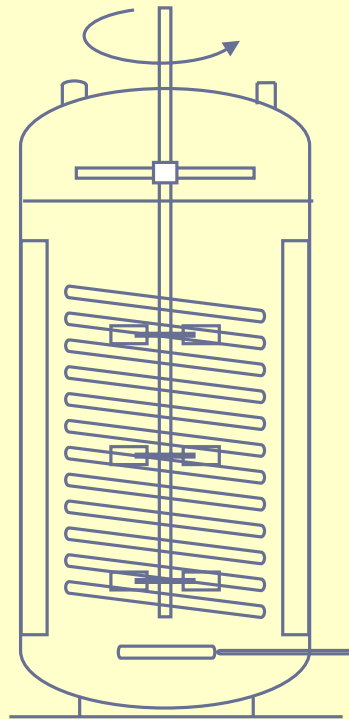


Biochemical Engineering

James M. Lee




eBook Version 2.1

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General Guide

Navigation

- You can navigate the whole book by using the navigation pane. To open the navigation pane click this button  of Acrobat Reader (Not working for this sample).
- Official web site for this ebook is <http://us.geocities.com/eBioChE>

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Chapter 2. Enzyme Kinetics

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Chapter 2.

Enzyme Kinetics

2.1. Introduction

Enzymes are biological catalysts that are protein molecules in nature. They are produced by living cells (animal, plant, and microorganism) and are absolutely essential as catalysts in biochemical reactions. Almost every reaction in a cell requires the presence of a specific enzyme. A major function of enzymes in a living system is to catalyze the making and breaking of chemical bonds. Therefore, like any other catalysts, they increase the rate of reaction without themselves undergoing permanent chemical changes.

The catalytic ability of enzymes is due to its particular protein structure. A specific chemical reaction is catalyzed at a small portion of the surface of an enzyme, which is known as the active site. Some physical and chemical interactions occur at this site to catalyze a certain chemical reaction for a certain enzyme.

Enzyme reactions are different from chemical reactions, as follows:

1. An enzyme catalyst is highly specific, and catalyzes only one or a small number of chemical reactions. A great variety of enzymes exist, which can catalyze a very wide range of reactions.
2. The rate of an enzyme-catalyzed reaction is usually much faster than that of the same reaction when directed by nonbiological catalysts. Only a small amount of enzyme is required to produce a desired effect.
3. The reaction conditions (temperature, pressure, pH, and so on) for the enzyme reactions are very mild.
4. Enzymes are comparatively sensitive or unstable molecules and require care in their use.

2.1.1. Nomenclature of Enzymes

Originally enzymes were given nondescriptive names such as:

rennin curding of milk to start cheese-making process

pepsin hydrolyzes proteins at acidic pH

2-4 Enzyme Kinetics

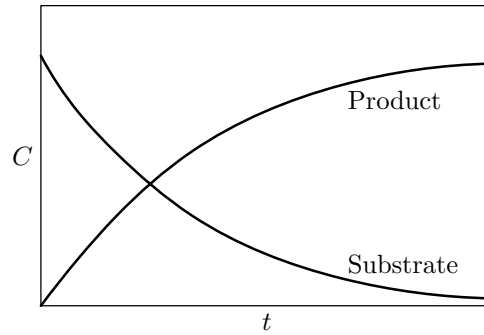


Figure 2.1 The change of product and substrate concentrations with respect to time.

2.2. Simple Enzyme Kinetics

Enzyme kinetics deals with the rate of enzyme reaction and how it is affected by various chemical and physical conditions. Kinetic studies of enzymatic reactions provide information about the basic mechanism of the enzyme reaction and other parameters that characterize the properties of the enzyme. The rate equations developed from the kinetic studies can be applied in calculating reaction time, yields, and optimum economic condition, which are important in the design of an effective bioreactor.

Assume that a substrate (S) is converted to a product (P) with the help of an enzyme (E) in a reactor as



If you measure the concentrations of substrate and product with respect to time, the product concentration will increase and reach a maximum value, whereas the substrate concentration will decrease as shown in Figure 2.1

