Ch. 7 Reaction Mechanisms, Pathways, Bioreactions and Bioreactors

Active Intermediates and Non-elementary Rate Laws:

Usually we have seen that rate can be expressed as:

\[-r_A = k_c A^\alpha\] where \(\alpha = 1, 2, 3, 0\)

However, a large # of rxns, the orders are non-integers

Rate laws may also involve a # of elementary rxns and at least one active intermediate.

Active intermediate is a high energy molecule that reacts virtually as fast as it is formed.

It is found in small amounts and shown as \(A^*\).

\(A + M \rightarrow A^* + M\)

The high E is stored in the chemical bonds where high-amplitude oscillations lead to bond ruptures, molecular rearrangement and decomposition

- Collision
- Free radicals, unpaired e-
- Ionic intermediates
- Enzyme-substrat complexes

Pseudo-steady-state Hypothesis (PSSH)

The PSSH assumes that the net rate of species \( A^* \) is zero.

\[ r_{A^*} \approx 0 \]

The reaction

\[ 2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2 \]

has an elementary rate law

\[ r_{\text{NO}_2} = kC_{\text{NO}}^2C_{\text{O}_2} \]

However

Why does the rate law decrease with increasing temperature?
Mechanism:

\[ \begin{align*} 
    & \text{NO} + \text{O}_2 \xrightarrow{k_1} \text{NO}_2^* \\
    & \text{NO}_2^* \xrightarrow{k_2} \text{NO} + \text{O}_2 \\
    & \text{NO}_2^* + \text{NO} \xrightarrow{k_3} 2\text{NO}_2 
\end{align*} \]

(1)  
(2)  
(3)

Assume that reactions (1) and (2) are elementary reactions, such that

\[ r_1 = k_1 [\text{NO}] [\text{O}_2] \]
\[ r_2 = k_2 [\text{NO}]^2 \]
\[ r_3 = k_3 [\text{NO}_2^*] [\text{NO}] \\
 r_3 = k_3 [\text{NO}_2^*] [\text{NO}] = k_4 [\text{NO}_3^*] [\text{NO}] \]

The net reaction rate for \( \text{NO}_2^* \) is the sum of the individual reaction rates for \( \text{NO}_2^* \):

\[ r_{\text{NO}_2^*} = r_{1, \text{NO}_2^*} + r_{2, \text{NO}_2^*} + r_{3, \text{NO}_2^*} \]
\[ r_{\text{NO}_2^*} = k_1 [\text{NO}] [\text{O}_2] - k_2 [\text{NO}]^2 + \frac{1}{2} k_3 [\text{NO}_3^*] [\text{NO}] \]

The PSSH assumes that the net rate of \( \text{NO}_3^* \) is zero:

\[ r_{\text{NO}_3^*} = 0 \]

0 = \( k_3 [\text{NO}] [\text{O}_2] - k_2 [\text{NO}]^2 - \frac{1}{2} k_3 [\text{NO}_3^*] [\text{NO}] \)

0 = \( k_3 [\text{NO}] [\text{O}_2] - [\text{NO}_3^*] \left( k_3 + \frac{k_3}{2} [\text{NO}] \right) \)

\[ [\text{NO}_3^*] = \frac{k_3 [\text{NO}] [\text{O}_2]}{k_2 + \frac{k_3}{2} [\text{NO}]} \]

if \( \frac{k_3}{2} [\text{NO}] << k_2 \)  \( \text{NO}_3^* \) = \( \frac{k_1}{k_2} [\text{NO}] [\text{O}_2] \)

if \( \frac{k_3}{2} [\text{NO}] >> k_2 \)  \( \text{NO}_3^* \) = \( \frac{2 k_1}{k_3} [\text{O}_2] \)

if \( k_2 >> \frac{k_3}{2} [\text{NO}] \) at low NO conc'n since \( r_{\text{NO}_2} = k_3 [\text{NO}_3] C_{\text{NO}} \)

\[ r_{\text{NO}_2} = \frac{k_1 k_3}{k_2} [\text{NO}]^2 [\text{O}_2] = \frac{A_1 A_3}{A_2} e^{-\frac{E_{\text{NO}_2} (E_1 + E_3)}{RT}} [\text{NO}]^2 [\text{O}_2] \]
if \( E_2 > (E_1 + E_3) \) as \( T \) increases \( r \) decreases.

