Biochemical Engineering

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Preface

Biochemical engineering embraces aspects of biochemistry, cell and molecular biology, bioorganic and bioinorganic chemistry, and has at its core the discipline of chemical engineering. Biochemical engineering may be loosely defined as the use of living organisms, or part of them (for example, enzymes), for the production of chemical or biological materials or the development of new processes. In this sense, the practice of biochemical engineering has a long history, including such early applications as the fermentation of grapes for wine, brewing of beer, cheese manufacture, leavening of bread, and effluent disposal. In more recent times, biochemical engineers have been involved in antibiotic fermentations, production of industrial solvents, organic acids, vaccines, blood and tissue products, animal feedstuffs, commercial enzymes and in the treatment of effluents and wastes.

The advent of recombinant DNA and hybridoma technology in the late 1970s opened new avenues for the production of enzymes and therapeutic proteins, and the modification of metabolic pathways for the production of biochemicals. Today, biochemical engineers work with a variety of cells from microbial, mammalian and plant sources, in the form of single cells and as tissues. Many issues that were vitally important earlier have been resolved in the past decades; these include sterile air filtration, medium and equipment sterilization, aseptic equipment design, and instrumentation. Our knowledge of microbial and cellular physiology has expanded rapidly, together with our understanding of enzyme structure and catalytic function. New biological catalysts, such as ribozymes, catalytic antibodies, and modified enzymes provide new tools for the production of chemicals, biochemicals, materials, and therapeutic agents. Exploitation of advances in recombinant DNA technology relies on early studies of microbial physiology, scale-up and reactor design, heat and mass transfer in fermentations, and on separation and purification processes.

One of the most important tools in biochemical engineering has been continuous culture (the chemostat); it provided a technique to study microbial physiology under well-defined conditions, and a means to develop kinetic models of microbial growth and

product formation. The theoretical and experimental basis of chemostat operation was originally proposed by Monod[1] and Novik and Szilard[2] and it was extensively developed by researchers at the Microbiological Research Establishment at Porton Downs (U.K.)[3] and at the Institute of Microbiology of the Czechoslovak Academy of Sciences[4]. The annual review of publications on continuous culture, appearing in Folia Microbiologia[5], included developments in the theory of continuous cultivation, microbial physiology and product formation and applications of the method. In the past twenty years, the number of publications involving chemostats has grown tremendously. Continuous culture has played an important role in understanding the stability of plasmids in recombinant cells, in following events in the cell cycle, and in understanding monoclonal antibody production by hybridoma cells.

Paralleling these advances in fermentation, the area of separation and recovery of biological products has seen the development of techniques such as ion exchange, affinity and gel permeation chromatography, electrophoresis, aqueous two-phase extraction, and membrane separation processes. Most of these techniques rely on newly-developed materials with suitable properties. The high purity requirement of recombinant proteins has been a significant factor in developing highly-efficient small-scale separation processes. The theoretical underpinnings of these separation processes are based on biophysical chemistry, colloidal and interfacial phenomena and mass transfer. Appropriate descriptions of intermolecular interactions are important in understanding processes such as precipitation, crystallization, extraction and interactions of biological materials with surfaces.

There have been several texts that have had a profound influence on the development of biochemical engineering. One of the first texts, written from a chemical engineering perspective, is F.C. Webb's book, "Biochemical Engineering", developed from courses at University College, London[6]. It emphasizes physical chemistry, kinetics, and heat and mass transfer. The first text to integrate microbiology, enzymology and bioprocess engineering was Aiba, Humphrey and Millis' "Biochemical Engineering"[7]. It served as the standard reference for many years and provided a valuable introduction to biochemistry and biology for chemical engineers. Bailey and Ollis' text "Biochemical Engineering

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(1) Monod, J., Annals Inst. Pasteur **79**, 390 (1950)

(2) Novick, A. and L. Szilard, Science, **112**, 715 (1950)

(3) Herbert, D., Ellsworth, R. and R.C. Telling, J. Gen. Microbiol. **14**, 601 (1956)

(4) Malek, I., Beran K. and Z. Fencl, Theoretical and Methodological Basis of Continuous Culture of Microorganisms, Publishing House of Czech. Academy of Sciences, Prague (1966)

(5) Malek, I. and Z. Fencl, Folia Microbiologia, **6**, 142 (1961) and annual reviews thereafter

(6) Webb, F.C., Biochemical Engineering, van Nostrand (1964)

(7) Aiba, S., Humphrey, A.E. and N.F. Millis, Biochemical Engineering, Academic Press, 1st edition (1964) and 2nd edition (1973)

Fundamentals"[8] incorporated modern biology and included sections on the commercial applications of biochemical engineering. It provided students with sufficient background in the life sciences to enable them to address problems in microbial growth, enzyme kinetics and metabolic control.