The rate of reaction can be expressed in terms of either the change of the substrate C_S or the product concentrations C_P as follows:

$$r_S = -\frac{dC_S}{dt} \quad (2.2)$$

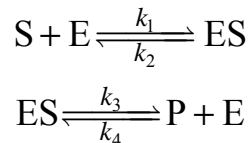
$$r_S = \frac{dC_P}{dt} \quad (2.3)$$

In order to understand the effectiveness and characteristics of an enzyme reaction, it is important to know how the reaction rate is influenced by reaction conditions such as substrate, product, and enzyme concentrations. If we measure the initial reaction rate at different levels of substrate and

equal to the dissociation constant k_2/k_1 , while in the Briggs-Haldane approach, it is equal to $(k_2 + k_3)/k_1$. Eq. (2.18) can be simplified to Eq. (2.11) if $k_2 \gg k_3$, which means that the product-releasing step is much slower than the enzyme-substrate complex dissociation step. This is true with many enzyme reactions. Since the formation of the complex involves only weak interactions, it is likely that the rate of dissociation of the complex will be rapid. The breakdown of the complex to yield products will involve the making and breaking of chemical bonds, which is much slower than the enzyme-substrate complex dissociation step.

Example 2.1

When glucose is converted to fructose by glucose isomerase, the slow product formation step is also reversible as:



Derive the rate equation by employing (a) the Michaelis-Menten and (b) the Briggs-Haldane approach. Explain when the rate equation derived by the Briggs-Haldane approach can be simplified to that derived by the Michaelis-Menten approach.

Solution:

(a) Michaelis-Menten approach: The rate of product formation is

$$r_p = k_3 C_{ES} - k_4 C_P C_E \quad (2.19)$$

Since enzyme is preserved,

$$C_{E_0} = C_E + C_{ES} \quad (2.20)$$

Substitution of Eq. (2.20) into Eq. (2.19) for C_E yields

$$r_p = (k_3 + k_4 C_P) C_{ES} - k_4 C_P C_{E_0} \quad (2.21)$$

Assuming the first reversible reaction is in equilibrium gives

$$C_{ES} = \frac{k_1}{k_2} C_E C_S \quad (2.22)$$

Substituting Eq. (2.22) into Eq. (2.20) for C_E and rearranging for C_{ES} yields

2.7. Experiment: Enzyme Kinetics

Objectives:

The objectives of this experiment are:

1. To give students an experience with enzyme reactions and assay procedures
2. To determine the Michaelis-Menten kinetic parameters based on initial-rate reactions in a series of batch runs
3. To simulate batch and continuous runs based on the kinetic parameters obtained

Materials:

1. Spectrophotometer
2. 10g/L glucose standard solution
3. Glucose assay kit (No. 16-UV, Sigma Chemical Co., St. Louis, MO)
4. Cellobiose
5. Cellobiase enzyme (Novozym 188, Novo Nordisk Bioindustrials Inc., Danbury, CT) or other cellulase enzyme
6. 0.05M (mol/L) sodium acetate buffer (pH 5)
7. 600 mL glass tempering beaker (jacketed) (Cole-Parmer Instrument Co., Chicago, IL) with a magnetic stirrer
8. Water bath to control the temperature of the jacketed vessel

Calibration Curve for Glucose Assay:

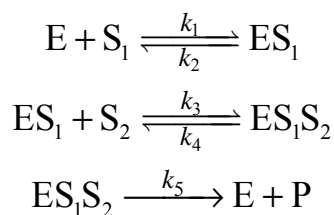
1. Prepare glucose solutions of 0, 0.5, 1.0, 3.0, 5.0 and 7.0g/L by diluting 10g/L glucose standard solution.
2. Using these standards as samples, follow the assay procedure described in the brochure provided by Sigma Chemical Co.
3. Plot the resulting absorbances versus their corresponding glucose concentrations and draw a smooth curve through the points.

2.9. Problems

- 2.1 In order to measure the enzyme activity and the initial rate of reaction, 5 mL of cellobiose (100 $\mu\text{mol/mL}$) and 44 mL of buffer solution were placed in a stirred vessel. The reaction was initiated by adding 1 mL of enzyme (beta-glucosidase) solution which contained 0.1 mg of protein per mL. At 1, 5, 10, 15, and 30 minutes, 0.1 mL of sample was removed from the reaction mixture and its glucose content was measured. The results were as follows:

Time Min	Glucose Concentration $\mu\text{mol/mL}$
1	0.05
5	0.23
10	0.38
15	0.52
30	1.03

- a. What is the activity of the β -glucosidase in units/mL of enzyme solution and in units/mg protein? A unit is defined as the enzyme activity which can produce 1 μmol of product per minute.
- b. What is the initial rate of reaction?
- 2.2 Suppose that the following sequence describes the reactions of two different substrates catalyzed by one enzyme:



- a. Derive the rate equation by making the Michaelis-Menten assumption.
- b. If the concentration of S_1 is much higher than that of S_2 , how can the rate equation be simplified?
- 2.3 In some enzyme-catalyzed reactions, multiple complexes are involved as follows: