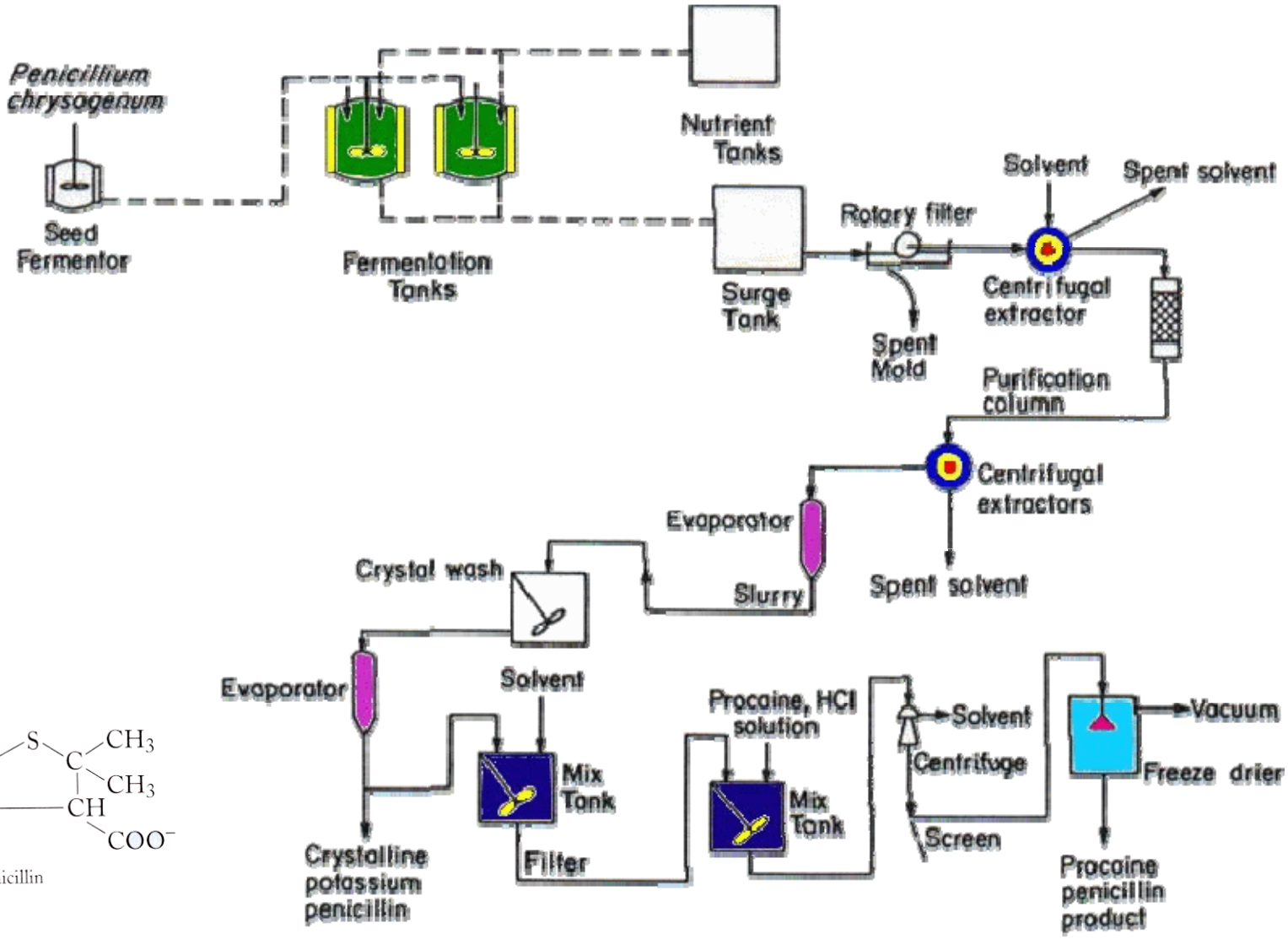
Biotechnology

- The use of living cells to produce marketable chemical products is becoming increasingly important. The number of chemicals, agricultural products and food products produced by biosynthesis has risen dramatically. In 2003, companies in this sector raised over $16 billion of new financing.

- Both microorganisms and mammalian cell are being used to produced a variety of products, such as insulin, most antibiotics and polymers.

- It is expected that in the future a number of organic chemicals currently derived from petroleum will be produced by living cells.

Biotechnology



Penicillin

Advantages of Bioconversions

- Mild reaction conditions

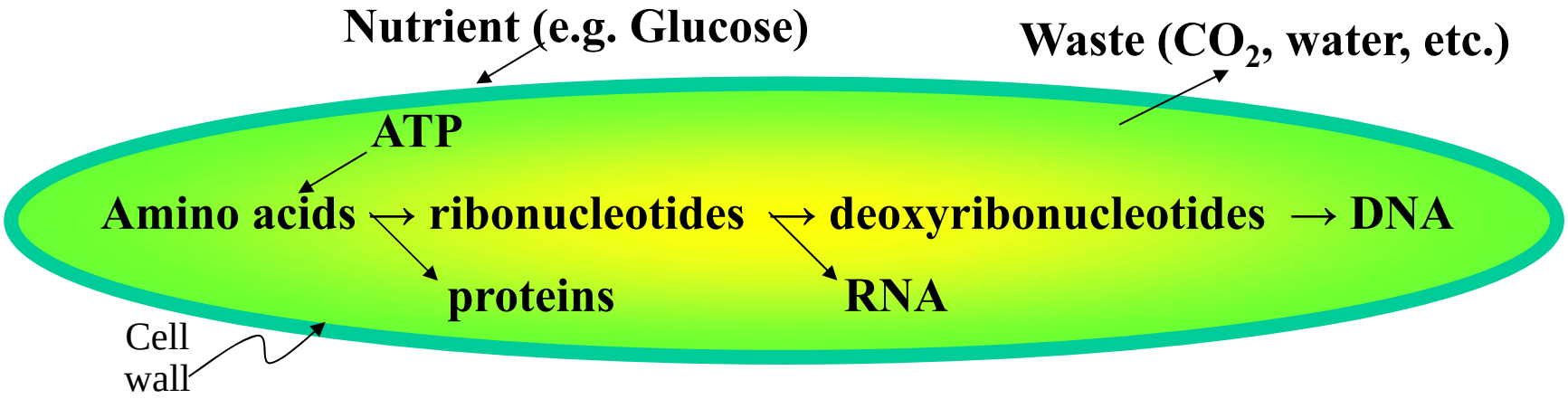
- High yields
  - 100% conversion of glucose to gluconic acid with *Aspergillus niger*

- Enzyme act as stereospecific catalysts
  - produce a single desired isomer
  - conversion of cis-proenylphosphonic acid to antibiotic (-) cis-1,2-epoxypropyl-phosphonic acid

- Bacteria can also be modified and turned into living chemical factories. (using recombinant DNA, a bacterial produces fertilizer by turning nitrogen into nitrates.

Reactions in the cell

- In biosynthesis, the cell consume nutrients to grow and produce more cells and important products with a series of enzyme reaction.
- The reactions take place simultaneously and are classified as either
  class (I) nutrient degradation (fueling reactions),
  class (II) synthesis of small molecules (amino acids) or
  class (III) synthesis of large molecules (polymerization, e.g., RNA, DNA)



- ATP also transfers the energy: it releases when it loses a phosphonate group to form adenosine diphosphate (ADP).

$$ATP + H_2O \rightarrow ADT + P + H_2O + Energy$$

Cell Growth and Division

- The cell growth and division typical mammalian cells (Fig. 7-17)

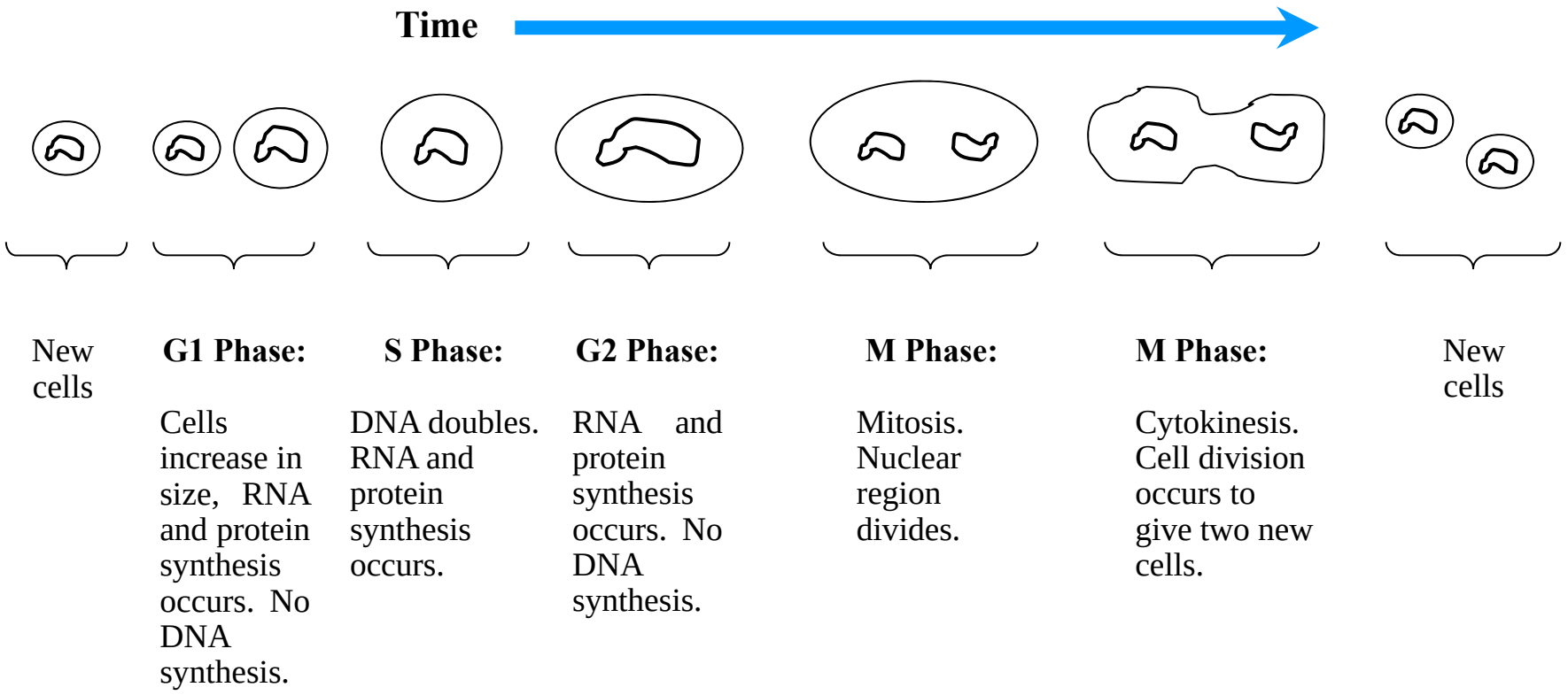


Figure 7-17 Phases of cell division.

Cytokinesis: 세포질분열

Growth of an aerobic organism

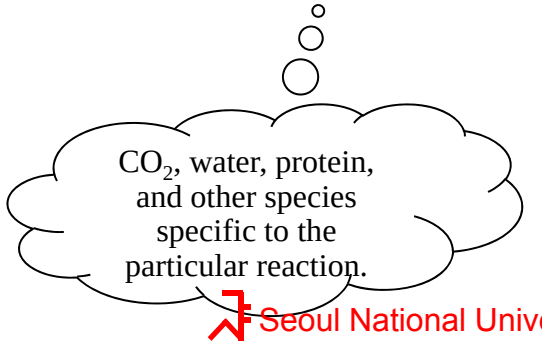
[cells] + [carbon source] + [nitrogen source]
+ [oxygen source] + [phosphate source]

Culture media conditions
(pH, temperature, etc.)

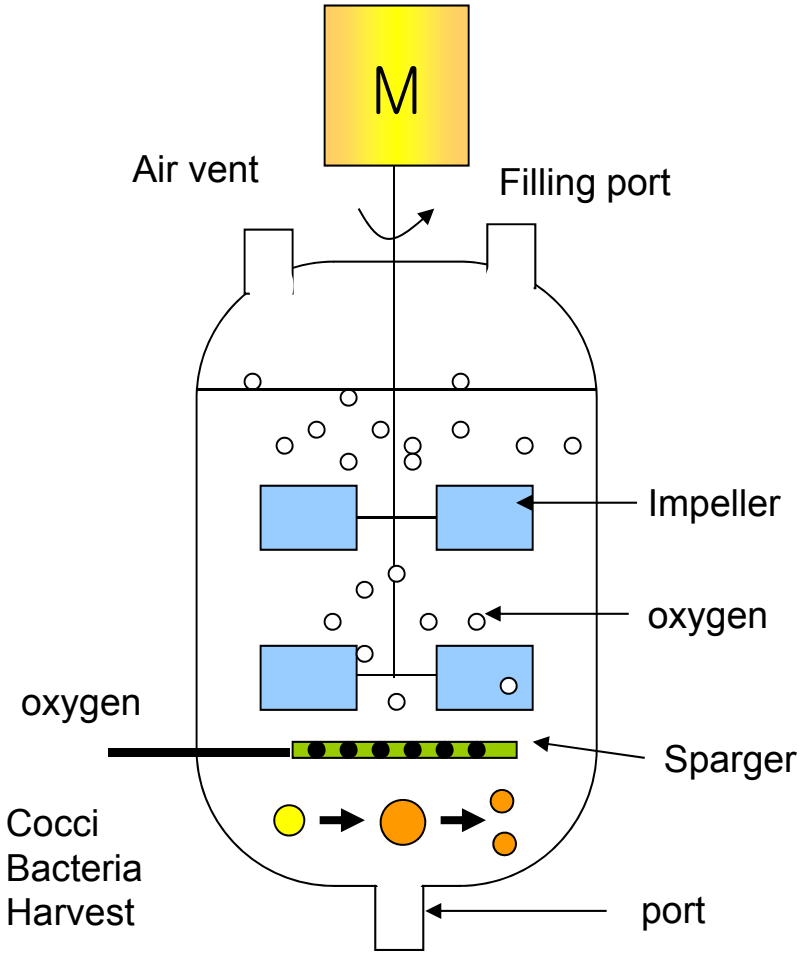
$$[CO_2] + [H_2O] + [\text{products}] + [\text{more cells}] \quad (7\text{-}49)$$

Simple form of (7-49)

$$\textbf{Substrate} \xrightarrow{\text{cells}} \textbf{More cells + Product} \quad (7\text{-}50)$$

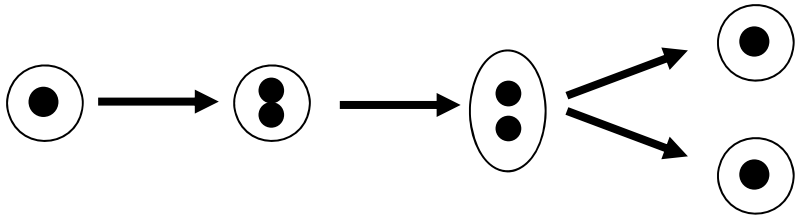


Batch bioreactor

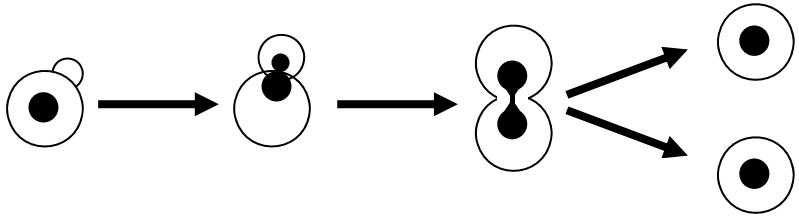


Growth

Bacteria



Yeast Budding



Yeast Budding: 효모발아

7.4.1 Cell Growth

Stages of cell growth in a batch reactor are shown schematically in Figure 7-19 and 7-20.

Initially, a small number of cells is inoculated into (i.e., added to) the batch reactor containing the nutrients and the growth process begins as shown in Figure 7-19.

In Figure 20, the number of living cells is shown as a function of time.