Apparent rxn order at low conc'ns: 2nd order with NO
Apparent rxn order at high conc'ns: 1st order with NO

you can say

**Rxns consist of**

a) Activation step
b) Deactivation step
c) Decomposition step

If you have

\[
A + M \xrightarrow{k_1} A^* + M \quad \text{(for } A \rightarrow P) \\
A^* + M \xrightarrow{k_2} A + M \\
A^* \xrightarrow{k_3} P
\]

write the rate of formation of \( P \):  
\[
r_p = k_3 C_A^* \\
r_p = r_A = \frac{k_1 k_A C_A C_M}{k_1 C_M + k_1}
\]

if \( C_M \) is constant (inert)  
\[
r_p = k_c C_A \text{ 1st order rxn}
\]
Searching for a Mechanism

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

If you have

$$r_P = \frac{k_1 k_2 C_A^2}{k_A + k_3}$$

$$r_P = \frac{k_t C_A^2}{1 + k' C_A}$$

Steps in Mechanism Search

1. Species having conc'ns appearing in the denominator of the rate law probably collide with the active intermediate

   $$A + A^* \rightarrow \text{Products}$$

2. If a constant appears in the denominator, one of the rxn steps is probably the spontaneous decomposition of the active intermediate

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

3. Species having conc'ns in the numerator probably produce the active intermediate

   $$\text{Reactant} \rightarrow A^* + \text{Other Products}$$

Example:

Chain rxns will have steps:

1. Initiation: Formation of active intermediate
2. Propagation: Interaction of $A^*$ with reactant
3. Termination: deactivation of $A^*$

   Active Intermediate $C_2H_6 \rightarrow 2CH_3^-$

   Reaction pathways help us to see the inter-connections for multiple rxns.
   Metabolic pathways are examples.
**Enzymatic Rxn Fundamentals**

Enzymes (E) are proteins with catalytic properties. They act on substrates (S). They increase the rate by $10^3$ to $10^{17}$ times!

The rxn coordinate for enzyme catalysis:

$$S \underset{E}{\overset{S}{\rightleftharpoons}} E \cdot S \rightarrow P + E$$

Active intermediate (enzyme substrate complex)

Enzymes are highly specific (1 enzyme $\rightarrow$ 1 rxn), remain unchanged and can be used over and over. A typical enzyme will catalyze the rxn of about 1000 substrate molecules every second.

Although conc'n are low, how do they bind specifically, motions are enormously fast at molecular level. It takes a small molecule 0.2 sec to diffuse a distance of 10 $\mu$m. Active site will be bombarded by about 500,000 random collisions with the substrate molecule per second.

They are named by the type of rxn they catalyze (-ase).

If the enzyme is exposed to extreme T or pH $\rightarrow$ it will denature.
(unfolding will make the active site disappear)

**Two models for SE interactions.**

1. Lock & Key Model

$$E + S \rightarrow E \cdot S \rightarrow P + E$$

2. Induced fit model

$$E + S \rightarrow E \cdot S \rightarrow E + P$$
Mechanisms

As an example, we will look at urea decomposition by urease.

\[ S + E \xrightarrow{\text{reactions}} P + E \]

The corresponding mechanism:

1. \[ E + S \xrightarrow{k_1} E \cdot S \quad (1) \]
2. \[ E \cdot S \xrightarrow{k_2} E + S \quad (2) \]
3. \[ E \cdot S + W \xrightarrow{k_3} P + E \quad (3) \]

Corresponding rate laws are

\[ \begin{align*}
    r_{E} &= -k_1 (E) (S) \\
    r_{S} &= k_2 (E \cdot S) \\
    r_{P} &= k_3 (E \cdot S) (W)
\end{align*} \]

Net rate of disappearance of \( S \) is: \((-r_{S})\)

\[ r_{S} = k_1 (E) (S) - k_2 (E \cdot S) \quad (*) \quad \text{from (1)} \]

