10.37 Chemical and Biological Reaction Engineering, Spring 2007
Prof. K. Dane Wittrup
Lecture 3: Kinetics of Cell Growth and Enzymes

This lecture covers: cell growth kinetics, substrate uptake and product formation in microbial growth, enzyme kinetics, and the Michaelis-Menten rate form.

Biological Rate Laws- Enzymes and Cell Growth

Rate Law: $-r_A = f(C_A, C_B, C_P, T, pH, ...)$

Why would you need a rate law?
- -Predictive description of a production process
- -Design tool for forming a desired product
- -Consistency or inconsistency with alternative mechanism

For a hypothesized mechanism, we can often derive an exact, closed form analytical solution.
If not, it's used to approximate:
- -some reactions go rapidly to equilibrium
- -the concentrations of some species rapidly reach their steady state values
- -the rate-limiting step

Or, as a last resort- numerical solution

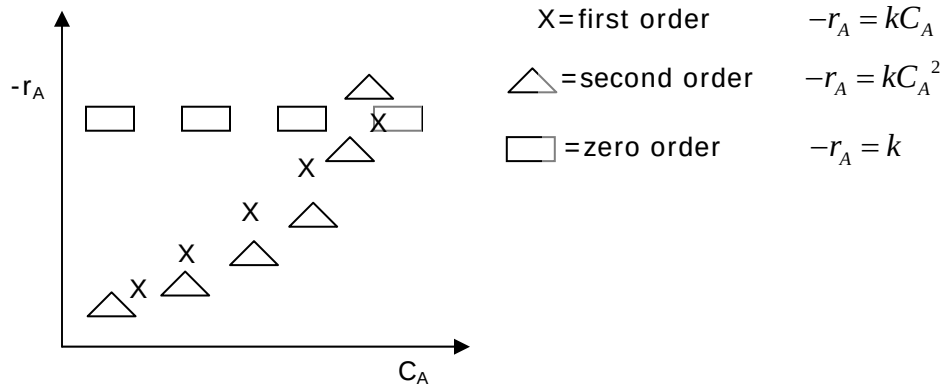


Figure 1. Rate versus concentration graphs for zero, first, and second order reactions.

Enzymes-Biological Catalysts

S=Substrate
E=Enzyme

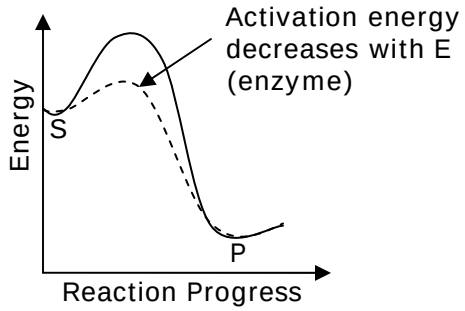


Figure 2. An energy diagram for a reaction with and without an enzyme. The activation energy is lower with an enzyme, so the reaction proceeds faster.

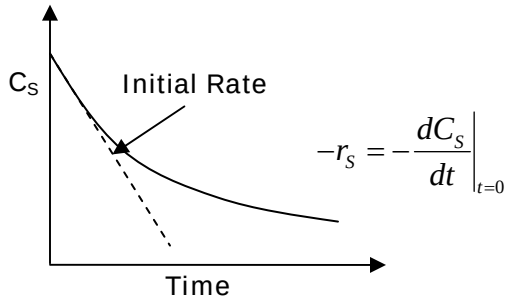


Figure 3. The slope of the concentration versus time curve at time = 0 can give the initial rate.

Michaelis Menten

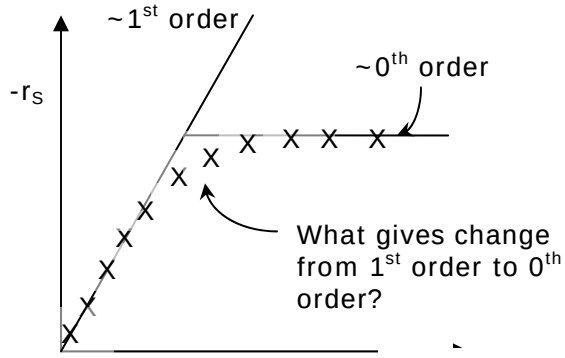


Figure 4. Michaelis-Menten kinetics.

Hypothesized Mechanism:
-Encounter complex ES formed
-Irreversible reaction occurs
-Rapid release of product from complex } In more complex cases, these are not always true
-Assume rapid equilibrium is reached in the formation of ES

Later, Briggs-Haldane derived a law with steady state assumption for ES and they got the same rate law as Michaelis-Menten. However, the Briggs-Haldane method is more generally applicable.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

$$ES \xrightarrow{k_{cat}} E + P$$

Steady state assumption on ES $\rightarrow \dfrac{dC_{ES}}{dt} = 0$

Material Balance on ES:

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - k_{-1} C_{ES} - k_{cat} C_{ES} \approx 0 \text{ (steady state)}$$

C_E: Free enzyme concentration $\neq C_{E,0}$

because $C_{E,0} \ll C_{S,0}$

$$C_{E,0} = C_E + C_{ES}$$

$$0 = k_1 (C_{E,0} - C_{ES}) C_S - C_{ES} (k_{-1} + k_{cat})$$

$C_s \approx C_{S,0}$

Solve for C_{ES} (steady state solution)

$$C_{ES} = \frac{C_{E,0} C_S}{\dfrac{k_{-1} + k_{cat}}{k_1} + C_S}$$

$$-r_S = r_p = \frac{dC_p}{dt} = k_{cat} C_{ES} = \frac{k_{cat} C_{E,0} C_S}{\dfrac{k_{-1} + k_{cat}}{k_1} + C_S} = \frac{V_{\max} C_S}{K_m + C_S}$$