7.4.1 Cell Growth

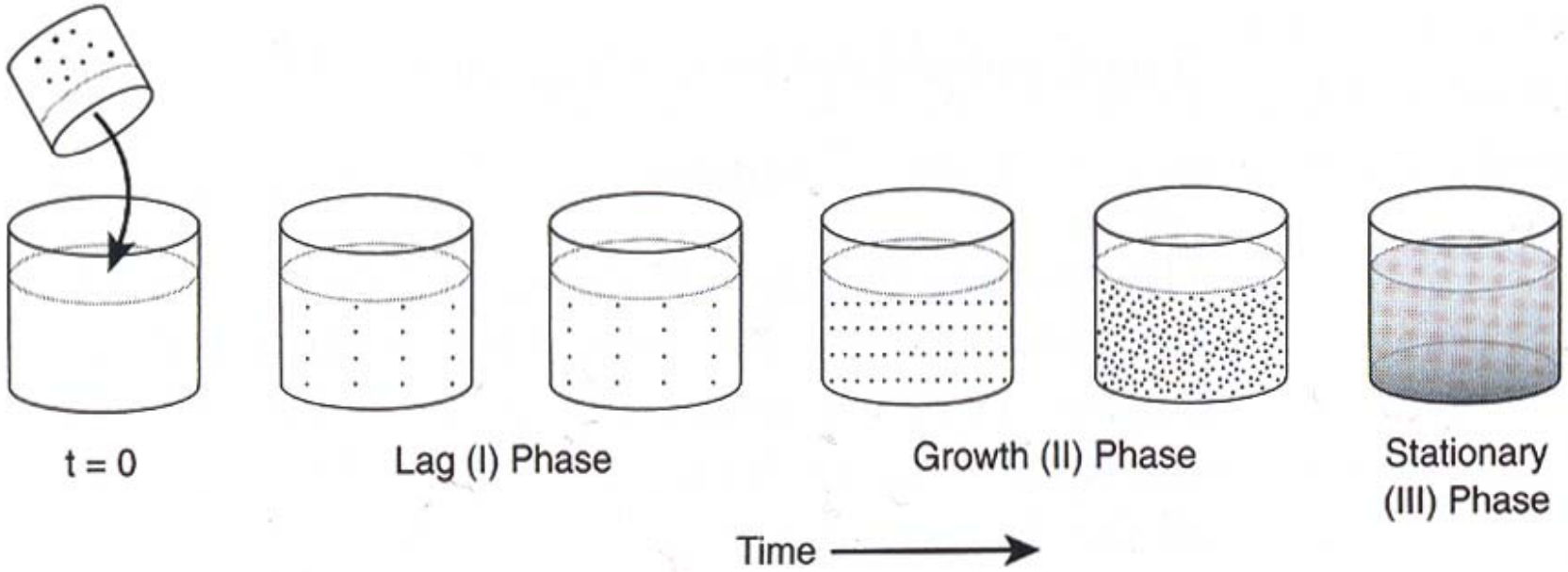


Figure 7-19 Increase in cell concentration.

Lag phase: 지연기
Growth phase: 성장기
Stationary phase: 정체기

7.4.1 Cell Growth

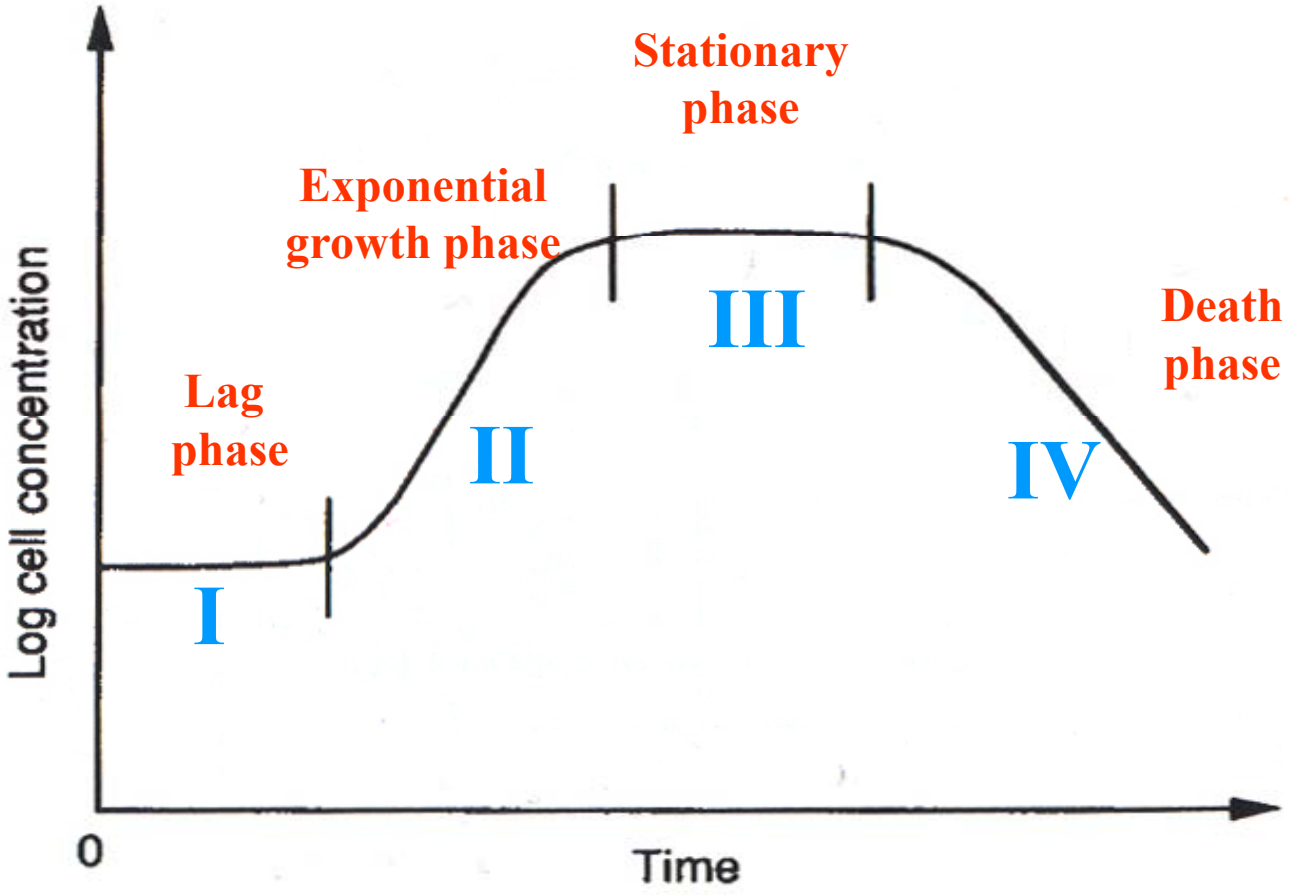
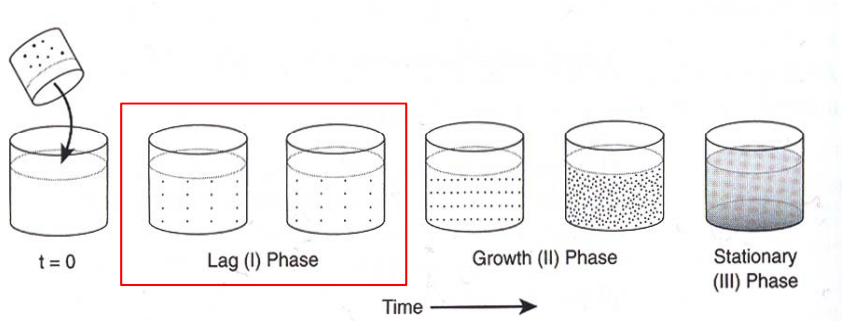


Figure 7-20 Phases of bacteria cell growth.

7.4.1 Cell Growth



In phase I (lag phase)

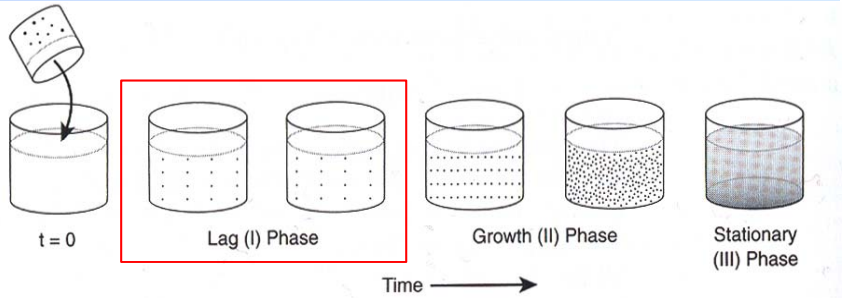
- There is little increase in cell concentration.
- The cells are adjusting to their new environment,

  synthesizing enzymes, and getting ready to begin reproducing.
- The cells carry out such functions as

  - synthesizing transport proteins for moving the substrate into the cell,
  - synthesizing enzymes for utilizing the new substrate, and
  - beginning the work for replicating the cells' genetic material.

7.4.1 Cell Growth

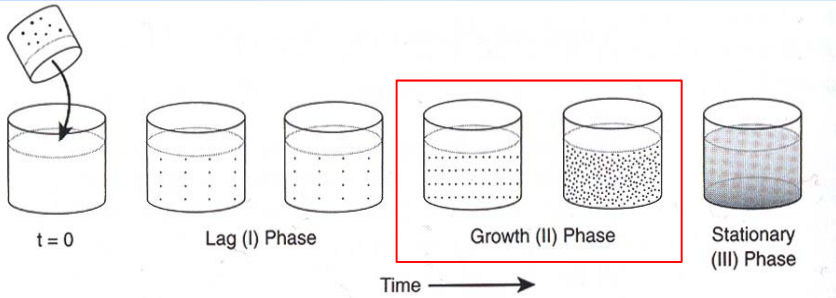
In phase I (lag phase)



- The duration of the lag phase depends upon the growth medium from which the inoculum was taken relative to the reaction medium in which it is placed.
- If the inoculum is similar to the medium of the batch reactor, the lag phase will be almost nonexistent.
- If, however, the inoculum were placed in a medium with a different nutrient or other constants, or if the inoculum culture were in the stationary or death phase, the cells would have to readjust their metabolic path to allow them to consume the nutrients in their new environment.

Inoculum: 접종 미생물

7.4.1 Cell Growth



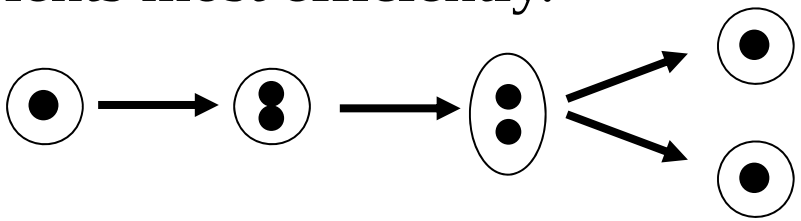
Phase II (Exponential growth phase)

$$r_g = \mu C_c$$

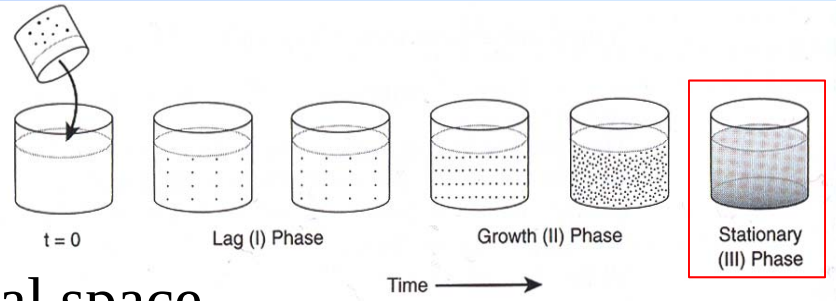
- The cell's growth rate is proportional to the cell concentration.
- The cells are dividing at the maximum rate

  because all the enzyme's pathways for metabolizing the substrate

  are in place as a result of the lag phase.
- The cells are able to use the nutrients most efficiently.



7.4.1 Cell Growth

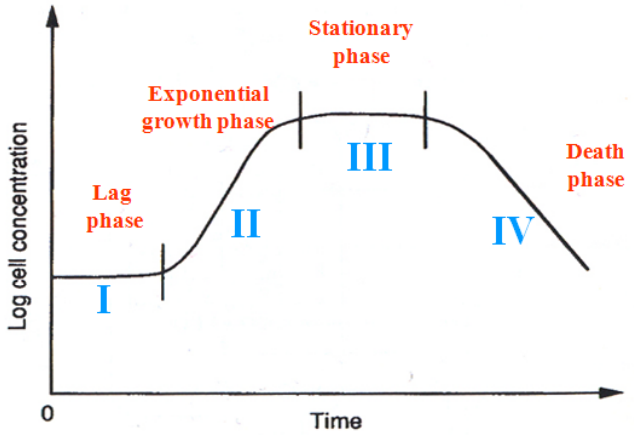


Phase III (Stationary phase)

- The cells reach a minimum biological space where the lack of one or more nutrients limits cell growth.
- The net growth rate is zero as a result of the depletion of nutrients and essential metabolites.
- Many important fermentation products, including most antibiotics, are produced. For example, penicillin produced commercially using *Penicillium chrysogenum* is formed only after cell growth has cease.
- Cell growth is also slowed by the buildup of organic acids and toxic materials generated during the growth phase.

7.4.1 Cell Growth

Phase IV (Death phase)



- The decrease in live cell concentration occurs.
- This decline is a result of the
  - (1) toxic by-products,
  - (2) harsh environments, and/or
  - (3) depletion of nutrient supply.

7.4.2 Rate Law

Cells + Substrate → More cells + Product

the cell growth rate of new cells (exponential growth)

Monod Equation of Cell growth rate

$$r_a = \mu C_c \qquad (7\text{-}51)$$

where r_g = cell growth rate, g/dm^3·s
C_c = cell concentration, g/dm^3
μ = specific growth rate, s^{-1}

7.4.2 Rate Law

The specific cell growth rate can be expressed as

$$\mu = \mu_{max} \frac{C_s}{K_s + C_s} \quad s^{-1} \tag{7-52}$$

where μ_{max} = a maximum specific growth reaction rate, s^{-1}
K_s = the *Monod* constant, g/dm^3
C_s = substrate (i.e., nutrient) concentration, g/dm^3

Representative values of μ_{max} and K_s are 1.3 h^{-1} and 2.2×10^{-5} mol/dm^3, respectively, which are the parameter values for the *E. coli* growth on glucose.

7.4.2 Rate Law

Combining Equations (7-51) and (7-52), we arrive at the ***Monod equation*** for bacterial cell growth rate

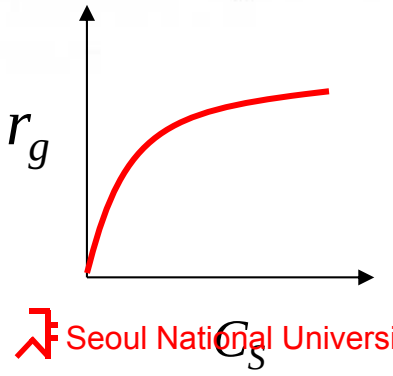
Bacterial cell growth rate

$$r_g = \frac{\mu_{max} C_s C_c}{K_s + C_s} \tag{7-53}$$

For a number of different bacteria, the constant K_s is small, in which case the rate law reduces to

$$r_g = \mu_{max} C_c \tag{7-54}$$

The growth rate, r_g, often depends on more than one nutrient concentration; however, the nutrient that is limiting is usually the one used in Equation (7-53).



7.4.2 Rate Law

In many systems the product inhibits the rate of growth. A classic example of this inhibition is in wine-making, where the fermentation of glucose to produce ethanol is inhibited by the product ethanol. There are a number of different equations to account for inhibition; one such rate law takes the empirical form

$$r_g = k_{\text{obs}} \frac{\mu_{\max} C_s C_c}{K_s + C_s} \tag{7-55}$$

where

$$k_{\text{obs}} = \left(1 - \frac{C_p}{C_p^*}\right)^n \tag{7-56}$$

with

C_p^* = product concentration at which all metabolism ceases, g/dm^3
n = empirical constant

For the glucose-to-ethanol fermentation, typical inhibition parameters are

$$n = 0.5 \quad \text{and} \quad C_p^* = 93 \text{ g/dm}^3$$

7.4.2 Rate Law

In addition to the Monod equation, two other equations are also commonly used to describe the cell growth rate; they are the ***Tessier*** equation,

$$r_g = \mu_{max}\left[1 - \exp\left(-\frac{C_s}{k}\right)\right] C_c \qquad (7\text{-}57)$$

and the ***Moser*** equation,

$$r_g = \frac{\mu_{max} C_c}{(1 + kC_s^{-\lambda})} \qquad (7\text{-}58)$$

The Moser and Tessier growth laws are often used because they have been found to better fit experimental data at the beginning or end of fermentation. Other growth equations can be found in Dean.[19]

7.4.2 Rate Law

The cell death rate is a result of harsh environments, mixing shear forces, local depletion of nutrients and the presence of toxic substances. The rate law is

$$r_d = (k_d + k_t C_t) C_c \qquad (7\text{-}59)$$

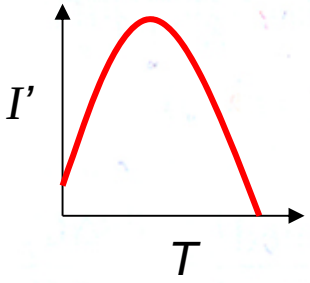
where C_t is the concentration of a substance toxic to the cell. The specific death rate constants k_d and k_t refer to the natural death and death due to a toxic substance, respectively. Representative values of k_d range from 0.1 h^{-1} to less than 0.0005 h^{-1}. The value of k_t depends on the nature of the toxin.

Microbial growth rates are measured in terms of *doubling times*. Doubling time is the time required for a mass of an organism to double. Typical doubling times for bacteria range from 45 minutes to 1 hour but can be as fast as 15 minutes. Doubling times for simple eukaryotes, such as yeast, range from 1.5 to 2 hours but may be as fast as 45 minutes.

Eukaryote: 진핵세포
Yeast: 효모

7.4.2 Rate Law

Effect of Temperature. As with enzymes (cf. Figure 7-9), there is an optimum in growth rate with temperature owing to the competition of increased rates with increasing temperature and denaturizing the enzyme at high temperatures. An empirical law that describes this functionality is given in Aiba et al.[20] and is of the form



$$\mu(T) = \mu(T_m)I'$$

$$I' = \frac{aTe^{-E_1/RT}}{1 + be^{-E_2/RT}} \qquad (7\text{-}60)$$

where I' is the fraction of the maximum growth rate, T_m is the temperature at which the maximum growth occurs, and $\mu(T_m)$ the growth at this temperature. For the rate of oxygen uptake of *Rhizobium trifollic*, the equation takes the form

$$I' = \frac{0.0038Te^{[21.6 - 6700/T]}}{1 + e^{[153 - 48,000/T]}} \qquad (7\text{-}61)$$

The maximum growth occurs at 310K.