Net rate of formation of \( E \cdot S \) complex

\[ r_{ES} = k_1 (E) (S) - k_2 (E \cdot S) - k_3 (W) (E \cdot S) \quad \text{(combine 1 with 2 and 3)} \]

Using PSSH, \( r_{ES} = 0 \)

\[ (E \cdot S) = \frac{k_1 (E) (S)}{k_2 + k_3 (W)} \quad (***) \]

Substitute into (*)

\[ r_{S} = k_1 (E) (S) \cdot k_2 \frac{k_1 (E) (S)}{k_2 + k_3 (W)} \]

Rearranging:

\[ -r_{S} = k_1 k_2 (E) (S) (W) \]

\[ k_2 + k_3 (W) \]

But still there is \((E)\): unbound enzyme conc'n, but we can measure total enzyme conc'n:

\[ (E_{t}) = (E) + (E \cdot S) \]

Solving for \((E)\) using (***)

\[ E = \frac{(E_{t}) (k_2 + k_3 (W))}{k_2 + k_3 (W) + k_1 (S)} \]

Substituting back in - \( r_{S} = k_1 k_3 \ldots \)

\[ \frac{k_2 + \ldots}{k_2 + \ldots} \]

\[ -r_{S} = \frac{k_1 k_3 (E) (S) (W)}{k_1 (S) + k_2 + k_3 (W)} \]
**Michaelis – Menten Eq’n:**

For a given enzyme conc’n, a sketch of rate of disappearance of the substrate is shown as a function of substrate conc’n:

![Michaelis – Menten Plot](image)

where $V_{\text{max}}$ is the maximum rate of rxn for a given total enzyme conc’n.

$K_m$ is called the Michaelis – Menten constant, it is the measure of attraction of the enzyme for its substrate (~affinity constant)

The $K_m$ is equal to substrate conc’n at which the rate of rxn is equal to one-half of its max rate.

\[ t_r = \frac{V_{\text{max}}}{K_m + [S]} \]

At low substrate conc’n, $K_m \gg [S]$

\[ t_r = \frac{V_{\text{max}}}{K_m} \]

the rxn is 1st order

At high conc’n, $K_m \ll [S]$

\[ t_r \approx V_{\text{max}} \]

the rxn is zero order

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**Product – Enzyme Complex:**

In many rxns, the Enzyme and Product Complex (E-P) is formed directly from the (E-S) complex as:

\[ E + S \leftrightarrow E \cdot S \leftrightarrow E \cdot P \leftrightarrow E + P \]

Applying PSSH to (E \cdot S) and (E \cdot P), we obtain:

\[ -t_r = \frac{V_{\text{max}} (C_i - C_p / K_p)}{C_i + K_{\text{max}} + K_p C_p} \]

[Briggs - Haldane Rate Law]

**Batch Reactor Calculations for Enzyme Rxns:**

Let’s start writing the mole balance on urea in a batch reactor.

\[ \text{Urea} + \text{Urease} \rightarrow 2\text{NH}_3 + \text{CO}_2 + \text{Urease} \]

\[ \frac{-dN_{\text{max}}}{dt} = -t_r_{\text{max}} \cdot V \quad (1) \]

In liq. phase \{C V = N\} and V is constant

\[ \frac{-dC_{\text{max}}}{dt} = -t_{\text{max}} \quad (2) \]
Rate Law:
\[-r_{\text{urea}} = \frac{V_{\text{max}} C_{\text{urea}}}{K_m + C_{\text{urea}}} \]  \hspace{1cm} (3)

Substitute (3) into (2):
\[ t = \int \frac{dC_{\text{urea}}}{C_{\text{urea}} - r_{\text{urea}}} = \int \frac{C_{\text{urea}}}{V_{\text{max}} C_{\text{urea}}} dC_{\text{urea}} \]

Integrate
\[ t = \frac{K_m}{V_{\text{max}}} \ln \left( \frac{C_{\text{urea},0} + C_{\text{urea},0} - C_{\text{urea}}}{V_{\text{max}}} \right) \]

Rewrite Eqn (3) in terms of X:
\[ C_{\text{urea}} = C_{\text{urea},0} (1 - X) \]
\[ t = \frac{K_m}{V_{\text{max}}} \ln \left( \frac{1}{1 - X} + \frac{C_{\text{urea},0} - X}{V_{\text{max}}} \right) \]  \hspace{1cm} (6)

Determine $K_m$ and $V_{\text{max}}$ from batch reactor data using integral method. Divide both sides of (6) by $(t K_m / V_{\text{max}})$
\[ \frac{1}{t} \ln \frac{1}{1 - X} = \frac{V_{\text{max}} - C_{\text{urea},0} X}{K_m} \]  \hspace{1cm} (7)

where $a = \frac{1}{t} \ln \frac{1}{1 - X}$

Draw \( a \) vs \( (X / t) \): slope = \(- C_{\text{urea},0} / K_m \)
intercept = \( V_{\text{max}} / K_m \)

From a different approach, rewrite M-M Eq'n in terms of \( S \):

Multiply by \( S_0 \) (LHS of (7))
\[ S_0 (1 - X) = S \text{ and } C_{\text{urea},0} = S_0 \]
\[ S_0 X = S_0 - S \]
\[ \frac{1}{t} \ln \frac{S_0}{S} = \frac{V_{\text{max}} - S_0 - S}{K_m} \]  \hspace{1cm} \[ \frac{1}{t} \ln \frac{S_0}{S} = \frac{V_{\text{max}} - S_0 - S}{K_m t} \]

\( S_0 \): initial conc'n of substrate

\[ \frac{1}{t} \ln \frac{S_0}{S} \text{ intercept } = V_{\text{max}} / K_m \]
slope = \(- 1 / K_m \)

\[ S_0 - S / t \]
Effect of temperature on Enzymatic Rxns
A very complex issue!
If the enzyme did not unfold, the rate would probably follow a Arrhenius temperature dependence.
However, at a certain temperature the enzyme will denature and its activity will be lost!
Therefore, we will first have an increase and then decrease:

\[ V_{\text{max}} \rightarrow \text{temp. Inactivation (thermal denaturation)} \]

Inhibition of Enzyme Reactions:
Temperature, pH and the presence of an inhibitor influences the rates. Even the inhibition of a single (critical) enzyme may be fatal:
E.g.: Cyanide will cause the aerobic oxidation to stop when bound to cytochrome oxidase \( \rightarrow \) death (the whole pathway will be affected)
Aspirin inhibits the enzyme used in synthesis of pain-producing reactants

Types of Enzyme Inhibition

- **Competitive**
  \[ E + I \rightleftharpoons I \cdot E \text{ (Inactive)} \]

- **Uncompetitive**
  \[ E \cdot S + I \rightleftharpoons I \cdot E \cdot S \text{ (Inactive)} \]

- **Non-competitive**
  \[ E \cdot S + I \rightleftharpoons I \cdot E \cdot S \text{ (Inactive)} \]
  \[ I \cdot E + S \rightleftharpoons I \cdot E \cdot S \text{ (Inactive)} \]

Let's look at them individually
Competitive Inhibition

Very important in drug design:

I competes with the substrate for the enzyme

\[ E + S \rightarrow E \cdot S \rightarrow E \cdot P \]
\[ E + I \rightarrow E \cdot I \]

[1] \[ E + S \overset{k_1}{\longrightarrow} E \cdot S \]
[2] \[ E \cdot S \overset{k_2}{\longrightarrow} E + S \]
[3] \[ E \cdot S \overset{k_3}{\longrightarrow} E + P \]
[4] \[ I + E \overset{k_4}{\longrightarrow} E \cdot I \text{ (inactive)} \]
[5] \[ E \cdot I \overset{k_5}{\longrightarrow} E + I \]

\[ r_p = k_1 (E \cdot S) \quad \text{(1)} \]

Applying PSSH

\[ r_{E,S} = 0 = k_1 (E) (S) - k_2 (E \cdot S) + k_3 (E \cdot S) \quad \text{(2)} \]

Similarly:

\[ r_{E,I} = 0 = k_4 (E) (I) - k_5 (E \cdot I) \]

The enzyme conc'n:

\[ E_\text{t}=E + (E \cdot S) + (E \cdot I) \]

Combining (1), (2), (3) and (4):

\[ r_p = -r_s = \frac{V_{\text{max}} S}{S + K_m \left(1 + \frac{1}{K_i}\right)} \]

\[ V_{\text{max}} = k_3 E_i \quad \text{and} \quad K_m = \frac{k_2 + k_3}{k_1} \quad \text{and} \quad K_i = \frac{k_5}{k_4} \]

Say \( K_m' = K_m \left(1 + \frac{1}{K_i}\right) \Rightarrow K_m' > K_m \) (apparent M - M constants)

\( \Rightarrow \) means a larger S is needed for the decomposition of S.

To reach it \( \frac{1}{2} V_{\text{max}} \)
Rearranging:

\[
\frac{1}{-r_s} = \frac{1}{V_{\text{max}}} \left( \frac{1}{S} \frac{K_m}{V_{\text{max}}} \left( 1 + \frac{1}{K_i} \right) \right) \quad \text{(Lineweaver Burk)}
\]

\[\Rightarrow \text{As } I \text{ increases the slope increases and } -r_s \text{ decreases.}\]

Uncompetitive Inhibition

I has no affinity for the enzyme itself but for (E S) complex.

\[
\begin{align*}
E + S & \leftrightarrow E \cdot S \rightarrow E + P \\
+ & \\
I & \\
\downarrow k_2 & \\
E \cdot S \cdot I &
\end{align*}
\]

\[
\begin{align*}
E + S & \xrightarrow{k_1} E \cdot S \\
E \cdot S & \xrightarrow{k_2} E + S \\
E \cdot S & \xrightarrow{k_3} P + E \\
I + E \cdot S & \xrightarrow{k_4} I \cdot E \cdot S \text{ (inactive)} \\
I \cdot E \cdot S & \xrightarrow{k_5} I + E \cdot S
\end{align*}
\]
we have:

\[
\frac{1}{1 - r_s} = \frac{1}{S \cdot V_{max}} \left( \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \left( 1 + \frac{1}{K_i} \right) \right)
\]

where \( K_i = \frac{k_4}{k_4} \)

Slope remains constant, I increases, Intercept increases, \(-r_s\) decreases.

**Non – Competitive Inhibition (Mixed Inhibition)**

I and S bind to different sites on E!

\[
\begin{align*}
E + S & \leftrightarrow E \cdot S \rightarrow E + P \\
+ & + \\
I & I \\
\beta K_1 & \beta K_1' \\
E \cdot I + S & \leftrightarrow E \cdot S \cdot I
\end{align*}
\]

\[
\begin{align*}
E + S & \leftrightarrow E \cdot S \\
E + I & \leftrightarrow I \cdot S \text{ (inactive)} \\
I + E \cdot S & \leftrightarrow I \cdot E \cdot S \text{ (inactive)} \\
S + I \cdot E & \leftrightarrow I \cdot E \cdot S \text{ (inactive)} \\
E \cdot S & \rightarrow P
\end{align*}
\]
We have:

\[
\frac{1}{I_s} = \frac{1}{V_{\text{max}}} \left( \frac{1 + \frac{1}{K_t}}{S} + \frac{1}{S} \frac{K_m}{V_{\text{max}}} \left( 1 + \frac{1}{K_t} \right) \right)
\]

Both the slope and intercept increase as \( I \) increases.

**Summary** to compare three types of inhibition

Substrate Inhibition

In some cases, \( S \) can act as an inhibitor. If we have uncompetitive substrat inhibition:

\[
S + E \cdot S \rightarrow S \cdot E \cdot S
\]

\[
-r = \frac{V_{\text{max}}}{K_m + S + S^2/K_1}
\]  
(1)

(replace \( I \) with \( S \) in normal uncomp inh.)
at low conc’n \( K_m >> S + S^2 / K_1 \Rightarrow -r_s = \frac{V_{max} S}{K_m} \)

at high conc’n \( S^2 / K_1 >> K_m + S \Rightarrow -r_s = \frac{V_{max} K_1}{S} \)

\[ S_{max} = \sqrt{\frac{K_{m1} K_{m2}}{K_1 K_2}} \quad \text{(from} \quad \frac{dr_s}{dS} = 0) \]

**Multiple E and S Systems:**
We will look at multiple E and multiple S systems in which cyclic regeneration of the activated E occurs.

**Enzyme Regeneration:**
Glucose (\( S_R \)) is oxidized to \( \delta \)-gluconolactone (\( P \)) by glucose oxidase (\( E_0 \))

\[
S_R + E_0 \leftrightarrow S_R \cdot E_0 \leftrightarrow P \cdot E_R \leftrightarrow P + E_R
\]

\( E_R \) cannot be used in further catalysis rxns, to regenerate:

\[
E_R + O_2 \rightarrow E_0 + H_2O_2
\]

Overall

\[
S_R + O_2 \xrightleftharpoons{E} \rightarrow H_2O_2 + P
\]

**Enzyme Cofactors**
In enzymatic rxns, a second substrate is needed to activate the enzyme. Cofactor / coenzyme bind to the enzyme

**Unbound enzyme:** apo-enzyme  \quad **Bound enzyme:** holo-enzyme
Bioreactors

A bioreactor is a special reactor that sustains and supports life for cells and tissue cultures. The use of living cells to produce marketable chemical products made the biotechnology a growing industry. Chemicals, agricultural products, food, etc.

Some organic chemicals are thought to be produced by living cells instead of petroleum.

The advantages are mild rxn conditions, high yields and stereospecific compounds.

Bacteria can be modified to be used as chemical factories.

In biosynthesis, the cells are called as “biomass”.

\[
\text{Nutrients} \rightarrow \text{Cell} \rightarrow \text{Products}
\]

The chemical rxns are simultaneous in the cell

We have

i) Nutrient degradation (fueling) rxns, ATP molecules are used to provide energy.

ii) Synthesis of small molecules (like aa, nucleotides)

iii) Synthesis of large molecules (like polymerization off aa to form proteins)

Cell Growth & Division

The four phases of cell division G1, S, G2 and M

The cell division eq’n can be written as

\[
\text{Substrate} \xrightarrow{\text{cells}} \text{More cells} + \text{Product}
\]

Carbon, Nitrogen, Oxygen and Phosphate are some of the substrates.
Cell Growth

The number of living cells as a function of time can be shown in a batch reactor as:

**In Phase I**: lag phase, there is little increase in cell conc’n. Cells are adjusting to their new environment, synthesizing enzymes, getting ready to reproduce.

**Phase II**: exponential growth phase. Cell divide at maximum rate, all of the enzyme’s pathways are in place.

**Phase III**: Stationary phase: Cells reach a minimum biological place where the one or more of the nutrient are limiting.

**Phase IV**: Death phase: A decrease in live cell conc’n occurs.

Rate Laws

The most commonly used rate expression for

\[ \text{Cell + Substrate} \rightarrow \text{More cells + Product} \]

is the Monod eq’n for exp growth.

\[ r_g = \mu C_s \]

\( r_g \): Cell Growth Rate (g/dm³/s)

\( \mu \): Specific growth rate (s⁻¹)

\( C_s \): Cell conc’n (g/dm³)

\( \mu_{\text{max}} \): Max spec. growth rate

\( K_s \): Monod constant (g/dm³)

\( C_s \): Substrate (nutrient) conc’n (g/dm³)

\[ \mu = \frac{\mu_{\text{max}} C_s}{K_s + C_s} \]

\[ r_g = \mu_{\text{max}} \frac{C_s C_s}{K_s + C_s} \]

For most of the bacteria \( K_s \) is small (~10⁻⁵ mol/dm³) \( \rightarrow r_g = \mu_{\text{max}} C_s \)
Growth rate, \( r_g \), depends on the nutrient conc’n (Cs)

\[
\begin{align*}
\text{\( r_g \)} &= \text{constant} \times \left( \frac{\text{Cs}}{K_s + \text{Cs}} \right)^n
\end{align*}
\]

In many systems, product inhibits the growth rate. Wine production is an example, fermentation of glucose to produce ethanol is inhibited by the product ethanol (ethanol kills the yeast)

\[
\begin{align*}
r_g &= k_{\text{obs}} \frac{\mu_{\text{max}} \text{Cs}}{K_s + \text{Cs}} \quad \text{where} \quad k_{\text{obs}} = \left( 1 - \frac{\text{Cp}^*}{\text{Cp}} \right)^n
\end{align*}
\]

\( \text{Cp}^* \): product conc’n at which metabolism stops (g/dm³)

\( n \): empirical constant

There are also Tessier & Moser eq’ns that fit experimental data better.