This book is a comprehensive textbook of modern biochemical engineering, intended for students in engineering and applied science. With the increasing emphasis on life sciences in engineering curricula, many students interested in biochemical engineering already have backgrounds in biochemistry or cell biology. This text therefore does not include sections on biochemistry, microbiology, and molecular and cell biology. There are a number of excellent texts available that can be used as a supplement to this text, permitting the student to cover biology to any depth required[9]. Material is presented at levels appropriate for both undergraduate and graduate courses in biochemical engineering, and sample course outlines are provided below for courses taken by students with and without backgrounds in the life sciences. Each major topic follows a logical progression from basic principles to more advanced concepts. The major topics include enzyme kinetics and biocatalysis, microbial growth and product formation, bioreactor design, transport in bioreactors, bioproduct recovery, and bioprocess economics and design. Problems are included at the end of each chapter, ranging from straightforward exercises testing knowledge of fundamental concepts to more complex problems dealing with real systems and real bioprocesses. Although the primary aim of the book is to serve as an instructional text for students of biochemical engineering, it should also be useful to practicing biochemical engineers who are concerned with the basic concepts underlying the design and behavior of bioprocesses.

Acknowledgments

We are very grateful to Peter Michels and Jeanne George for reviewing preliminary versions of each chapter. Their critical comments on content and presentation were extremely useful. Suggestions offered by teaching assistants and students as the text progressed to the classroom were also invaluable, especially those of Andrew Shuler, M. Scott Clarke, and Mareia Frost. Thoughtful and useful reviews of selected chapters were furnished by Jonathan Dordick, Charles Goochee, and Timothy Oolman. Thanks are also owed to Larry Erikson, Doug Lauffenburger, and Jay Bailey for generously supplying several homework problems. We are especially grateful to John Martens for his assistance with the illustrations throughout the text.

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(8) Bailey, J.E. and D.F. Ollis, Biochemical Engineering Fundamentals, McGraw Hill, 1st edition (1977) and 2nd edition (1986)

(9) Stryer, L., Biochemistry, W.H. Freeman, N.Y., 4th edition (1995); Glazer, A.N., and H. Nikaido, *Microbial Biotechnology*, W.H. Freeman & Co. (1995)

Course Outlines

Undergraduate course on biochemical engineering

Given below is the outline for a course on biochemical engineering, structured for a 15-week semester, assuming three 50-minute lectures per week, and three lecture periods for examinations or review. This outline is intended for students who have had an introductory course in biochemistry or a related biological science, or who are taking such a course concurrently. A course outline for students with no previous experience in the biological sciences follows.

Lecture	Reading Assignment
1. Overview of biotechnology	Handouts
2. Principles of enzyme catalysis	pp. 1 - 5; 18 - 27 (Chapter 1)
3. Principles of enzyme catalysis (cont.)	pp. 27 - 39
4. Kinetics of single substrate reactions	pp. 5 - 18
5. Burst kinetics of chymotrypsin. Enzyme inhibition	pp. 50 - 52; 39 - 45
6. Enzyme inhibition (cont.) Enzyme denaturation and inactivation	pp. 39 - 45; 77 - 81
7. Methods of enzyme and cell immobilization	pp. 103 - 116 (Chapter 2)
8. Electrostatic and external mass transfer effects on immobilized enzyme kinetics	pp. 116 - 127
9. Internal mass transfer effects on immobilized enzyme kinetics	pp. 127 - 140
10. Internal mass transfer effects (cont.)	pp. 127 - 140
11. Stoichiometry and energetics of microbial growth	pp. 162 - 181 (Chapter 3)
12. Unstructured models of microbial growth	pp. 181 - 209
13. Unstructured models (cont.)	pp. 181 - 209
14. Structured models of microbial growth	pp. 225 - 230
15. Structured models (cont.)	pp. 230 - 236; 244 - 246
16. Continuous stirred tank bioreactors	pp. 276 - 291 (Chapter 4)
17. Enzyme catalysis in a CSTR Chemostats in series	pp. 292 - 297
18. Plug-flow and packed bed bioreactors Imperfect mixing	pp. 297 - 305
19. Fed batch bioreactors	pp. 305 - 309
20. Gas-liquid mass transfer in bioreactors	pp. 353 - 361 (Chapter 5)
21. Mass balances for two-phase bioreactors	pp. 361 - 364
22. Mass transfer coefficient $k_l a$	pp. 390 - 403
23. Power requirements for bioreactors	pp. 403 - 415
24. Sterilization	pp. 415 - 426

25. Introduction to bioproduct recovery. Centrifugation	pp. 453 - 467 (Chapter 6)
26. Filtration and ultrafiltration	pp. 467 - 470
27. Filtration and ultrafiltration	pp. 482 - 490
28. Precipitation of proteins	pp. 491 - 502
29. Basic concepts of chromatography	pp. 502 - 511
30. Mathematical analysis of chromatography	pp. 512 - 520
31. Fixed-bed adsorption and affinity chromatography. Plate theory of chromatography	pp. 523 - 533
32. Plate theory of chromatography (cont.)	pp. 526 - 533
33. Electrophoresis and crystallization	pp. 533 - 542
34. Review	Chapters 4 - 6
35. Microbial interactions and competition	pp. 578 - 590 (Chapter 7)
36. Competition (cont.)	pp. 581 - 590
37. Predation	pp. 592 - 597
38. Manufacture of biological products	pp. 609 - 643 (Chapter 8)
39. Economic analysis of bioprocesses	pp. 643 - 648
40. Capital costs and manufacturing costs	pp. 648 - 657
41. Case studies	pp. 657 - 677
42. Final Review	Chapters 1 - 8

Additional or alternate topics for a graduate course:

Extended coverage of enzyme kinetics (limiting conditions, multi-substrate reactions, allosteric interactions, pH and temperature effects)	Sections 1.7 - 1.10
Intraparticle diffusion and immobilized enzyme stability	Section 2.5
Growth of eukaryotic cells	Section 3.4
Models of product formation	Section 3.5.3
Gene expression and regulation. Plasmid expression and regulation	Sections 3.6 and 3.7
Further analysis of bioreactors (transient behavior, recycle systems)	Sections 4.6 and 4.7
Expanded survey of transport processes (rheology of fermentation broths, bubble columns, coupled mass transfer and reaction)	Sections 5.1, 5.3, and 5.6
More on heat transfer	Section 5.5.2
Additional separation methods (e.g., extraction)	Section 6.3.1
Microbial interactions in greater depth (chemotaxis, stability)	Sections 7.3, 7.5, and 7.6

For students without background in biological sciences, the following outline is suggested.

Lecture	**Reading Assignment**
1. Introduction to microorganisms and biological molecules	Supplemental reading
2. Chemistry of amino acids and proteins	Supplemental reading
3. Protein structure and function	Supplemental reading
4. Molecular genetics and protein synthesis	Supplemental reading
5. Recombinant DNA and genetic engineering	Supplemental reading
6. Basic microbiology and cellular structure	Supplemental reading
7. Energetics and metabolism	Supplemental reading
8. Overview of biotechnology	Handouts
9. Principles of enzyme catalysis	pp. 1 - 5, 18-27 (Chapter 1)
10. Kinetics of single substrate reactions	pp. 5 - 18
11. Enzyme inhibition	pp. 39 - 45
12. Enzyme denaturation and inactivation	pp. 77 - 81
13. Methods of enzyme and cell immobilization. External mass transfer and immobilized enzyme kinetics	pp. 103 - 116; 121-125 (Chapter 2)
14. Internal mass transfer effects on immobilized enzyme kinetics	pp. 127 - 140
15. Stoichiometry and energetics of microbial growth	pp. 162 - 181 (Chapter 3)
16. Unstructured models of microbial growth	pp. 181 - 190
17. Structured models of microbial growth	pp. 225 - 230 pp. 244 - 246
18. Continuous stirred tank bioreactors	pp. 276 - 291 (Chapter 4)
19. Enzyme catalysis in a CSTR. Chemostats in series	pp. 292 - 297
20. Plug-flow and packed bed bioreactors. Fed batch bioreactors	pp. 297 - 300 pp. 305 - 308
21. Gas-liquid mass transfer in bioreactors	pp. 353 - 361 (Chapter 5)
22. Mass balances for two-phase bioreactors	pp. 361 - 364
23. Mass transfer coefficient k_La	pp. 390 - 403
24. Power requirements for bioreactors	pp. 403 - 415
25. Sterilization	pp. 415 - 426
26. Introduction to bioproduct recovery; Centrifugation	pp. 453 - 467 (Chapter 6)
27. Filtration and ultrafiltration	pp. 467 - 470
28. Filtration and ultrafiltration	pp. 482 - 490
29. Precipitation of proteins	pp. 491 - 502
30. Basic concepts of chromatography	pp. 502 - 511

31. Mathematical analysis of chromatography	pp. 512 - 520
32. Fixed-bed adsorption and affinity chromatography. Plate theory of chromatography	pp. 523 - 533
34. Plate theory of chromatography (cont.)	pp. 526 - 533
35. Electrophoresis and crystallization	pp. 533 - 542
36. Review	Chapters 3 - 6
37. Microbial interactions and competition	pp. 578 - 590 (Chapter 7)
38. Competition (cont.)	pp. 581 - 590
39. Manufacture of biological products	pp. 609 - 643 (Chapter 8)
40. Economic analysis of bioprocesses	pp. 643 - 648
41. Capital costs and manufacturing costs	pp. 648 - 657
42. Case studies	pp. 657 - 677
43. Final Review	Chapters 1 - 8

Contents

Chapter 1. Enzyme Catalysis

Enzymes are one of the essential components of all living systems. These macromolecules have a key role in catalyzing the chemical transformations that occur in all cell metabolism. The nature and specificity of their catalytic activity is primarily due to the three-dimensional structure of the folded protein, which is determined by the sequence of the amino acids that make up the enzyme. The activity of globular proteins may be regulated by one or more small molecules, which cause small conformational changes in the protein structure. Catalytic activity may depend on the action of these non-protein components (known as *cofactors*) associated with the protein. If the cofactor is an organic molecule, it is referred to as a *coenzyme*. The catalytically inactive enzyme (without cofactor) is termed an *apoenzyme;* when coenzyme or metal ion is added, the active enzyme is then termed a *holoenzyme*. Many cofactors are tightly bound to the enzyme and cannot be easily removed; they are then referred to as *prosthetic groups*.

In this chapter we shall examine the nature of enzyme catalysis, first by examining the types of reactions catalyzed and the mechanisms employed by enzymes to effect this catalysis, and then by reviewing the common constitutive rate expressions which describe the kinetics of enzyme action. As we shall see, these can range from simple rate expressions to complex expressions that involve several reactants and account for modification of the enzyme structure.

1.1 Specificity of Enzyme Catalysis

Enzymes have been classified into six main types, depending on the nature of the reaction catalyzed. A numbering scheme for enzymes has been developed, in which the main classes are distinguished by the first of four digits. The second and third digits describe the type of reaction catalyzed, and the fourth digit is employed to distinguish between enzymes of the same function on the basis of the actual substrate in the reaction catalyzed. This scheme has proven useful in clearly delineating many enzymes that have similar activities. It was developed by the Enzyme Commission and the prefix E.C. is generally employed with the numerical scheme.

We shall examine the six main classes of enzymes primarily to illustrate the broad range of reactions which can be catalyzed by enzymes and the types of substrates they can act on.

Class 1. Oxidoreductases

These enzymes catalyze the transfer of hydrogen or oxygen atoms or electrons from one substrate to another. They are often called oxidases or dehydrogenases, and reference is made to the substrate of the reaction, e.g. lactate dehydrogenase. The first digit (1.) of the classification thus indicates the class oxidoreductase; the second digit indicates the donor of the hydrogen atom or electron (i.e., reducing equivalent) involved. The third digit describes the hydrogen atom or electron acceptor.

First digit	*Second digit*	*Third digit*
1.	1 alcohol	1 NAD^+ or $NADP^+$
(Oxidoreductase)	2 aldehyde or ketone	2 Fe^{3+}
	3 alkene -CH=CH-	3 O_2
	4 primary amine	4 otherwise unclassified
	5 secondary amine	
	6 NADH or NADPH	

Class 2. Transferases

Transferases catalyze the group transfer reactions, with a general form given below. However hydrolase and oxidoreductase reactions are excluded, as they are classified above.

$$AX + B \Leftrightarrow BX + A$$

The second digit indicates the general type of group transferred (these are listed below), and the third digit provides details on the exact nature of the group transferred (e.g., 2.1.2 are hydroxymethyl-, formyl and related transferases).