7.4.3 Stoichiometry

The stoichiometry for cell growth is very complex and varies with microorganism/nutrient system and environmental conditions such as pH, temperature, and redox potential. This complexity is especially true when more than one nutrient contributes to cell growth, as is usually the case. We shall focus our discussion on a simplified version for cell growth, one that is limited by only one nutrient in the medium. In general, we have

$$\text{Cells} + \text{Substrate} \longrightarrow \text{More cells} + \text{Product}$$

In order to relate the substrate consumed, new cells formed, and product generated, we introduce the *yield* coefficients. The yield coefficient for cells and substrate is

$$Y_{c/s} = \frac{\text{Mass of new cells formed}}{\text{Mass of substrate consumed}} = -\frac{\Delta C_C}{\Delta C_S} \tag{7-62}$$

with

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

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

A representative value of $Y_{c/s}$ might be 0.4 (g/g). See Chapter 3, Problem P3-14$_B$ where the value of $Y_{c/s}$ was calculated.

Product formation can take place during different phases of the cell growth cycle. When product formation only occurs during the exponential growth phase, the rate of product formation is

Growth associated Product formation

$$r_p = Y_{p/c} r_g = Y_{p/c} \mu C_C = Y_{p/c} \frac{\mu_{max} C_c C_s}{K_s + C_s} \tag{7-63}$$

where

$$Y_{p/c} = \frac{\text{Mass of product formed}}{\text{Mass of new cells formed}} = -\frac{\Delta C_p}{\Delta C_c} \tag{7-64}$$

The product of $Y_{p/c}$ and μ, that is, ($q_P = Y_{p/c}\ \mu$) is often called the specific rate of product formation, q_P, (mass product/volume/time). When the product is formed during the stationary phase where no cell growth occurs, we can relate the rate of product formation to substrate consumption by

Non-growth associated Product formation

$$r_p = Y_{p/s}\ (-r_s) \tag{7-65}$$

The substrate in this case is usually a secondary nutrient, which we discuss in more detail later.

The stoichiometric yield coefficient that relates the amount of product formed per mass of substrate consumed is

$$\boxed{Y_{p/s} = \frac{\text{Mass of product formed}}{\text{Mass of substrate consumed}} = -\frac{\Delta C_p}{\Delta C_s}} \qquad (7\text{-}66)$$

In addition to consuming substrate to produce new cells, part of the substrate must be used just to maintain a cell's daily activities. The corresponding maintenance utilization term is

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

A typical value is

$$m = 0.05 \frac{\text{g substrate}}{\text{g dry weight}} \frac{1}{\text{h}} = 0.05\ \text{h}^{-1}$$

The rate of substrate consumption for maintenance whether or not the cells are growing is

$$\boxed{r_{sm} = mC_c} \qquad (7\text{-}67)$$

When maintenance can be neglected, we can relate the concentration of cells formed to the amount of substrate consumed by the equation

$$\boxed{C_c = Y_{c/s}[C_{s0} - C_s]} \tag{7-68}$$

This equation can be used for both batch and continuous flow reactors.

If it is possible to sort out the substrate (S) that is consumed in the presence of cells to form new cells (C) from the substrate that is consumed to form product (P), that is,

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

the yield coefficients can be written as

$$Y'_{c/s} = \frac{\text{Mass of substrate consumed to form new cells}}{\text{Mass of new cells formed}} \tag{7-69A}$$

$$Y'_{s/p} = \frac{\text{Mass of substrate consumed to form product}}{\text{Mass of product formed}} \tag{7-69B}$$

Substrate Utilization. We now come to the task of relating the rate of nutrient consumption, $-r_s$, to the rates of cell growth, product generation, and cell maintenance. In general, we can write

$$\begin{bmatrix}\text{Net rate of}\\ \text{substrate}\\ \text{consumption}\end{bmatrix} = \begin{bmatrix}\text{Rate}\\ \text{consumed}\\ \text{by cells}\end{bmatrix} + \begin{bmatrix}\text{Rate}\\ \text{consumed to}\\ \text{form product}\end{bmatrix} + \begin{bmatrix}\text{Rate}\\ \text{consumed for}\\ \text{maintenance}\end{bmatrix}$$

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

In a number of cases extra attention must be paid to the substrate balance. If product is produced during the growth phase, it may not be possible to separate out the amount of substrate consumed for cell growth from that consumed to produce the product. Under these circumstances all the substrate consumed is lumped into the stoichiometric coefficient, $Y_{s/c}$, and the rate of substrate disappearance is

$$-r_s = Y_{s/c}r_g + mC_c \tag{7-70}$$

The corresponding rate of product formation is

$$r_p = r_g Y_{p/c} \tag{7-63}$$

Because there is no growth during the stationary phase, it is clear that Equation (7-70) cannot be used to account for substrate consumption, nor can the rate of product formation be related to the growth rate [e.g., Equation (7-63)]. Many antibiotics, such as penicillin, are produced in the stationary phase. In this phase, the nutrient required for growth becomes virtually exhausted, and a different nutrient, called the secondary nutrient, is used for cell maintenance and to produce the desired product. Usually, the rate law for product formation during the stationary phase is similar in form to the Monod equation, that is,

$$\boxed{r_p = \frac{k_p C_{sn} C_c}{K_{sn} + C_{sn}}} \tag{7-71}$$

where k_p = specific rate constant with respect to product, ($dm^3/g \cdot s$)
C_{sn} = concentration of the secondary nutrient, g/dm^3
C_c = cell concentration, g/dm^3 (g ≡ gdw = gram dry weight)
K_{sn} = Monod constant, g/dm^3
$r_p = Y_{p/sn}(-r_{sn})$ ($g/dm^3 \cdot s$)

The net rate of secondary nutrient consumption during the stationary phase is

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

$$= mC_c + \frac{Y_{sn/p} k_p C_{sn} C_c}{K_{sn} + C_{sn}} \tag{7-72}$$

Because the desired product can be produced when there is no cell growth, it is always best to relate the product concentration to the change in secondary nutrient concentration. For a batch system the concentration of product, C_p, formed after a time t in the stationary phase can be related to the substrate concentration, C_s, at that time.

$$C_p = Y_{p/s}(C_{sn0} - C_{sn}) \tag{7-73}$$

We have considered two limiting situations for relating substrate consumption to cell growth and product formation; product formation only during the growth phase and product formation only during the stationary phase. An example where neither of these situations applies is fermentation using lactobacillus, where lactic acid is produced during both the logarithmic growth and stationary phase.

The specific rate of product formation is often given in terms of the Luedeking–Piret equation, which has two parameters α (growth) and β (non-growth)

$$q_p = \alpha\mu_g + \beta \tag{7-74}$$

with

$$r_p = q_p C_C$$

The assumption here in using the β-parameter is that the secondary nutrient is in excess.

Example 7-5 Estimate the Yield Coefficients

The following data was determined in a batch reactor for the yeast *Saccharomyces cerevisiae*

$$\text{Glucose} \xrightarrow{\text{cells}} \text{More cells} + \text{Ethanol}$$

Time t(hr)	Cells C_C(g/dm^3)	Glucose C_S(g/dm^3)	Ethanol C_P(g/dm^3)
0	1	250	0
1	1.37	245	2.14
2	1.87	238.7	5.03
3	2.55	229.8	8.96

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

Determine $\boldsymbol{Y_{S/C}}$, $\boldsymbol{Y_{C/S}}$, $\boldsymbol{Y_{S/P}}$, $\boldsymbol{Y_{P/S}}$, $\boldsymbol{Y_{P/C}}$, $\boldsymbol{\mu_{max}}$, and $\boldsymbol{K_S}$. Assume no lag and neglect maintenance at the start of the growth where there are just a few cells.

Example 7-5 Estimate the Yield Coefficients

Solution

(a) Calculate the **substrate and cell** yield coefficient $Y_{S/C}$ and $Y_{C/S}$

$$0 < t < 1\ \text{h}: \quad Y_{S/C} = \frac{-\Delta C_S}{\Delta C_C} = \frac{-(245-250)}{(1.37-1)} = 13.51\ \text{g/g}$$

$$2 < t < 3\ \text{h}: \quad Y_{S/C} = \frac{-\Delta C_S}{\Delta C_C} = \frac{-(229.8-238.7)}{(2.55-1.87)} = 13.1\ \text{g/g}$$

Average value

$$Y_{S/C} = 13.3\ \text{g/g}$$

$$Y_{C/S} = \frac{1}{Y_{S/C}} = \frac{1}{13.3\ \text{g/g}} = 0.075\ \text{g/g}$$

Polymath regression → 13.3 g/g

Example 7-5 Estimate the Yield Coefficients

Solution

(b) Calculate the **substrate and product** yield coefficients $\boldsymbol{Y}_{S/P}$ and $\boldsymbol{Y}_{P/S}$

$$1< t < 2\ \text{h}: \qquad \boldsymbol{Y}_{S/P} = \frac{-\Delta C_S}{\Delta C_P} = \frac{-(238.7-245)}{(5.03-2.14)} = 2.18\ \text{g/g}$$

Average value : $\boldsymbol{Y}_{S/P} = 2.12\ \text{g/g}$

$$\boldsymbol{Y}_{P/S} = \frac{1}{\boldsymbol{Y}_{S/P}} = \frac{1}{2.12\ \text{g/g}} = 0.459\ \text{g/g}$$

Example 7-5 Estimate the Yield Coefficients

Solution

(c) Calculate the **product/cell** yield coefficients $\boldsymbol{Y}_{S/P}$ and $\boldsymbol{Y}_{P/S}$

$$1< t < 2\ \text{h}: \quad \boldsymbol{Y}_{P/C} = \frac{\Delta C_P}{\Delta C_C} = \frac{(5.03\text{-}2.14)}{(1.87-1.37)} = 5.78\ \text{g/g}$$

Average value : $\boldsymbol{Y}_{P/C} = 5.78\ \text{g/g}$

$$\boldsymbol{Y}_{C/P} = \frac{1}{\boldsymbol{Y}_{P/C}} = \frac{1}{5.78\ \text{g/g}} = 0.173\ \text{g/g}$$

Example 7-5 Estimate the Yield Coefficients

Solution

Determine the rate law parameter μ_{max} and K_S in the Monod equation

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

For a batch system

$$r_a = \frac{dC_C}{dt}$$

To find the rate law parameters μ_{max} and K_S, we first apply the differential formulas in Chapter 5 to columns 1 and 2 of Table E7-5.1 to find r_g . Because $C_S >> K_S$ initially, it is best to regress the data using the Henes-Woolf form of the Monod equation

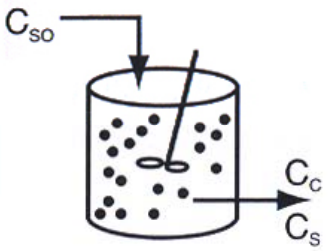
$$\frac{C_C}{r_g} = \frac{K_S}{\mu_{max}}\left(\frac{1}{C_s}\right) + \frac{1}{\mu_{max}}$$

Polymath regression → $\mu_{max} = 0.33\ h^{-1}$ and $K_S = 1.7\ /dm^3$

7.4.4 Mass Balances

There are two ways that we could account for the growth of microorganisms. One is to account for the number of living cells, and the other is to account for the mass of the living cells. We shall use the latter. A mass balance on the microorganism in a CSTR (chemostat) (shown in Figure 7-21) of constant volume is

Cell Balance



$$\left[\begin{array}{c}\text{Rate of}\\ \text{accumulation}\\ \text{of cells,}\\ \text{g/s}\end{array}\right] = \left[\begin{array}{c}\text{Rate of}\\ \text{cells}\\ \text{entering,}\\ \text{g/s}\end{array}\right] - \left[\begin{array}{c}\text{Rate of}\\ \text{cells}\\ \text{leaving,}\\ \text{g/s}\end{array}\right] + \left[\begin{array}{c}\text{Net rate of}\\ \text{generation}\\ \text{of live cells,}\\ \text{g/s}\end{array}\right] \tag{7-75}$$

$$V\frac{dC_c}{dt} = v_0 C_{c0} - vC_c + (r_g - r_d)V$$

Substrate Balance

The corresponding substrate balance is

$$\left[\begin{array}{c}\text{Rate of}\\ \text{accumulation}\\ \text{of substrate,}\\ \text{g/s}\end{array}\right] = \left[\begin{array}{c}\text{Rate of}\\ \text{substrate}\\ \text{entering,}\\ \text{g/s}\end{array}\right] - \left[\begin{array}{c}\text{Rate of}\\ \text{substrate}\\ \text{leaving,}\\ \text{g/s}\end{array}\right] + \left[\begin{array}{c}\text{Rate of}\\ \text{substrate}\\ \text{generation,}\\ \text{g/s}\end{array}\right] \tag{7-76}$$

$$V\frac{dC_s}{dt} = v_0 C_{s0} - vC_s + r_s V$$

In most systems the entering microorganism concentration C_{c0} is zero.

Batch Operation

For a batch system $v = v_0 = 0$ and the mass balances are as follows:

Cell

$$V\frac{dC_c}{dt} = \cancel{v_0 C_{c0}}^{\,0} - \cancel{vC_c}^{\,0} + (r_g - r_d)V$$

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

Dividing by the reactor volume V gives

$$\boxed{\frac{dC_c}{dt} = r_g - r_d} \qquad (7\text{-}77)$$

Substrate

$$V\frac{dC_s}{dt} = \cancel{v_0 C_{s0}}^{\,0} - \cancel{vC_s}^{\,0} + r_s V \qquad (7\text{-}76)$$

The rate of disappearance of substrate, $-r_s$, results from substrate used for cell growth and substrate used for cell maintenance, $\left(-r_s = Y_{s/c} r_g + mC_c\right)$

Growth Phase

$$V\frac{dC_s}{dt} = r_s V = Y_{s/c}(-r_g)V - mC_c V \qquad (7\text{-}78)$$

Dividing by V yields the substrate balance for the growth phase

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

For cells in the stationary phase, where there is no growth, cell maintenance and product formation are the only reactions to consume the substrate. Under these conditions the substrate balance, Equation (7-76), reduces to

Stationary Phase

$$\boxed{V\frac{dC_s}{dt} = -mC_c V + Y_{s/p}(-r_p)V} \qquad (7\text{-}79)$$

Typically, r_p will have the same form of the rate law as r_g [e.g., Equation (7-71)]. Of course, Equation (7-79) only applies for substrate concentrations greater than zero.

Product

The rate of product formation, r_p, can be related to the rate of substrate consumption through the following balance:

$$V\frac{dC_p}{dt} = r_pV = Y_{p/s}(-r_s)V \qquad (7\text{-}80)$$

During the growth phase we could also relate the rate of formation of product, r_p, to the cell growth rate, r_g. The coupled first-order ordinary differential equations above can be solved by a variety of numerical techniques.

Example 7-6 Bacteria Growth in a Batch Reactor

Glucose-to ethanol fermentation is to be carried out in a batch reactor using an organism such as *Saccharomyces cerevisiae*.

Plot the concentrations of cells, substrate, and product and growth rates as a function of time. The initial cell concentration is 1.0 g/L, and the substrate (glucose) concentration is 250 g/L.

Additional data:

C_P^*=93 g/L
n=0.52
μ_{max}=0.33 h^{-1}
K_s=1.7 g/L
m=0.03 (g substrate)/(g cells · h)

$Y_{c/s}$=0.08 g/g
$Y_{p/s}$=0.45g/g (est.)
$Y_{p/c}$=5.6 g/g (est.)
k_d=0.01 h^{-1}

Example 7-6 Bacteria Growth in a Batch Reactor

Solution

1. Mass balances:

Cells: $$V\frac{dC_C}{dt} = (r_g - r_d)V$$

Substrate: $$V\frac{dC_S}{dt} = Y_{S/C}(-r_gV) - r_{sm}V$$

Product: $$V\frac{dC_P}{dt} = Y_{P/C}(r_gV)$$

Example 7-6 Bacteria Growth in a Batch Reactor

Solution

2. Rate laws:

Cell growth: $$r_g = \mu_{\max}\left(1-\frac{C_P}{C_P^*}\right)^{0.52}\frac{C_C C_S}{K_S + C_S}$$

Cell death: $$-r_d = k_d C_C$$

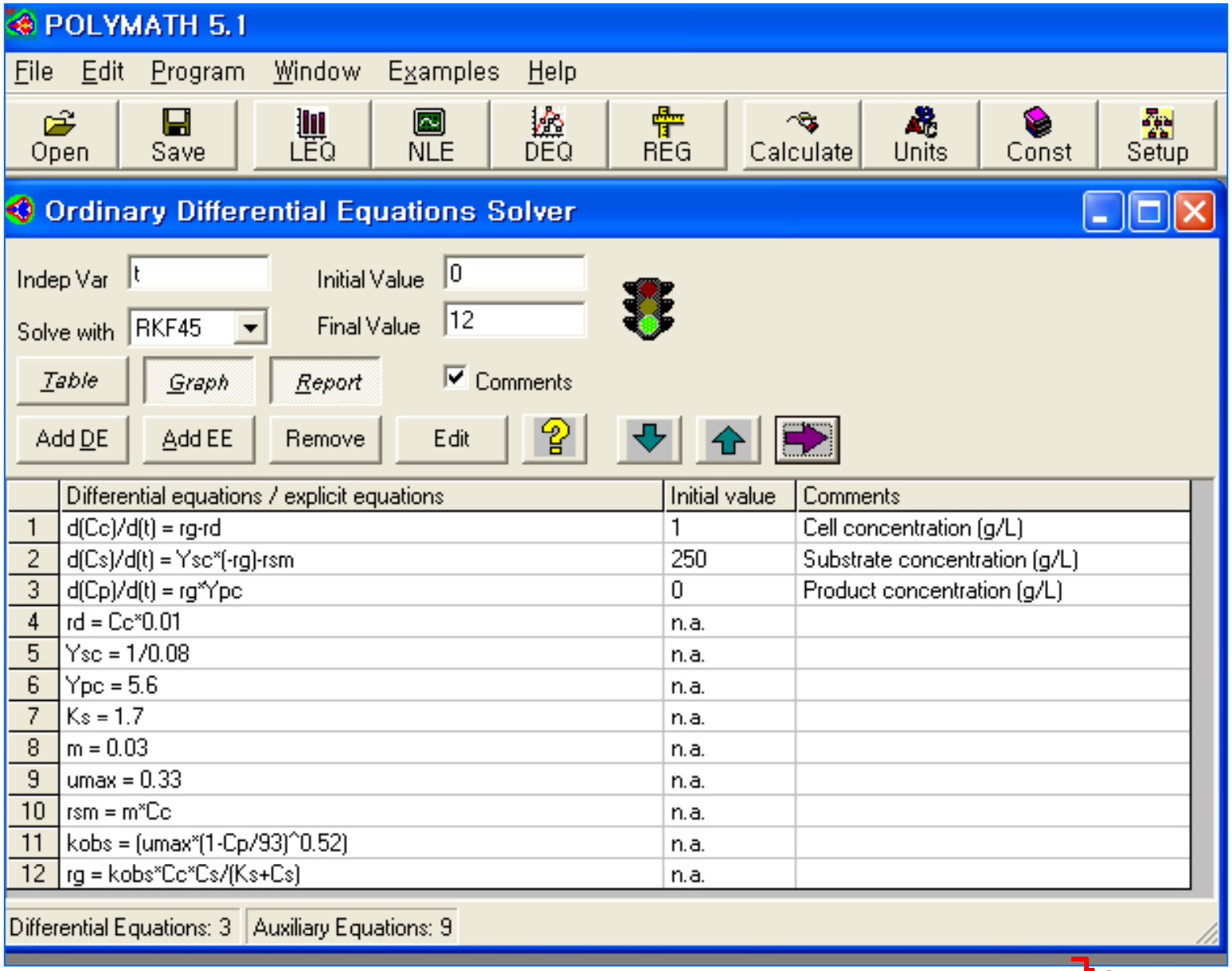
Cell maintain: $$-r_{sm} = mC_C$$

Example 7-6 Bacteria Growth in a Batch Reactor

Solution

3. Stoichometry: $r_P = Y_{P/C} r_g$

Example 7-6 Bacteria Growth in a Batch Reactor



	Differential equations / explicit equations	Initial value	Comments
1	d(Cc)/d(t) = rg-rd	1	Cell concentration (g/L)
2	d(Cs)/d(t) = Ysc*(-rg)-rsm	250	Substrate concentration (g/L)
3	d(Cp)/d(t) = rg*Ypc	0	Product concentration (g/L)
4	rd = Cc*0.01	n.a.	
5	Ysc = 1/0.08	n.a.	
6	Ypc = 5.6	n.a.	
7	Ks = 1.7	n.a.	
8	m = 0.03	n.a.	
9	umax = 0.33	n.a.	
10	rsm = m*Cc	n.a.	
11	kobs = (umax*(1-Cp/93)^0.52)	n.a.	
12	rg = kobs*Cc*Cs/(Ks+Cs)	n.a.	

Example 7-6 Bacteria Growth in a Batch Reactor

Differential Equations Solution #3

POLYMATH Results
06-11-2007, Rev5.1.233

Calculated values of the DEQ variables

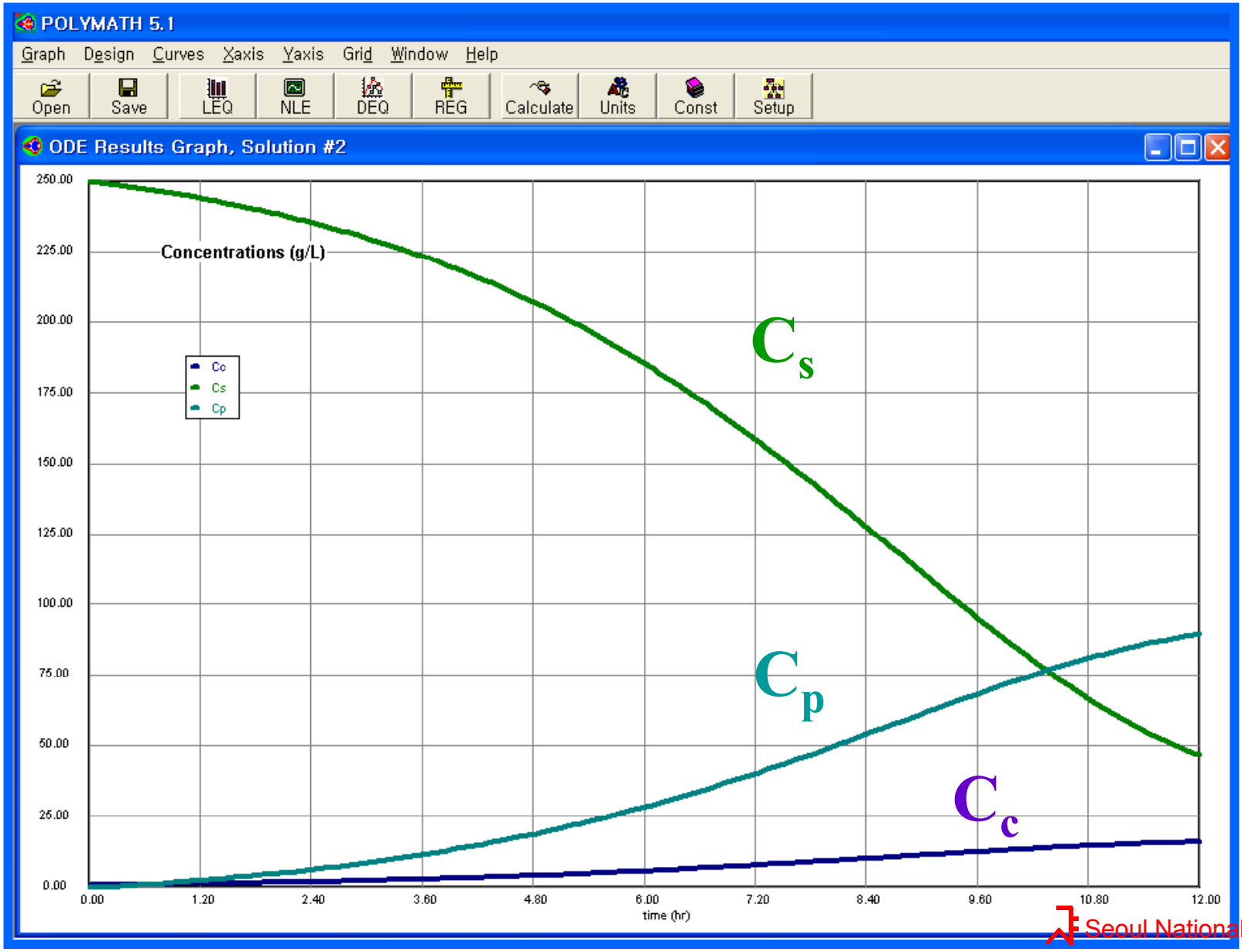
Variable	initial value	minimal value	maximal value	final value
t	0	0	12	12
Cc	1	1	16.184058	16.184058
Cs	250	46.935135	250	46.935135
Cp	0	0	89.82293	89.82293
rd	0.01	0.01	0.1618406	0.1618406
Ysc	12.5	12.5	12.5	12.5
Ypc	5.6	5.6	5.6	5.6
Ks	1.7	1.7	1.7	1.7
m	0.03	0.03	0.03	0.03
umax	0.33	0.33	0.33	0.33
rsm	0.03	0.03	0.4855217	0.4855217
kobs	0.33	0.0570107	0.33	0.0570107
rg	0.3277712	0.3277712	2.1454943	0.8904142

ODE Report (RKF45)

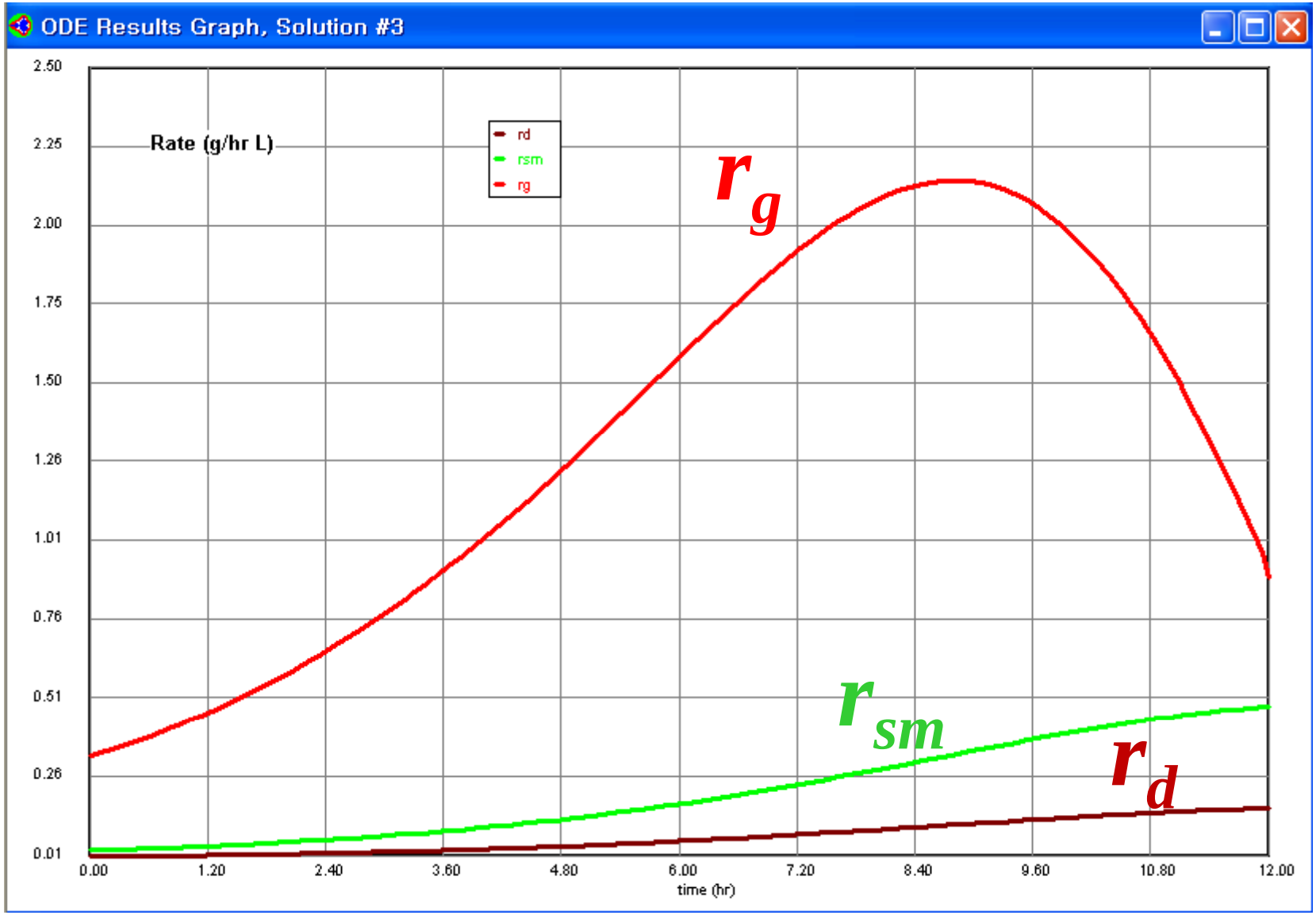
Differential equations as entered by the user
[1] d(Cc)/d(t) = rg-rd
[2] d(Cs)/d(t) = Ysc*(-rg)-rsm
[3] d(Cp)/d(t) = rg*Ypc

Explicit equations as entered by the user
[1] rd = Cc*0.01
[2] Ysc = 1/0.08
[3] Ypc = 5.6
[4] Ks = 1.7
[5] m = 0.03
[6] umax = 0.33
[7] rsm = m*Cc
[8] kobs = (umax*(1-Cp/93)^0.52)
[9] rg = kobs*Cc*Cs/(Ks+Cs)

Example 7-6 Bacteria Growth in a Batch Reactor

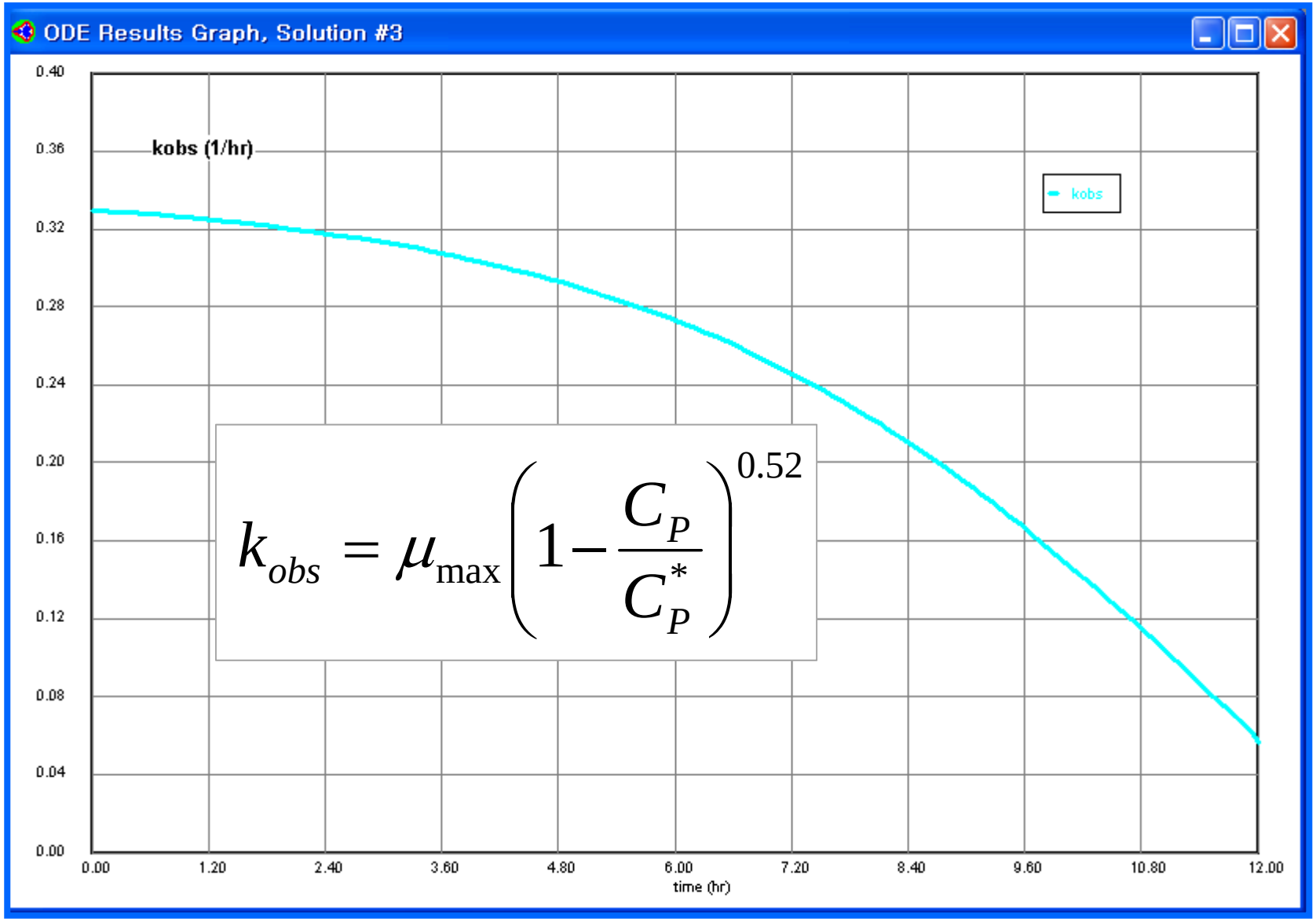


Example 7-6 Bacteria Growth in a Batch Reactor



$r_g > r_d$

Example 7-6 Bacteria Growth in a Batch Reactor



Example 7-6 Bacteria Growth in a Batch Reactor

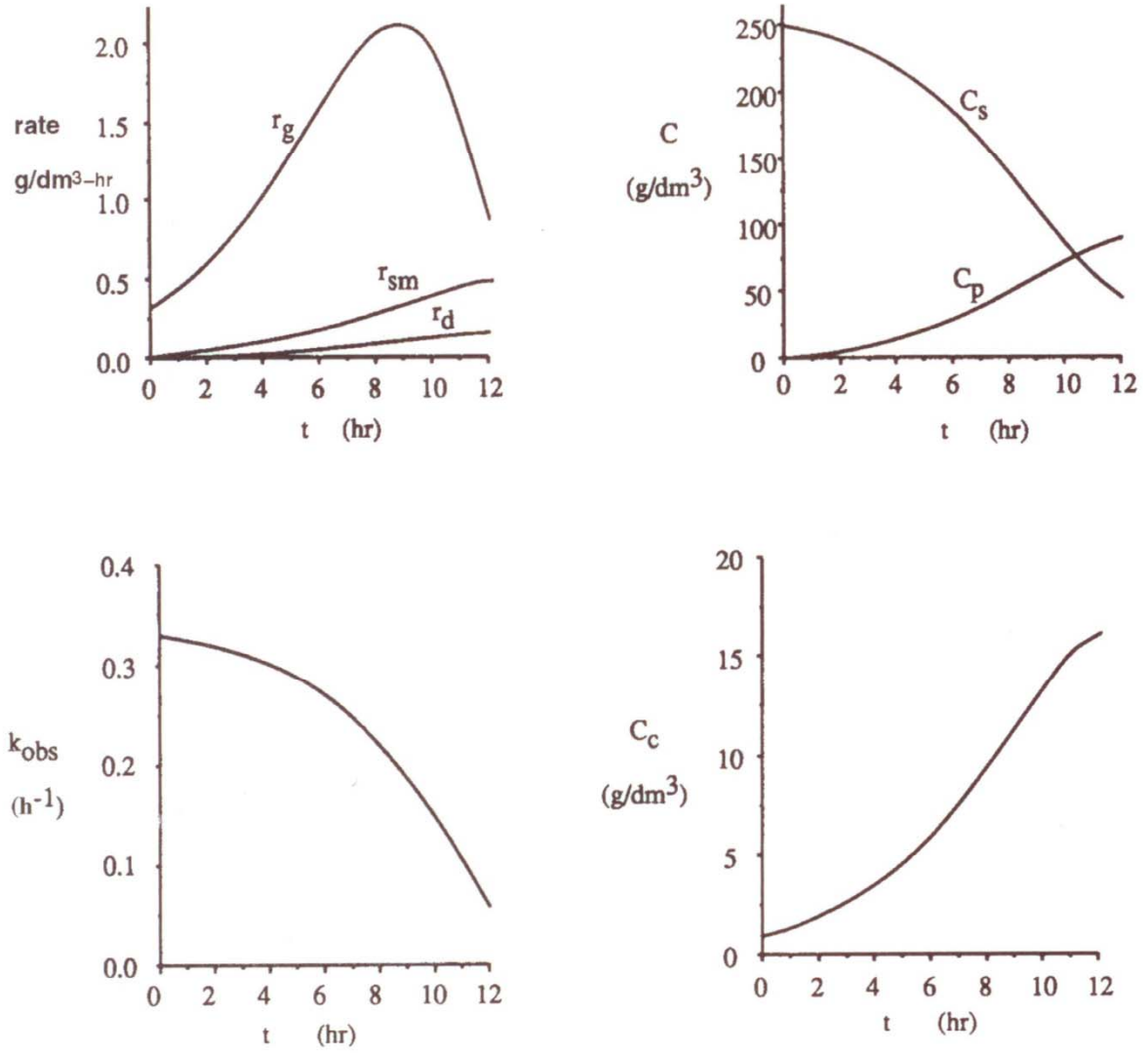


Figure E7-6.1 Concentrations and rates as a function of time.

7.4.5 Chemostats

Chemostats are essentially **CSTRs** that contain microorganisms. One of the most important features of the chemostats is that it allows the operator to control the cell growth rate. This control of the growth rate is achieved by adjusting the volumetric feed rate (dilution rate).

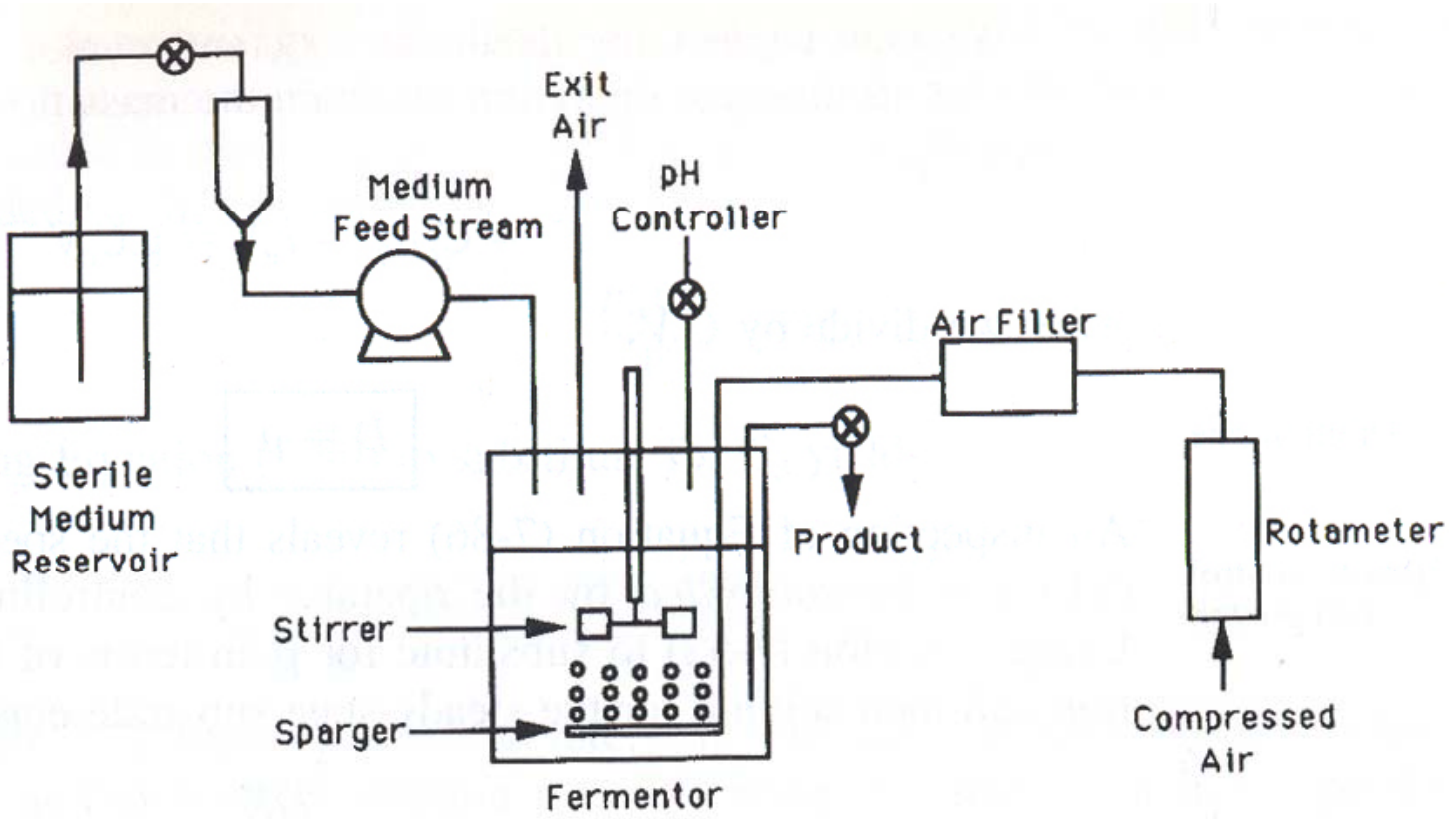


Figure 7-21 Chemostat system.

7.4.5 Chemostats

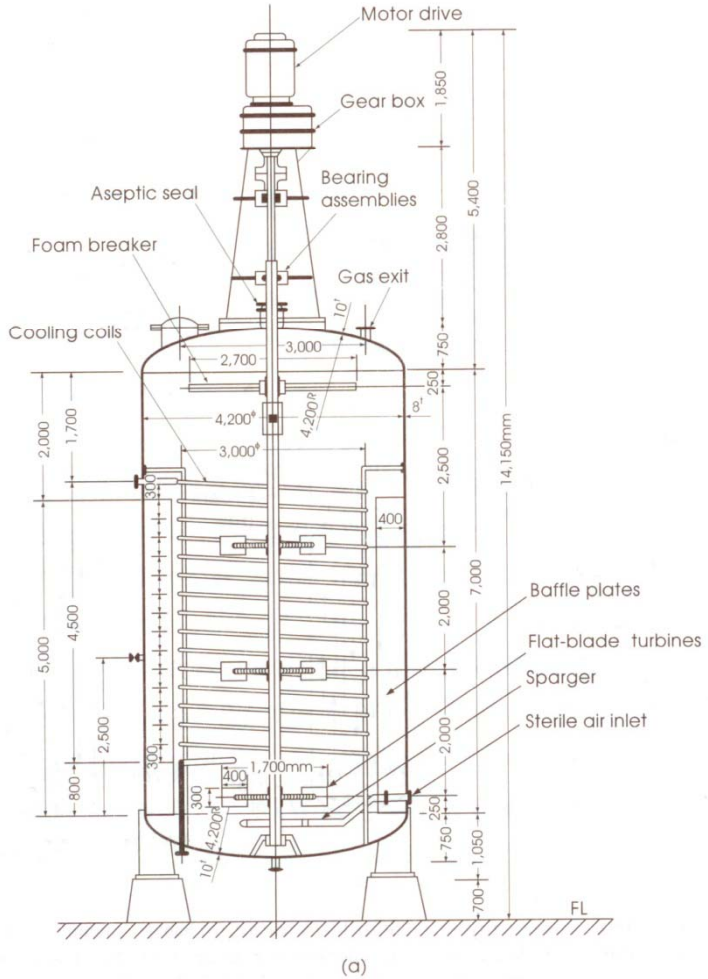


Figure 10.2 (a) Mechanically stirred 100,000-liter fermenter. (With permission, from S. Alba, A. E. Humphrey, and N. F. Millis, *Biochemical Engineering*, 2nd ed., University of Tokyo Press, Tokyo, 1973.)(b; see p. 289) Installation of mechanically stirred fermenter: S, steam, C, condensate; W, water, and A, air. The steam lines permit in-place sterilization of valves, pipes, and seals. The input air can be sterilized by both incineration and filtration. (With permission, from W. Crueger and A. Crueger, *Biotechnology: A Textbook of Industrial Microbiology*, R. Oldenbourg Verlag, München, Germany, 1984.)

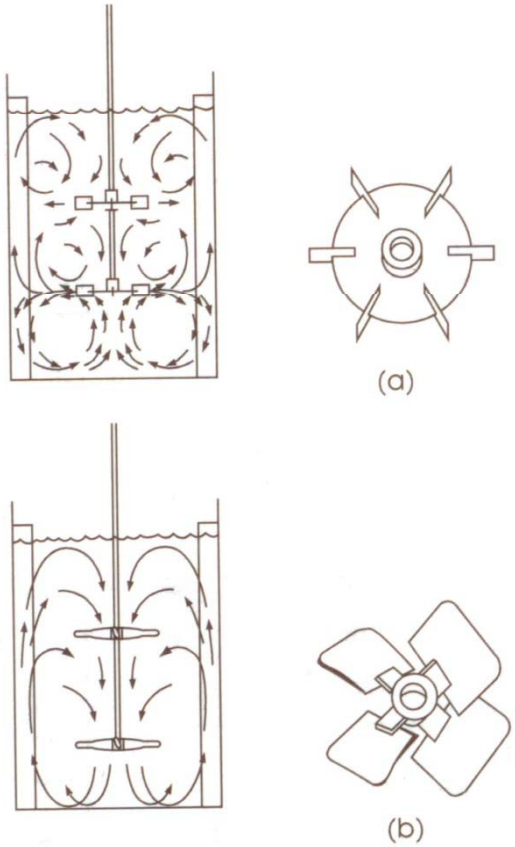


Figure 10.3 Liquid flow in bottled tanks with (a) Rushton radial flow impellers and with (b) axial flow hydrofoil impellers.

7.4.5 Chemostats

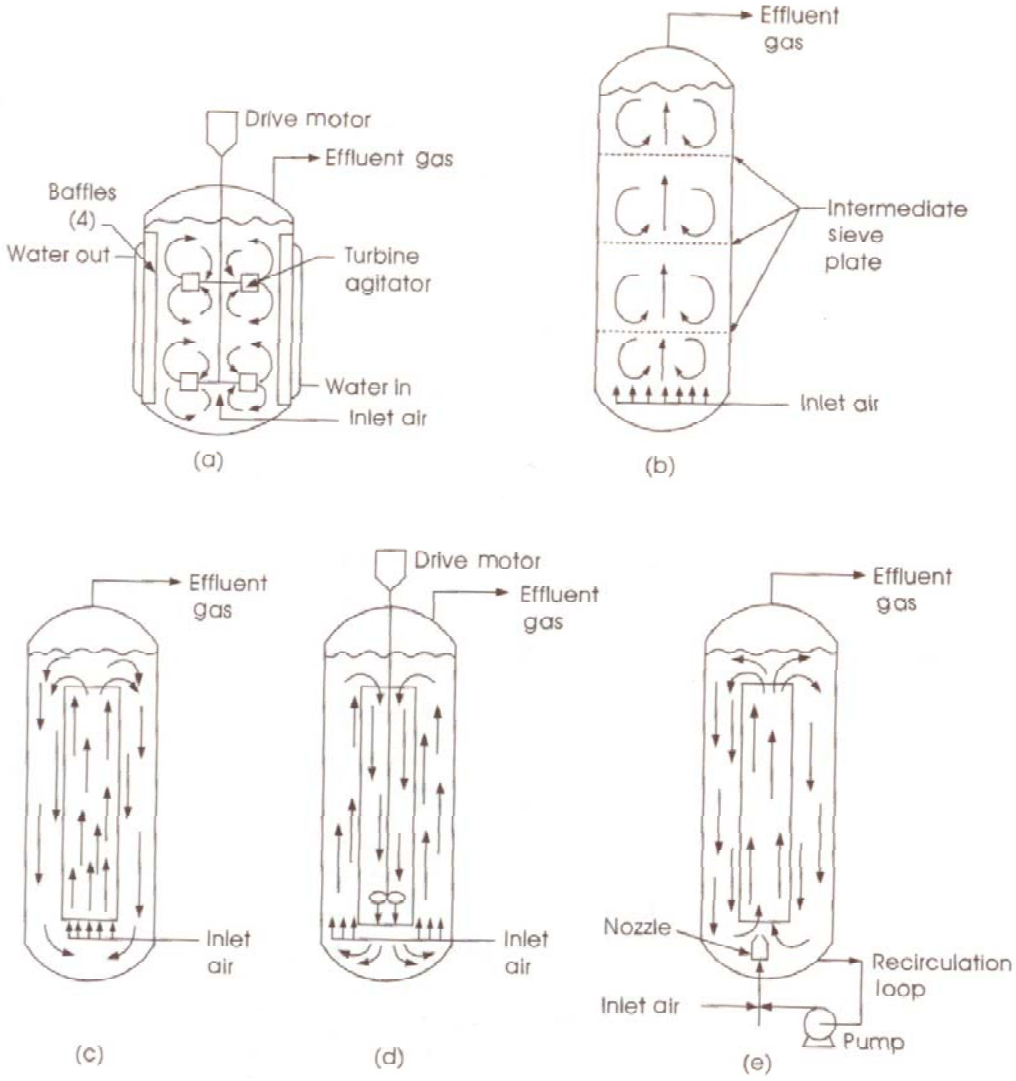


Figure 10.1 Bioreactor types. (a) Stirred-tank reactor, (b) bubble-column reactor, (c) airlift loop reactor with central draft tube, (d) propeller loop reactor, and (e) jet loop reactor. Arrows indicate fluid circulation patterns. (With permission, from D. N. Bull, R. W. Thoma, and T. E. Stinnett, *Adv. Biotechnol. Processes I*, 1, 1985, and Alan R. Liss, Inc., New York.)

7.4.6 Design Equations

CSTR

In this section we return to mass equations on the cells [Equation (7-75)] and substrate [Equation (7-76)] and consider the case where the volumetric flow rates in and out are the same and that no live (i.e., viable) cells enter the chemostat. We next define a parameter common to bioreactors called the dilution rate, D. The dilution rate is

$$D = \frac{v_0}{V}$$

CSTR mass balance

and is simply the reciprocal of the space time τ. Dividing Equations (7-75) and (7-76) by V and using the definition of the dilution rate, we have

$$\text{Accumulation} = \text{In} - \text{Out} + \text{Generation}$$

$$V\frac{dC_c}{dt} = v_0 C_{c0} - vC_c + (r_g - r_d)V$$

$$\text{Cell:} \quad \frac{dC_c}{dt} = 0 - DC_c + (r_g - r_d) \tag{7-81}$$

$$V\frac{dC_s}{dt} = v_0 C_{s0} - vC_s + r_s V$$

$$\text{Substrate:} \quad \frac{dC_s}{dt} = DC_{s0} - DC_s + r_s \tag{7-82}$$

Using the Monod equation, the growth rate is determined to be

$$r_g = \mu C_c = \frac{\mu_{max} C_s C_c}{K_s + C_s} \tag{7-53}$$

For steady-state operation we have

$$DC_c = r_g - r_d \tag{7-83}$$

and

$$D(C_{s0} - C_s) = r_s \tag{7-84}$$

$$V\frac{dC_c}{dt} = v_0 C_{c0} - vC_c + (r_g - r_d)V$$

We now neglect the death rate, r_d, and combine Equations (7-51) and (7-83) for steady-state operation to obtain the mass flow rate of cells out of the system, F_c.

$r_g = \mu C_c$

$$F_c = C_c v_0 = r_g V = \mu C_c V \tag{7-85}$$

After we divide by $C_c V$,

Dilution rate

$$\boxed{D = \mu} \tag{7-86}$$

An inspection of Equation (7-86) reveals that the specific growth rate of the cells *can be controlled* by the operator by controlling the dilution rate D. Using Equation (7-52) to substitute for μ in terms of the substrate concentration and then solving for the steady-state substrate concentration yields

$$\mu = \mu_{max}\frac{C_s}{K_s + C_s} \qquad C_s = \frac{DK_s}{\mu_{max} - D} \tag{7-87}$$

Assuming that a single nutrient is limiting, cell growth is the only process contributing to substrate utilization, and that cell maintenance can be neglected, the stoichiometry is

$$-r_s = r_g Y_{s/c} \tag{7-88}$$

$$C_c = Y_{c/s}(C_{s0} - C_s) \tag{7-68}$$

Substituting for C_s using Equation (7-87) and rearranging, we obtain

$$\boxed{C_c = Y_{c/s}\left[C_{s0} - \frac{DK_s}{\mu_{max} - D}\right]} \tag{7-89}$$

7.4.7 Wash-out

$$\frac{dC_c}{dt} = 0 \qquad -DC_c + (r_g - r_d) \qquad r_g = \mu_{max} C_c \qquad \mu = \mu_{max} \frac{C_s}{K_s + C_s}$$

To learn the effect of increasing the dilution rate, we combine Equations (7-81) and (7-54) and set $r_d = 0$ to get

$$\frac{dC_c}{dt} = (\mu - D)C_c \tag{7-90}$$

We see that if $D > \mu$, then dC_c/dt will be negative, and the cell concentration will continue to decrease until we reach a point where all cells will be washed out:

$$C_c = 0$$

The dilution rate at which wash-out will occur is obtained from Equation (7-89) by setting $C_c = 0$.

$$C_c = Y_{c/s}\left[C_{s0} - \frac{DK_s}{\mu_{max} - D}\right] = 0$$

Flow rate at which Wash-out occurs

$$\boxed{D_{max} = \frac{\mu_{max} C_{s0}}{K_s + C_{s0}}} \tag{7-91}$$

We next want to determine the other extreme for the dilution rate, which is the rate of maximum cell production. The cell production rate per unit volume of reactor is the mass flow rate of cells out of the reactor (i.e., $\dot{m}_c = C_c v_0$) divided by the volume V, or

$$\frac{v_0 C_c}{V} = DC_c$$

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

Using Equation (7-89) to substitute for C_c yields

Cell production rate / Volume of reactor

$$DC_c = DY_{c/s}\left(C_{s0} - \frac{DK_s}{\mu_{max} - D}\right) \tag{7-92}$$

Figure 7-22 shows production rate, cell concentration, and substrate concentration as functions of dilution rate. We observe a maximum in the production rate, and this maximum can be found by differentiating the production rate, Equation (7-92), with respect to the dilution rate D:

$$\frac{d(DC_c)}{dD} = 0 \tag{7-93}$$

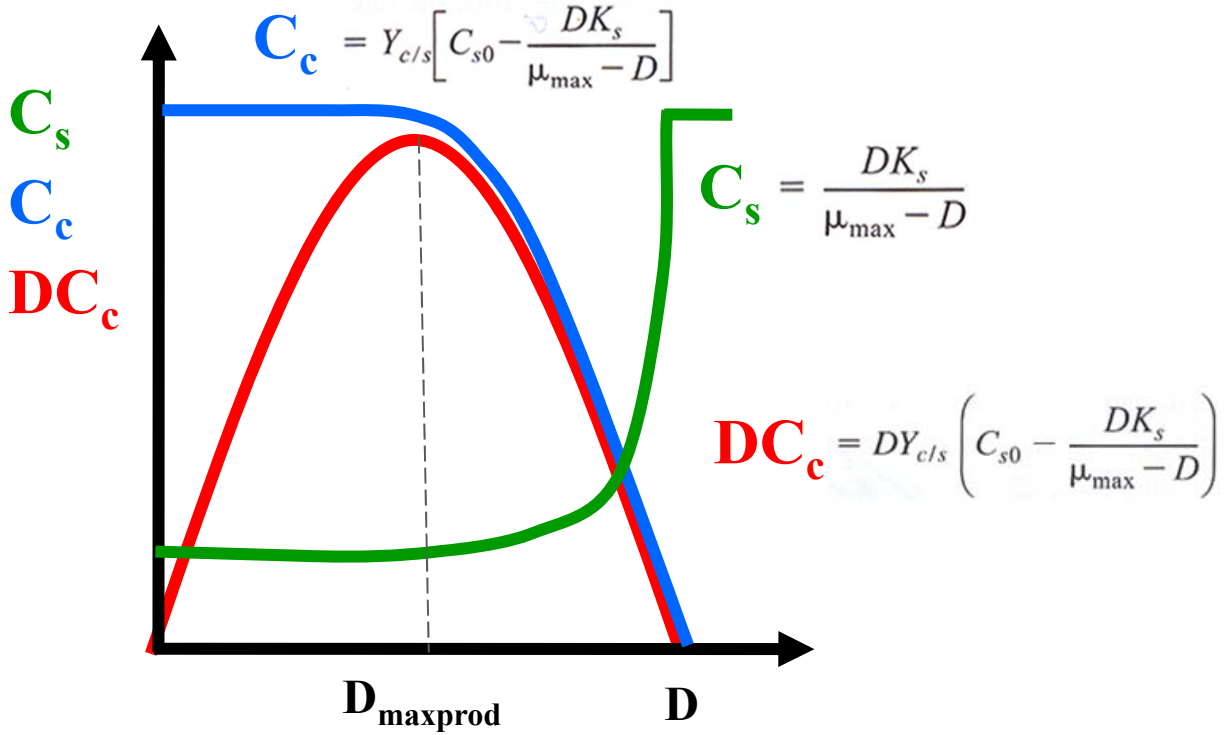


Figure 7-22 Cell concentration and production rate as a function of dilution rate.

Then

$$D_{maxprod} = \mu_{max}\left(1 - \sqrt{\frac{K_s}{K_s + C_{s0}}}\right) \tag{7-94}$$

The organism *Streptomyces aureofaciens* was studied in a 10 dm^3 chemostat using sucrose as a substrate. The cell concentration, C_c (mg/ml), the substrate concentration, C_s (mg/ml), and the production rate, DC_c (mg/ml/h), were

measured at steady state for different dilution rates. The data are shown in Figure 7-23.[21]

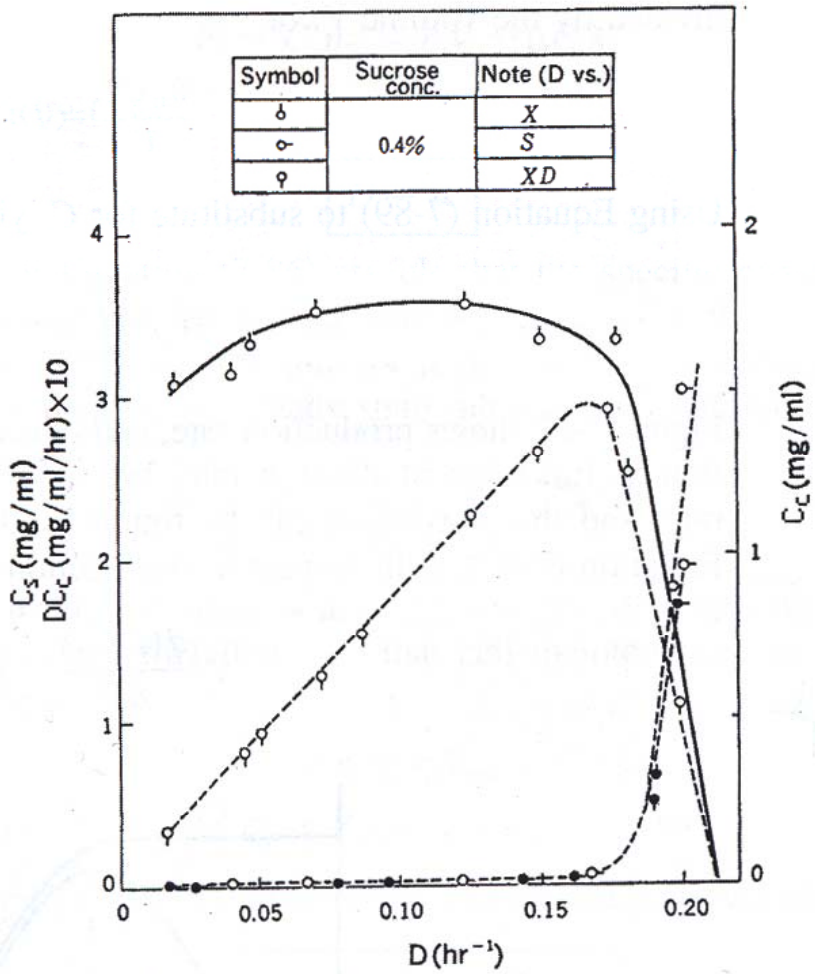


Figure 7-23 Continuous culture of *Streptomyces aureofaciens* in chemostats. (Note: $X \equiv C_c$) Courtesy of S. Aiba, A. E. Humphrey, and N. F. Millis, *Biochemical Engineering*, 2nd Ed. (New York: Academic Press, 1973).

7.4.8 Oxygen-Limited Growth

★ Oxygen is necessary for all aerobic growth (by definition). Maintaining the appropriate concentration of dissolved oxygen in the bioreactor is important for efficient operation of a bioreactor.

★ For oxygen-limited systems, it is necessary to design a bioreactor to maximize the oxygen transfer between the injected air bubbles and the cells. Typically, a bioreactor contains a gas sparger, heat transfer surfaces, and an impeller.

★ The oxygen transfer rate (OTR) is related to the cell concentration by

$$OTR = Q_{O2}\, C_C$$

Q_{O2}: Microbial respiration rate (=specific oxygen uptake rate)

7.4.9 Scale-up

★ Scale-up for the growth of microorganisms is usually based on maintaining a constant dissolved oxygen concentration in the liquid (broth), independent of reactor size.

★ One key to a scale-up is to have the speed of the tip of the impeller equal to the velocity in both the laboratory pilot reactor and the full-scale plant reactor.

★ If the impeller speed is too rapid, it can lyse the bacteria; if the speed is too slow, the reactor contents will not be well mixed. Typical tip speeds range from 5 to 7 m/s.

Lyse=분리하다

Physiologically Based Pharmacokinetic (PBPK) Model

★ We now apply the material we have been discussing on enzyme kinetics to modeling reactions in living systems. PBPK models are used to predict the distribution and concentration-time trajectories of medications, toxins, poisons, alcohol, and drugs in the body.

★ The approach is to model the body components (e.g., liver, muscle) as compartments consisting of PFRs and CSTRs connected to one another with in-flow and out-flow to each organ compartment as shown in Figure 7-24.

Physiologically Based Pharmacokinetic (PBPK) Model

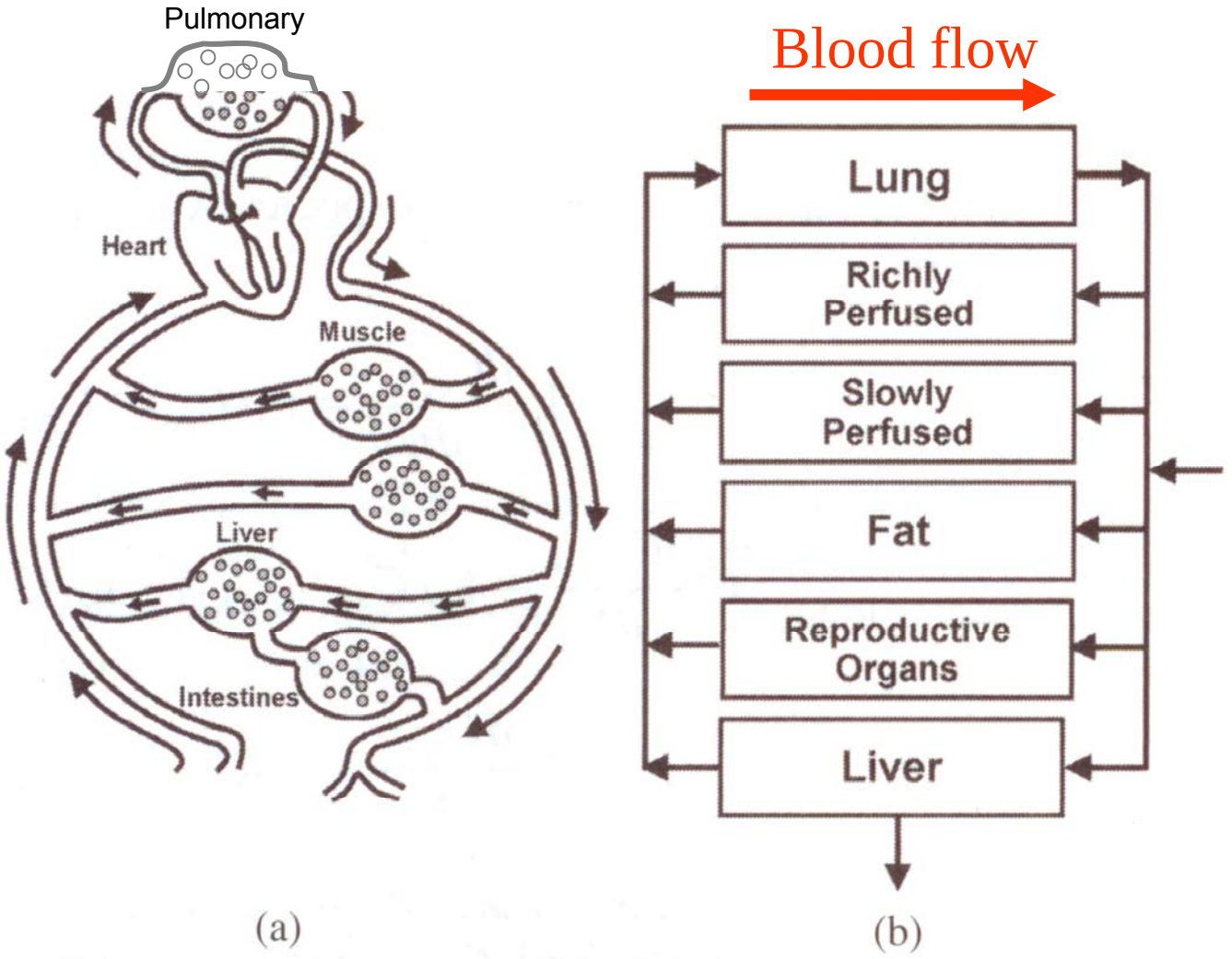


Figure 7-24 (a) Compartment model of human body. (b) Generic structure o PBPK models. Courtesy of *Chem. Engrg. Progress*, 100 (6) 38 (June 2004).

Physiologically Based Pharmacokinetic (PBPK) Model

★ Associated with each organ is a certain tissue water volume, **TWV**, which we will designate as the organ compartment.

★ The organ compartments will be modeled as an unsteady well-mixed CSTRs with the exception of the liver, which will be modeled as an unsteady PFR.

★ We will apply the chemical reaction engineering algorithm (mole balance, rate raw, stoichiometry) to the unsteady operation of each compartment.

★ Some compartments with similar fluid residence times are modeled to consist of several body parts (skin, lungs, etc) lumped into one compartment, such as the central compartment.

Physiologically Based Pharmacokinetic (PBPK) Model

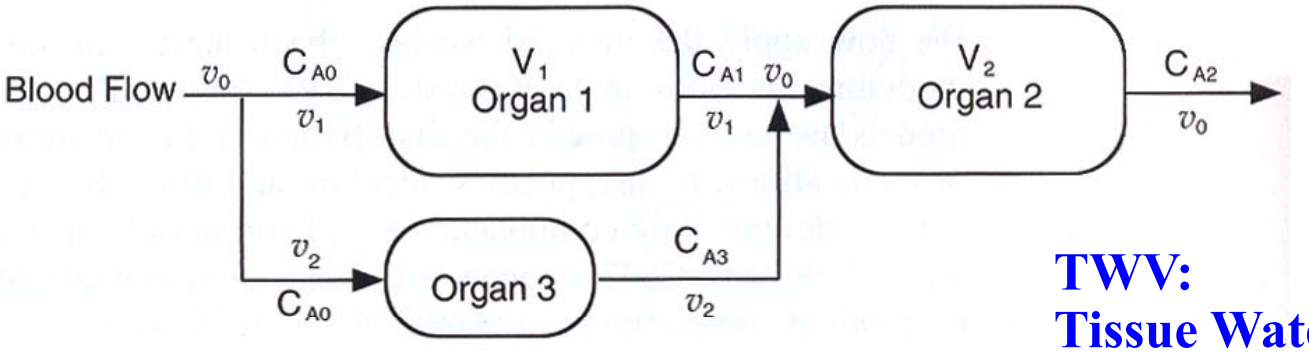
★ The interchange of material between compartments is primarily through blood flow to the various components.