The cell death rate is given by

\[
\begin{align*}
r_d &= (k_d + k_t \text{Cc}) \text{Cc}
\end{align*}
\]

Conc’n of the substance toxic to the cell

**Effect of Temperature** can be given as

\[
\begin{align*}
\mu(T) &= \mu(T_m) \ I' \\
I' &= \frac{a T e^{E_{\text{a}}/R T}}{1 + b e^{E_{\text{b}}/R T}} \\
\end{align*}
\]

where

\( I' \): fraction of max growth rate

\( T_m \): temp. at which max growth occurs.
**Stoichiometry** for cell growth is complex, and changes with microorganism/nutrient system, and pH, T, etc.

Let's look at the case where one nutrient is limiting:

\[
\text{Cell + Subs} \rightarrow \text{More cells + Products}
\]

\[
y_{CS} = \frac{\text{mass of new cells formed}}{\text{mass of substrate consumed}} = \frac{\Delta C_c}{\Delta C_S}; \quad y_{SC} = \frac{1}{y_{SC}}
\]

Product formation can take place during different phases of the cell growth cycle if it occurs during the exp growth phase:

\[
\hat{r} = \hat{y}_{PC} \quad \hat{r}_s = \hat{y}_{PC} \quad \mu = y_{PC} \frac{\mu_{\text{max}} C_s C_i}{K_i + C_i}
\]

where \( y_{PC} = \frac{\text{mass of product formed}}{\text{mass of new cells formed}} = \frac{-\Delta C_P}{\Delta C_c} \)

The stoichiometric yield coeff btw product & substrate is given by:

\[
y_{PS} = \frac{\text{mass of product formed}}{\text{mass of substrate consumed}}
\]

Another term is the maintenance utilization term: (to maintain a cell's daily activities)

\[
m = \frac{\text{mass of substrate consumed for maintenance}}{\text{mass of cells} \times \text{Time}}
\]

The rate of substrate consumption

\[
r_{sm} = m \quad C_c
\]

Neglecting the cell maintenance

\[
C_c = y_{PS} [C_{s0} - C_s]
\]
Substrate Utilization

\[
\begin{bmatrix}
\text{Net rate of Substrate Consumption} \\
\end{bmatrix}
= \begin{bmatrix}
\text{Rate consumed by cells} \\
\text{Rate consumed to form product} \\
\text{Rate consumed for maintenance}
\end{bmatrix}
\begin{bmatrix}
\text{Product form consumed rate}
\end{bmatrix}
\]

\[-r_s = y_{s;c} r_g + y_{s;p} r_p + m C_c
\]

If there is production during growth, it is difficult to separate the amount of substrate used in production and growth \( \rightarrow \)

\[-r_s = y_{s;c} + m C_c
\]

The corresponding rate law is

\[r_p = r_g y_{p;c} \quad \text{growth associated product formation in the growth phase.}
\]

Apparently, no net growth during stationary phase

\[r_p = \frac{k_p C_p C_s}{K_{ss} + C_{ss}} \quad \text{Non-growth-associated product formation in stationary phase}
\]

Concentration of a second nutrient which is used for maintenance and production (because the nutrient is used up for growth)
Mass Balances

There are two ways that we could account for the growth of microorganisms. Either # of living cells or mass of the living cells.