$V_{\max} = k_{cat} C_{E,0} =$ maximum reaction velocity

$K_m = \dfrac{k_{-1} + k_{cat}}{k_1} =$ Michaelis constant

This model fits the data:

-As $C_S \ll K_m$, $-r_S \rightarrow \dfrac{V_{\max}}{K_m} C_S$

-As $C_S \gg K_m$, $-r_S \rightarrow V_{\max}$

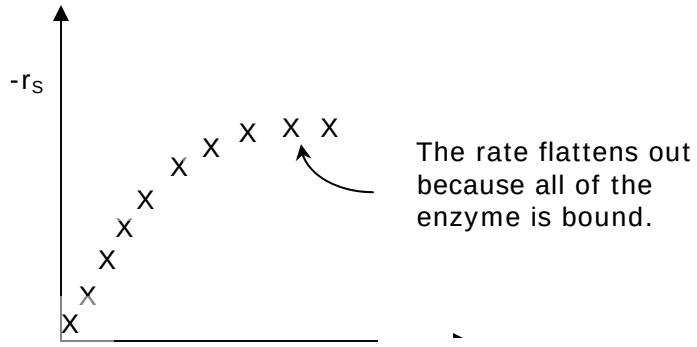


Figure 5. Michaelis-Menten kinetics.

-This rate law reappears in heterogeneous catalysis (Langmuir-Hinshelwood)
-In the actual Michaelis-Menten derivation K_{max} was an equilibrium constant
-Briggs and Haldane showed that this rate law still works for steady state
-Steady state approximation is more general
    -Steady state approximation is consistent with the data
    -Steady state is defined over a limited period of time. Because there is an irreversible step, eventually all of the intermediate will be consumed.
    -Equilibrium assumption is not exactly correct. The irreversible step prevents true equilibrium.

Growth Kinetics

A population of single cells, growing without limitations
N=#cells per volume

$\frac{dN}{dt} = \mu N$, $\mu =$ constant, specific growth rate

$N = N_0 e^{\mu t}$ – exponential growth while true, "Malthusian growth"

Growth Stops
    -Run out of nutrients
    -Accumulate toxic byproducts

Nutrient Effect on μ (Monod)

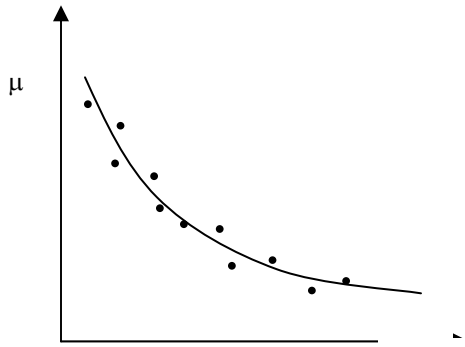
$$\mu = \frac{\mu_{\max} C_S}{K_S + C_S}$$

This expression is purely empirical (no mechanistic meaning). Even the fit is not that good.

$$\frac{dN}{dt} = \frac{\mu_{\max} C_S}{K_S + C_S} N$$

$$\frac{dC_S}{dt} = \frac{-1}{Y_{x/s}} \mu N$$

We will return to this when we look at bioreactors



I=inhibitor or toxin

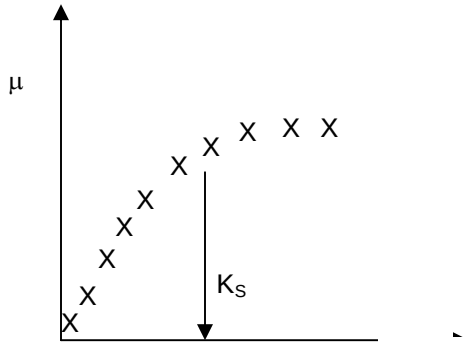
$$\mu \propto e^{-kC_I}$$

$$\mu \propto \frac{1}{(1 - kC_I)}$$

$$\mu \propto \frac{k_I}{k_I + C_I}$$

All forms fit the data

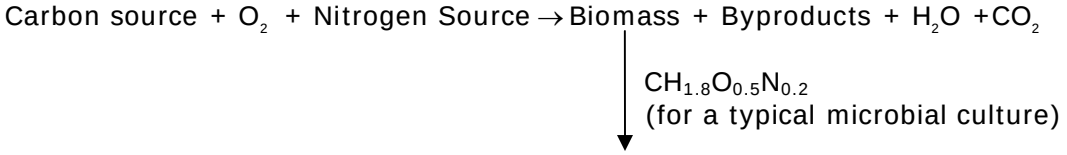
Figure 6. Growth rate versus concentration of inhibitor. Many functional forms fit the curve.



C_S=concentration of growth limiting substrate, often glucose

Cell Growth as a Chemical Reaction

Aerobic growth:



A yield coefficient can be defined:

$$Y_{A/B} = \frac{\Delta A}{\Delta B}$$

A=biomass, byproducts, CO_2, heat (any of these)
B=carbon source or oxygen

For glucose, $Y_{x/s} \approx 0.6 \pm 0.1 \frac{\text{g biomass}}{\text{g glucose}}$

For oxygen, $Y_{x/O_2} \approx 1.9 \pm 0.7 \frac{\text{g biomass}}{\text{g } O_2}$