★ The drug/medication concentrations are based on the tissue water volume (TWV) of a given compartment.

★ If we know the perfusion rate, we can determine the exchange of material between the bloodstream and that organ.

If organs are connected in series on one in parallel by blood flow as shown in Figure 7-25,

Physiologically Based Pharmacokinetic (PBPK) Model



TWV:
Tissue Water Volume

Figure 7-25 Physiologically based model.

then the balance equations on species A in the TWVs of the organs V_1, V_2, and V_3 are

$$V_1\frac{dC_{A1}}{dt} = v_1(C_{A0} - C_{A1}) + r_{A1}V_1 \tag{7-96}$$

$$V_2\frac{dC_{A2}}{dt} = v_1(C_{A1} - C_{A2}) + v_2(C_{A3} - C_{A2}) + r_{A2}V_2 \tag{7-97}$$

$$V_3\frac{dC_{A3}}{dt} = v_2(C_{A0} - C_{A3}) + r_{A3}V_3 \tag{7-98}$$

where r_{A1}, r_{A2}, and r_{A3} are the metabolism rates of species A in organs 1, 2, and 3, respectively, and C_{Aj} is the concentration of species A being metabolized in each of the organ compartments j = 1, 2, and 3.

Physiologically Based Pharmacokinetic (PBPK) Model

THE HUMAN REACTOR
(not to scale)

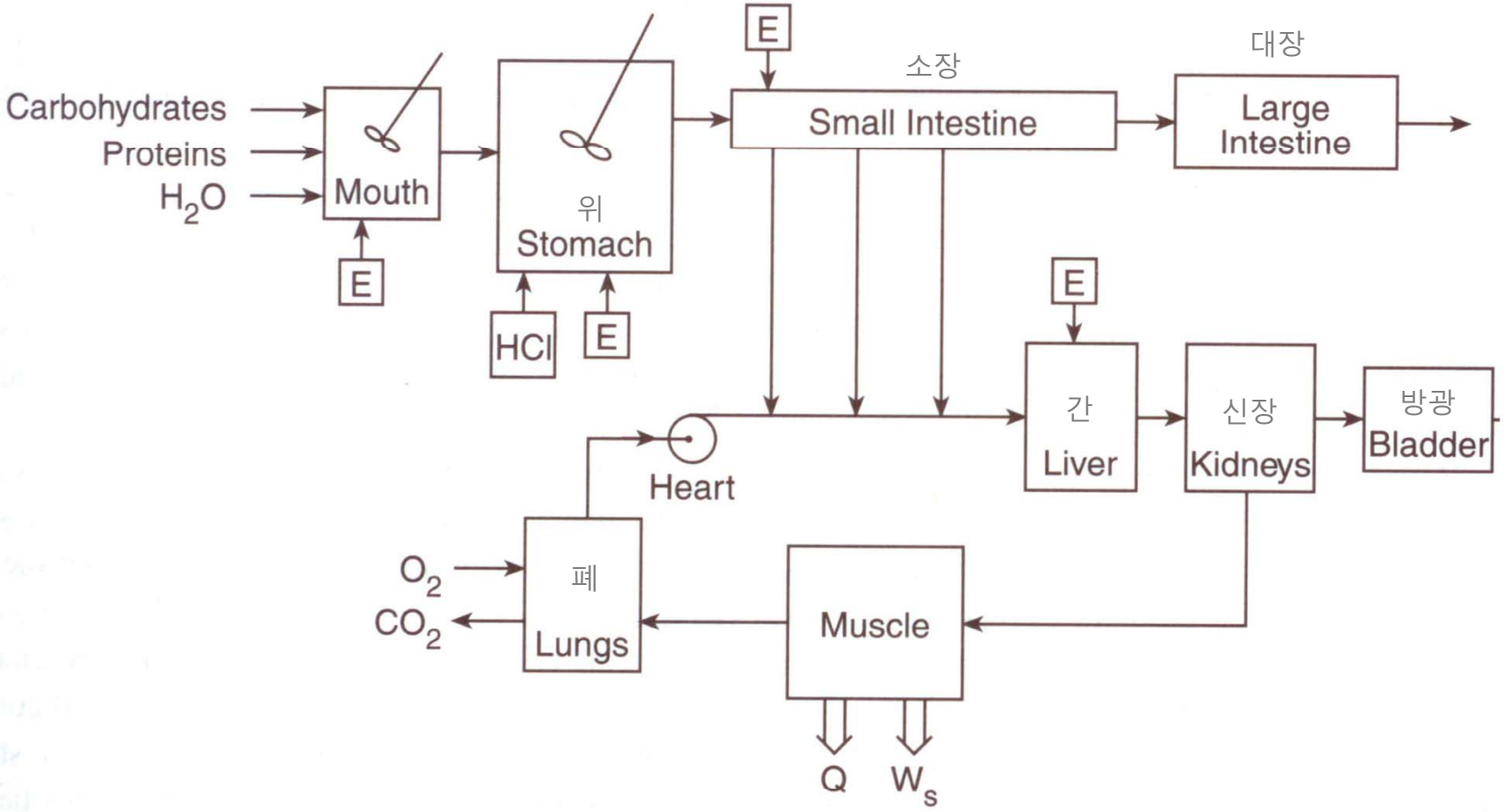


Figure 7–30 A simplified flow sheet of the digestive and circulatory systems of animals such as humans. HCl and enzyn (E) catalyze most reactions.

Alcohol Metabolism in the Body

Example 7–7 Alcohol Metabolism in the Body[22]

E7-7.A. General

We are going to model the metabolism of ethanol in the human body using fundamental reaction kinetics along with five compartments to represent the human body. Alcohol (Ac) and acetaldehyde (De) will flow between these compartments, but the alcohol and aldehyde will only be metabolized in the liver compartment. Alcohol and acetaldehyde are metabolized in the liver by the following series reactions.

$$C_2H_5OH \underset{ADH}{\rightleftharpoons} CH_3CHO \xrightarrow[ALDH]{} CH_3COOH$$

The first reaction is catalyzed by the enzyme alcohol dehydrogenease (ADH) and the second reaction is catalyzed by aldehyde dehydrogenease (ADLH).

Alcohol Metabolism in the Body

The reversible enzyme ADH reaction is catalyzed reaction in the presence of a cofactor, nicotinamide adenime dinucleotide (NAD^+)

$$C_2H_5OH + NAD^+ \underset{\text{ADH}}{\rightleftharpoons} CH_3CHO + H^+ + NADH$$

The rate law for the disappearance of ethanol follows Michaelis–Menton kinetics and is

$$-r_{AC} = \frac{[V_{\text{max ADH}} C_{Ac} - V_{\text{rev ADH}} C_{De}]}{K_M + C_{Ac} + K_{\text{rev ADH}} C_{De}} \qquad \text{(E7-7.1)}$$

where V_{max} and K_M are the Michaelis–Menten parameters discussed in Section 7.2, and C_{Ac} and C_{De} are the concentrations of ethanol and acetadehyde, respectively. For

Alcohol Metabolism in the Body

For the metabolism of acetaldehyde in the presence of acetaldehyde dehydrogenase, and NAD^+

$$NAD^+ + CH_3CHO + H_2O \xrightarrow[ALDH]{} CH_3COOH + NADH + H^+$$

the enzymatic rate law is

$$-r_{De} = \frac{V_{\max\,ALDH} C_{De}}{K_{MALDH} + C_{De}} \qquad \text{(E7-7.2)}$$

The parameter values for the rate laws are $V_{maxADH} = 2.2$ mM/(min • kg liver), $K_{MADH} = 0.4$ mM, $V_{revADH} = 32.6$ mmol/(min • kg liver), $K_{revADH} = 1$ mM, $V_{maxALDH} = 2.7$ mmol/(min • kg liver), and $K_{MALDH} = 1.2$ μM (see Summary Notes).

The concentration time trajectories for alcohol concentration in the central compartment are shown in Figure E7-7.1.

Alcohol Metabolism in the Body

E7-7.B The Model System

We are going to use as an example a five-organ compartment model for the metabolism of ethanol in humans. We will apply the CRE algorithm to the tissue water volume in each organ. The TWVs are lumped according to their perfusion rates and residence times. That is, those compartments receiving only small amounts of blood flow will be lumped together (e.g., fat and muscle) as will those receiving large blood flows (e.g., lungs, kidneys, etc.). The following organs will be modeled as single unsteady CSTRs: stomach, gastrointestinal tract, central system, and muscle and fat. The metabolism of ethanol occurs primarily in the liver, which is modeled as a PFR. A number of unsteady CSTRs in series approximate the PFR. Figure E7-7.1 gives a diagram showing the connection blood flow (perfusion), and mean residence, τ.

The residence times for each organ were obtained from the individual perfusion rates and are shown in the margin note next to Figure E7.7-1.

We will now discuss the balance equation on the tissue water volume of each of the organs/compartments.

Alcohol Metabolism in the Body

★ 69.4kg male human has 40.8ℓ total body water content

Organ Compartments
S=Stomach
G=G.I. Tract
C=Central
M=Muscle
L=Liver

TWV

Muscle & Fat=25.76 L
Central=11.56 L
Liver=1.11 L
G.I.=2.4 L

Residence time

Muscle & Fat=27 min
Central=0.9 min
Liver=2.4 min

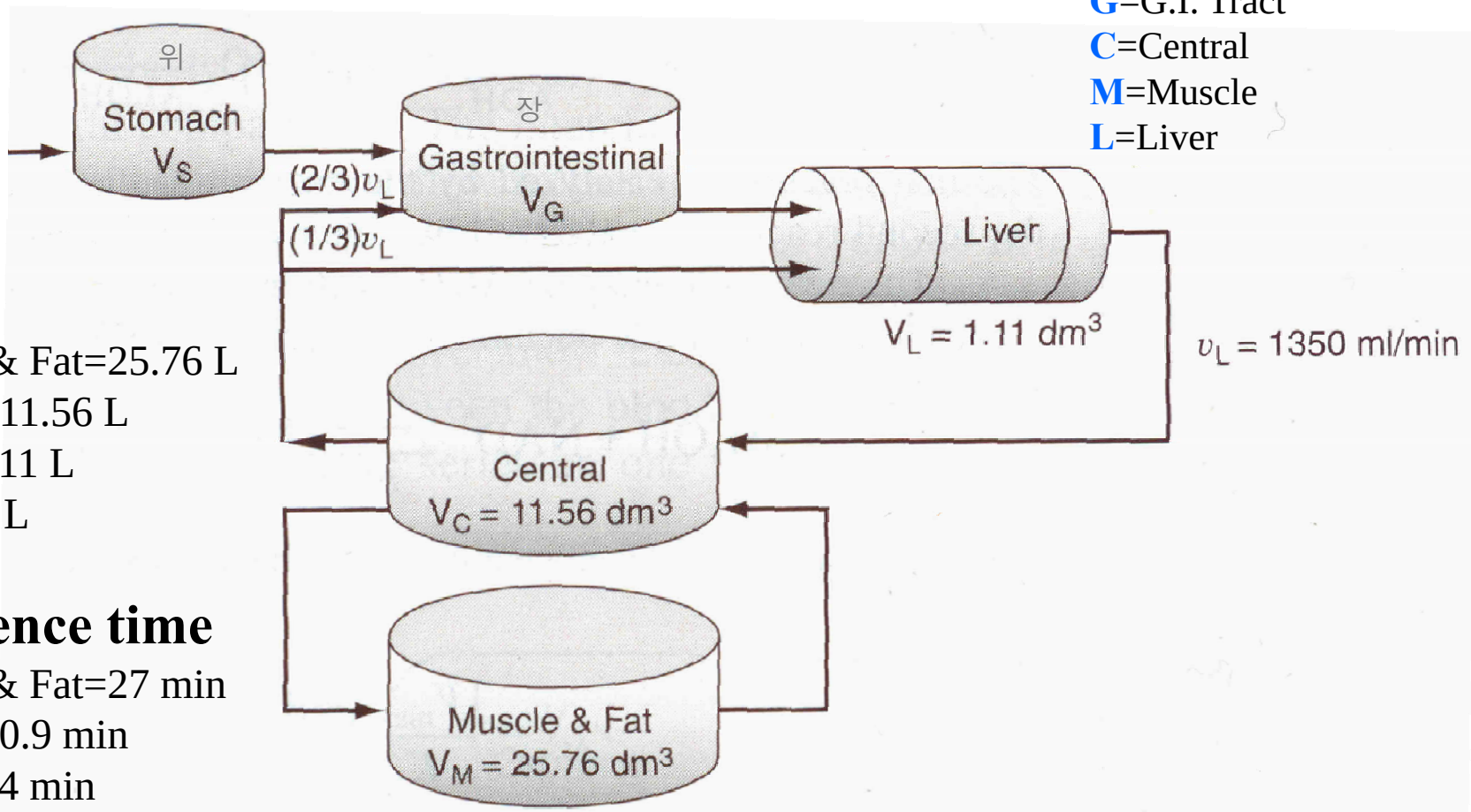


Figure E7-7.1 Compartment model of human body.

Alcohol Metabolism in the Body

Stomach

As a first approximation, we shall neglect the 10% of the total alcohol ingested that is absorbed in the stomach because the majority of the alcohol (90%) is absorbed at the entrance to the gastrointestinal (G.I.) tract. The contents of the stomach are emptied into the G.I. tract at a rate proportional to the volume of the contents in the stomach.

$$\frac{dV_S}{dt} = -k_S V_S \qquad \text{(E7-7.3)}$$

where V_S, is the volume of the contents of the stomach and k_S is the rate constant

$$k_S = \frac{k_{S\,max}}{1 + a(D)^2}$$

The flow of ethanol from the stomach into the G.I. tract, where it is absorbed virtually instantaneously, is

$$F_{Ac} = k_S V_S C_{S_{Ac}} \qquad \text{(E7-7.4)}$$

where $C_{S_{Ac}}$ is the ethanol concentration in the stomach, k_{Smax} is the maximum emptying rate, D is the dose of ethanol in the stomach in (mmol), and a is the emptying parameter in $(\text{mmol})^{-2}$.

Alcohol Metabolism in the Body

Duodenum=십이지장

Gastrointestinal (G.I.) Tract Component

Ethanol is absorbed virtually instantaneously in the duodenum at the entrance of the G.I. tract. In addition, the blood flow to the G.I. compartment from the central compartment to the G.I. tract is two-thirds of the total blood flow with the other third by-passing the G.I. tract to the liver, as shown in Figure E7-7.1. A mole mass balance on ethanol in the G.I. tract tissue water volume (TWV) V_G, gives

$$\begin{bmatrix}\text{In}\\ \text{from}\\ \text{Stomach}\end{bmatrix} + \begin{bmatrix}\text{In}\\ \text{from}\\ \text{Central}\end{bmatrix} - \begin{bmatrix}\text{Out}\\ \text{to}\\ \text{G.I.}\end{bmatrix} + \begin{bmatrix}\text{Generation}\\ \text{in}\\ \text{G.I.}\end{bmatrix} = \begin{bmatrix}\text{Accumulation}\\ \text{in}\\ \text{G.I.}\end{bmatrix}$$

$$k_s V_s C_{s_{Ac}} + \frac{2}{3} v_L C_{C_{Ac}} - \frac{2}{3} v_L C_{G_{Ac}} + 0 = \frac{d(V_G C_{G_{Ac}})}{dt} \qquad \text{(E7-7.5)}$$

where $C_{G_{Ac}}$ is the concentration of alcohol in the G.I. compartment. Because the TWV remains constant, the mass balance becomes

Ethanol:
$$V_G \frac{dC_{G_{Ac}}}{dt} = \frac{2}{3} v_L (C_{C_{Ac}} - C_{G_{Ac}}) + k_S V_S C_{S_{Ac}} \qquad \text{(E7-7.6)}$$

A similar balance on acetaldehyde gives

Acetaldehyde:
$$V_G \frac{dC_{G_{De}}}{dt} = \frac{2}{3} v_L (C_{C_{De}} - C_{G_{De}}) \qquad \text{(E7-7.7)}$$

Alcohol Metabolism in the Body

Central Compartment

second

The central volume has the largest TWV. Material enters the central compartment from the liver and the muscle/fat compartments. A balance on ethanol in this compartment is

$$[\text{Accumulation}] = \begin{bmatrix}\text{In}\\ \text{from}\\ \text{Liver}\end{bmatrix} + \begin{bmatrix}\text{In}\\ \text{from}\\ \text{Muscle}\end{bmatrix} - \begin{bmatrix}\text{Out to}\\ \text{Liver and}\\ \text{G.I.}\end{bmatrix} - \begin{bmatrix}\text{Out}\\ \text{to}\\ \text{Muscle}\end{bmatrix} + [\text{Generation}]$$

Ethanol: $$V_C \frac{dC_{C_{Ac}}}{dt} = v_L C_{L_{Ac}} + v_M C_{M_{Ac}} - v_L C_{C_{Ac}} - v_M C_{C_{Ac}} + 0 \quad \text{(E7-7.8)}$$

Similarly the acetaldehyde balance is

Acetaldehyde: $$V_C \frac{dC_{C_{De}}}{dt} = v_L(C_{L_{De}} - C_{C_{De}}) + v_M(C_{M_{De}} - C_{C_{De}}) \quad \text{(E7-7.9)}$$

Death by alcohol poisoning can occur when the central compartment concentration reaches 2 g/L.