For a CTSR: (called chemostats are the reactors that contain microorganisms)

\[
V \frac{dC_c}{dt} = V_0 C_{so} - V C_c + (r_g - r_d) V
\]

Cell balance  Accumulation = In – Out + Generation

Substrate Balance

\[
V \frac{dC_S}{dt} = V_0 C_{SO} - V S C_S + r_S V
\]

(In most cases Cco = 0)

For a Batch System:

\[
V = V_0 = 0
\]

Cell balance

\[
V \frac{dC_c}{dt} = r_g V - r_d V \Rightarrow \frac{dC_c}{dt} = r_g - r_d
\]

Substrate

\[
V \frac{dC_S}{dt} = r_S V = y_{S/C} (-r_g) V - m C_c V \Rightarrow \frac{dC_S}{dt} = y_{S/C} (-r_g) - m C_c
\]

(In growth phase)

\[
V \frac{dC_S}{dt} = r_S V = m C_c V + y_{S/P} (-r_p) V
\]

for stationary phase (no growth)
Design Equations

For a CSTR

\[ V_0 = V \]
\[ C_{CO} = 0 \]
\[ D = \frac{V_0}{V} \] is called the dilution rate (a parameter used in bioreactors)

\{ D = 1/T \}]

CSTR Mass Balance:

\[ Acc = \ln - Out + Gen' n \]
\[ \frac{dC_c}{dt} = 0 - DC_c + (r_g - r_d) \] \{ For Cell \}
\[ \frac{dC_S}{dt} = DC_{SO} - DC_S + r_s \] \{ For Substrate \}

Using Monod Eq'n; the growth rate is determined as:

\[ r_g = \mu C_c = \frac{(\mu_{max} C_S C_c)}{(K_S + C_S)} \]

For st−st operations

\[ DC_c = r_g - r_d \]
\[ D(C_{SO} - C_S) = r_s \]

If we neglect death rate

\[ F = C_c V_0 = r_g V = \mu C_c V \Rightarrow D = \mu \]

divide by \( C_c V \)

You can control specific growth rate by \( D \)!
We know \[ \mu = \mu_{\text{max}} \frac{C_s}{K_s + C_s} \] (Monod Eq'n)

Combining \[ C_s = \frac{DK_s}{\mu_{\text{max}} - D} \]

If a single nutrient is limiting,
- cell growth is the only process to substrate consumption.
- cell maintenance is neglected.

Then;

\[ -r_s = r_g \frac{y_s}{c} \]

\[ C_c = y_{c/s}(C_{so} - C_s) \]

\[ C_c = y_{c/s} \left[ C_{so} \left( \frac{DK_s}{\mu_{\text{max}} - D} \right) \right] \]

**wash-out**

Assume \[ r_d = 0 \]

\[ \frac{dC_c}{dt} = (\mu - D)C_c \quad r_g = \mu C_c \]

if \( D > \mu \) \[ \frac{dC_c}{dt} < 0 \Rightarrow C_c = 0 \] (at some time)

the dilution rate at which wash-out will occur \((C_c = 0)\) is:

\[ D_{\text{max}} = \frac{M_{\text{max}} C_{so}}{K_s + C_{so}} \]
D for the max. cell production:

\[ m_c = C_c \nu_o \]

\[ \frac{\nu_o C_c}{V} = DC_c \]

\{Cell production per unit volume is the mass flow rate of cells out of the reactor\}

Subs

\[ DC_c = Dv_{c/s} (C_{s/o} - \frac{DK_s}{\mu_{max} - D}) \]

\[ \frac{d(DF_c)}{dD} = 0 \quad \text{For max D!} \]

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**Physiologically Based Pharmacokinetic Models:** (PBPK)

You can model runs in living systems. You can find conc’n-time profiles for medications, toxins, alcohol and drugs in the body!

Compartments of body are either a PFR or CSTR.

Perfusion rate: Blood flow in/out of organs.

unst-st models are used as CSTR

liver: PFR(unst-st)

The interchange of material btw compartments is primarily through blood flow.
For example, if organs are connected in series as shown

Then the balance eq’ns on species A in the tissue water volumes of the organs $V_1$, $V_2$, and $V_3$ are:

$$V_1 \frac{dC_{A1}}{dt} = \nu_1 (C_{A0} - C_{A1}) + r_{A1} V_1$$

$$V_2 \frac{dC_{A2}}{dt} = \nu_1 (C_{A1} - C_{A2}) + \nu_2 (C_{A3} - C_{A2}) + r_{A2} V_2$$

$$V_3 \frac{dC_{A3}}{dt} = \nu_2 (C_{A0} - C_{A3}) + r_{A3} V_3$$

$r_{Ai}$: metabolism rate of A in organs