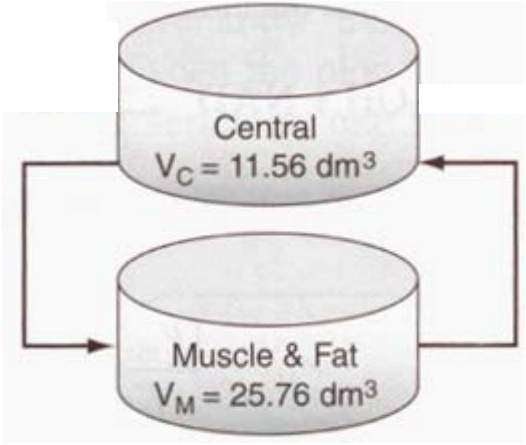
Alcohol Metabolism in the Body

Muscle/Fat Compartment

Very little material profuses in and out of the muscle and fat compartments compared to the other compartments. The muscle compartment mass balances on ethanol and acetaldehyde are

Ethanol:
$$V_M \frac{dC_{M_{Ac}}}{dt} = v_M (C_{C_{Ac}} - C_{M_{Ac}}) \qquad \text{(E7-7.10)}$$

Acetaldehyde:
$$V_M \frac{dC_{M_{De}}}{dt} = v_M (C_{C_{De}} - C_{M_{De}}) \qquad \text{(E7-7.11)}$$



Profuse=흐르다

Alcohol Metabolism in the Body

Liver Compartment

The liver will be modeled as a number of CSTRs in series to approximate a PFR with a volume of 1.1 dm^3. Approximating a PFR with a number of CSTRs in series was discussed in Chapter 2. The total volume of the liver is divided into four CSTRs.

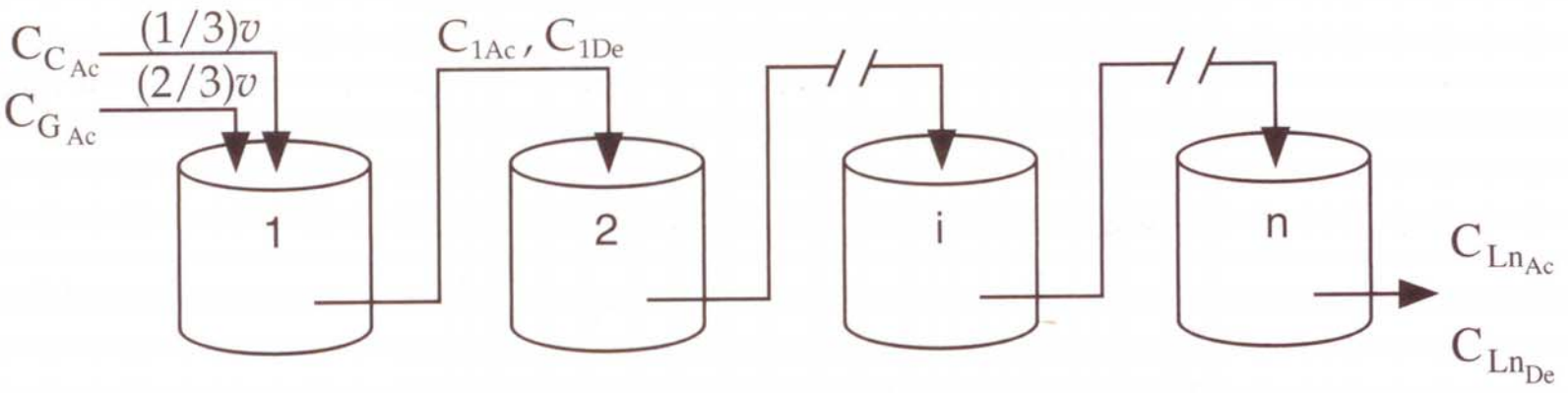


Figure E7-7.2 Liver modeled as a number of CSTRs in series.

Ethanol and acetaldehyde metabolism occurs within the liver which was considered as a tubular flow reactor

Alcohol Metabolism in the Body

Because the first CSTR receives in-flow from the central compartment (1/3 v) and from the G.I. compartment, it is treated separately. The balance on the first CSTR is

$$\text{Ethanol: } \Delta V_L \frac{dC_{L_{Ac}}}{dt} = v_L\left(\frac{1}{3}C_{C_{Ac}} + \frac{2}{3}C_{G_{Ac}} - C_{L_{Ac}}\right) + r_{L_{Ac}}(C_{L_{Ac}}, C_{L_{De}})\Delta V_L \quad \text{(E7-7.12)}$$

Acetaldehyde:

$$\Delta V_L \frac{dC_{L_{De}}}{dt} = v_L\left(\frac{1}{3}C_{C_{De}} + \frac{2}{3}C_{G_{De}} - C_{L_{De}}\right) - r_{L_{Ac}}(C_{L_{De}}, C_{L_{Ac}})\Delta V_L + r_{L_{De}}(C_{L_{De}})\Delta V_L \quad \text{(E7-7.13)}$$

where $C_{L_{Ac}}$ is the concentration of alcohol leaving the first CSTR. A balance on reactor i gives

$$\text{Ethanol: } \Delta V_L \frac{dC_{i_{Ac}}}{dt} = v_L[C_{(i-1)_{Ac}} - C_{i_{Ac}}] + r_{i_{Ac}}(C_{i_{Ac}}, C_{i_{De}})\Delta V_L \quad \text{(E7-7.14)}$$

Acetaldehyde:

$$\Delta V_L \frac{dC_{i_{De}}}{dt} = v_L[C_{(i-1)_{De}} - C_{i_{De}}] - r_{Ac_i}(C_{Ac_i}, C_{De_i})\Delta V_L + r_{i_{De}}(C_{i_{De}})\Delta V_L$$

Alcohol Metabolism in the Body

The concentrations exiting the last CSTR are $C_{Ln_{Ac}}$ and $C_{Ln_{De}}$. Equations (E7-7.1) through (E7-7.15) along with the parameter values are given on the CD-ROM summary notes and the Polymath living example problem. **The Polymath program can be loaded directly from the CD-ROM so that the reader can vary the model parameters.**† You can print or view the complete Polymath program and read the complete paper [*Alcohol* **35** (1), p.10, 2005] in the ***Summary Notes*** on the CD-ROM.

Polymath code

POLYMATH Results
Example 7-7 Alcohol Metabolism 08-18-2004, Rev5.1.232

Calculated values of the DEQ variables

Variable	initial value	minimal value	maximal value	final value
t	0	0	180	180
Vs	0.15	2.873E-05	0.1877742	2.873E-05
Cc	0	0	11.556606	4.2826187
Cca	0	0	0.0045311	0.0038191
Ct	0	0	10.504887	5.1906634
Cta	0	0	0.0044825	0.004054
CL1	0	0	20.98741	4.2199324
CL2	0	0	20.816457	4.0627945
CL3	0	0	20.645089	3.9062615
CL4	0	0	20.473294	3.7503741
CL5	0	0	20.301064	3.5951778
CL6	0	0	20.128388	3.4407227
CL7	0	0	19.955256	3.2870645
CL8	0	0	19.78166	3.1342649
CL9	0	0	19.60759	2.9823931
CL10	0	0	19.433034	2.8315261
CLa1	0	0	0.0051831	0.0039515
CLa2	0	0	0.0054428	0.0039121
CLa3	0	0	0.0054745	0.0038595
CLa4	0	0	0.0054744	0.0038035
CLa5	0	0	0.0054702	0.0037447
CLa6	0	0	0.0054654	0.0036832
CLa7	0	0	0.0054605	0.0036186
CLa8	0	0	0.0054555	0.0035507
CLa9	0	0	0.0054503	0.0034794
CLa10	0	0	0.0054451	0.0034044
Cg	0	0	27.5776	4.4251559
Cga	0	0	0.0045303	0.0038649
kd	0.013	0	0.013	0
vl	1.35	1.35	1.35	1.35
vt	1.5	1.5	1.5	1.5
Vc	11.56	11.56	11.56	11.56
Vt	25.8	25.8	25.8	25.8
Vg	2.4	2.4	2.4	2.4
Vl	1.1	1.1	1.1	1.1
dVl	0.11	0.11	0.11	0.11
VmAL	2.2	2.2	2.2	2.2
Vrev	29.1	29.1	29.1	29.1
KmAL	0.3898	0.3898	0.3898	0.3898
Krev	1	1	1	1
VmaxAc	2.74	2.74	2.74	2.74
KmAc	0.0015	0.0015	0.0015	0.0015
Cso	2170	2170	2170	2170
a1	1.5	1.5	1.5	1.5
a2	0.06	0.06	0.06	0.06
Vs1	0.15	0.15	0.15	0.15
Ds	325.5	325.5	325.5	325.5
ks	0.0517721	0.0517721	0.0517721	0.0517721

ODE Report (STIFF)

Differential equations as entered by the user
[1] d(Vs)/d(t) = -ks*Vs + kd
[2] d(Cc)/d(t) = (-vl*(Cc-CL10)-vt*(Cc-Ct))/Vc
[3] d(Cca)/d(t) = (-vl*(Cca-CLa10)-vt*(Cca-Cta))/Vc
[4] d(Ct)/d(t) = (vt*(Cc-Ct))/Vt
[5] d(Cta)/d(t) = (vt*(Cca-Cta))/Vt
[6] d(CL1)/d(t) = (vl*((1/3)*Cc+(2/3)*Cg-CL1)+(-VmAL*CL1+Vrev*CLa1)/(KmAL+CL1+Krev*CLa1)*dVl)/dVl
[7] d(CL2)/d(t) = (vl*(CL1-CL2)+(-VmAL*CL2+Vrev*CLa2)/(KmAL+CL2+Krev*CLa2)*dVl)/dVl
[8] d(CL3)/d(t) = (vl*(CL2-CL3)+(-VmAL*CL3+Vrev*CLa3)/(KmAL+CL3+Krev*CLa3)*dVl)/dVl
[9] d(CL4)/d(t) = (vl*(CL3-CL4)+(-VmAL*CL4+Vrev*CLa4)/(KmAL+CL4+Krev*CLa4)*dVl)/dVl
[10] d(CL5)/d(t) = (vl*(CL4-CL5)+(-VmAL*CL5+Vrev*CLa5)/(KmAL+CL5+Krev*CLa5)*dVl)/dVl

Alcohol Metabolism in the Body

Results

Figure E7-7.3 gives the predicted blood ethanol concentration trajectories and experimentally measured trajectories. The different curves are for different initial doses of ethanol. Note that the highest initial dose of ethanol reaches a maximum concentration of 16.5 mM of alcohol and that it takes between 5 and 6 hours to reach a level where it is safe to drive. A comparison of the model and experimental data of Jones et al. for the acetaldehyde concentration is shown in Figure E7-7.4. Because the acetaldehyde concentrations are three orders of magnitude smaller and more difficult to measure, there is a wide range of error bars. The model can predict both the alcohol and acetaldehyde concentration trajectories without adjusting any parameters.

In summary, physiologically based pharamacokinetic models can be used to predict concentration-time trajectories in the TWV of various organs in the body. These models find ever-increasing application of drug delivery to targeted organs and regions. A thorough discussion of the following data and other trends is given in the paper (Ulmulis, Gurmen, Singh, and Fogler).

Alcohol Metabolism in the Body

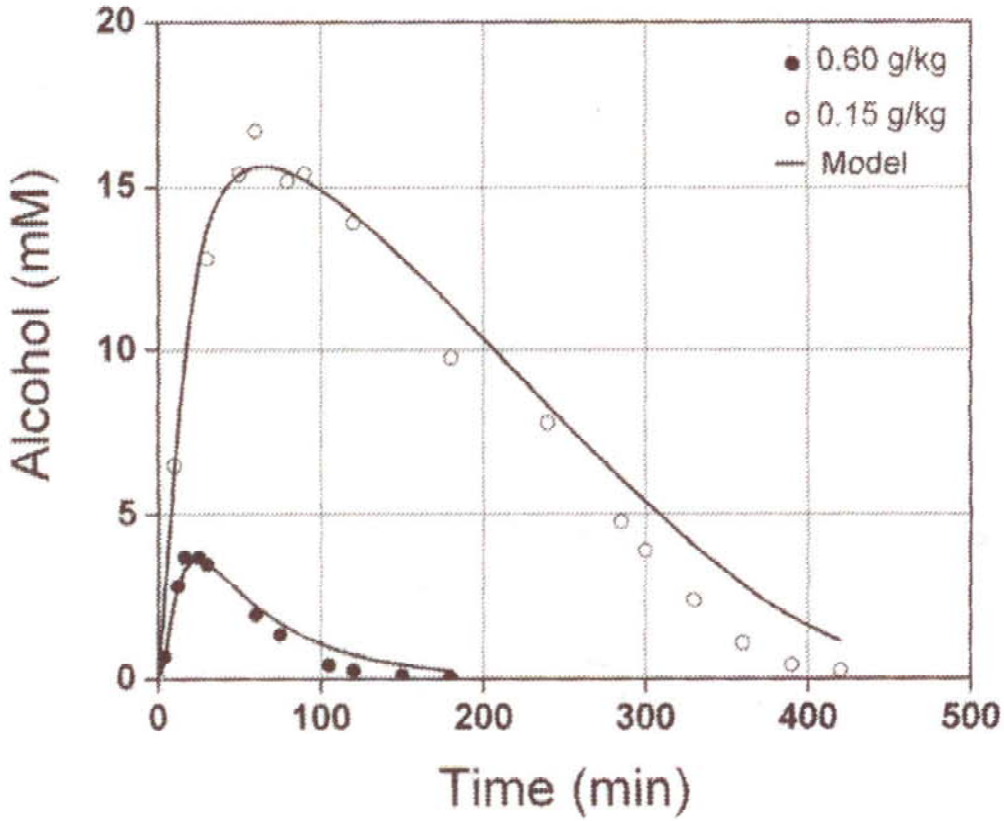


Figure E7-7.3. Blood alcohol–time trajectories from data of Wilkinson et al.[23]

Alcohol Metabolism in the Body

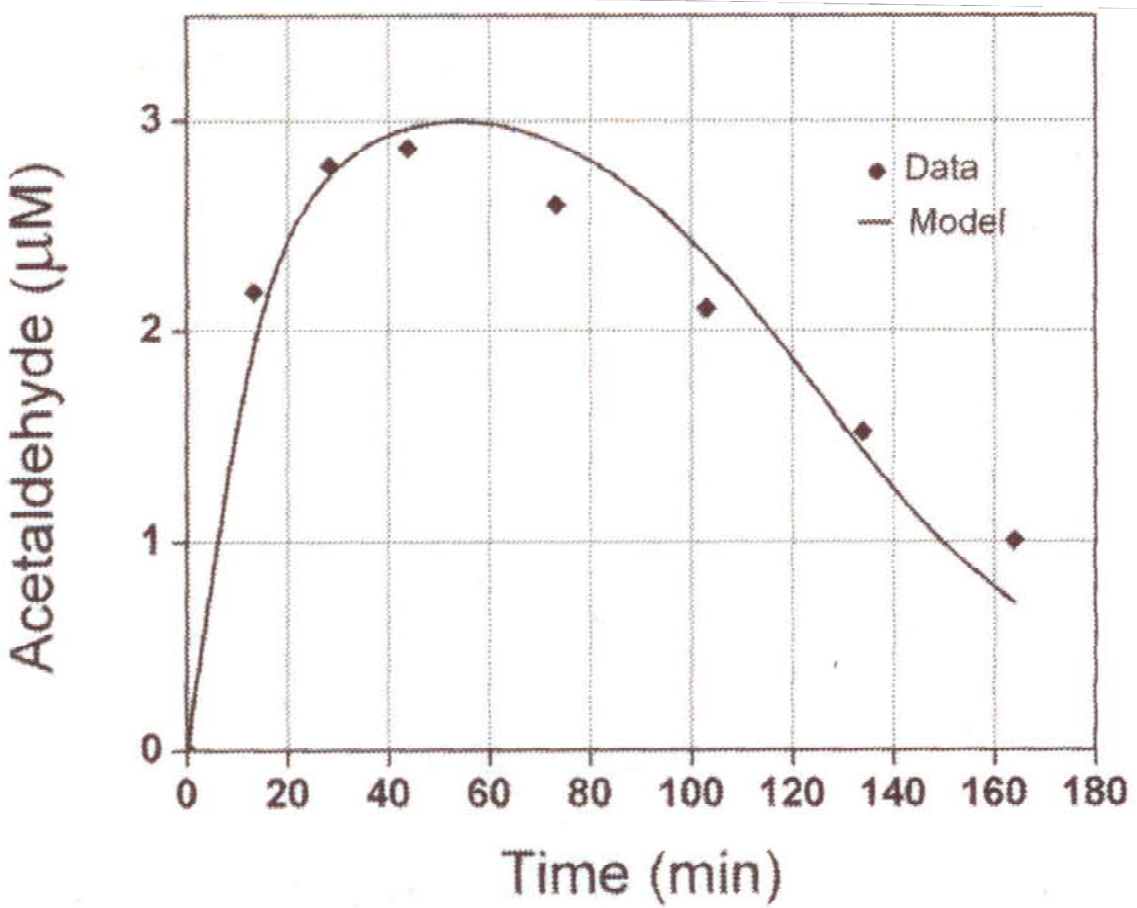


Figure E7-7.4. Blood alcohol–time trajectories from data of Jones et al.[24]