First digit	*Second digit*	*Third digit*
2.	1 1-carbon group	nature of group transferred
(Transferases)	2 aldehyde or ketone	
	3 acyl group (-CO-R)	
	4 glycosyl group	
	7 phosphate group	
	8 sulphur containing groups	

Class 3. Hydrolases

Hydrolases catalyze hydrolytic reactions, with the second digit indicating the type of bond hydrolyzed.

$$A - X + H_2O \Leftrightarrow X - OH + HA$$

First digit	*Second digit*	
3.	1	ester
(Hydrolases)	2	glycosidic (i.e., linking carbohydrate moieties)
	4	peptide
	5	C-N bonds other than peptides
	6	acid anhydrides

Class 4. Lyases

Lyases catalyze the non-hydrolytic removal of groups from substrates. Often the product contains a double bond. The second digit refers to the type of bond broken. The third digit describes the group removed. Also included in this class are enzymes which act in the *reverse* direction to group removal. These are often *synthases* or *hydratases*, e.g. reactions in which groups are added across a double bond.

First digit	*Second digit*		*Third digit*	
4.	1	C-C	1	carboxyl
(Lyases)	2	C-O	2	aldehyde
	3	C-N	3	ketoacid
	4	C-S		

Class 5. Isomerases

The second digit of the classification of isomerases describes the type of reaction involved. The third digit describes the type of molecule undergoing isomerization.

First digit	*Second digit*		*Third digit*	
5.	1	racemization or epimerization	1	amino acids
(Isomerases)	2	cis-trans isomerizations	2	hydroxyacids
	3	intramolecular oxidoreductases	3	carbohydrates
	4	intramolecular transfer reactions		

Class 6. Ligases

Ligases catalyze the synthesis of various types of bonds, where the reactions are coupled with breakdown of energy-containing materials, such as ATP or nucleoside triphosphates. For example;

$$X + Y + ATP \Leftrightarrow X - Y + ADP + P_i$$

or

$$X + Y + ATP \Leftrightarrow X - Y + AMP + PP_i$$

First digit	*Second digit (The second digit indicates the type of bond formed).*	
6.	1	C-O
(Ligases)	2	C-S
	3	C-N
	4	C-C

An example of this classification scheme is given below.

Recommended name (trivial name) : alcohol dehydrogenase
Systematic name: alcohol : NAD^+ oxidoreductase
(an alcohol is the electron donor and NAD^+ is the electon acceptor)

Enzyme number: EC

1.	1.	1.	1.
			number for further identification
		acceptor is NAD^+ or $NADP^+$	
	donor group is CH-OH		
number of the primary division (oxidoreductases)			

This classification scheme is useful as it unambiguously identifies the enzyme in question. Earlier nomenclature often resulted in one enzyme being identified by several names if its activity was broad.

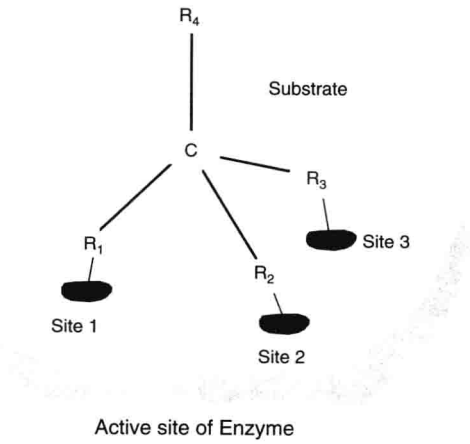

Figure 1.1 *Representation of the three-point interaction of substrate with enzyme.*

While enzymes are specific in function, the degree of specificity varies. Some may act on closely related substrates, and are said to exhibit *group specificity*; others are more exacting in their substrate requirements, and are said to be *absolutely specific*. The product formed from a particular enzyme and substrate is also unique. Enzymes are able to distinguish between stereochemical forms and only one isomer of a particular substrate may undergo reaction. Surprisingly, enzyme reactions may yield stereospecific products from substrates

that possess no asymmetric carbon atoms, as long as one carbon is *prochiral*. This chirality is a result of at least three-point interaction between substrate and enzyme in the active site of the enzyme. In Figure 1.1, sites 1, 2 and 3 are binding sites on the enzyme. When two of the R groups on the substrate are identical, the molecule has a *prochiral* center and a chiral center can result from the enzymatic reaction, as the substrate can only "fit" into the active site in one configuration if the site has binding selectivity for three of the R-group substituents. If the substrate has four different R groups, then chirality can be preserved in the reaction as a result of the multipoint attachment.

1.2 Kinetics of Single Substrate Reactions

In this section we shall review various simple constitutive rate expressions which have been applied to describe enzyme-catalyzed reactions. As we saw in Section 1.1, the reaction of an enzyme with a substrate involves the formation of an intermediate which then reacts further with other substrates or decomposes to form products. With recent developments in enzymology, it has been possible to identify many of the elementary reaction steps involved in enzymatic catalysis, and from experimental data on rates of formation of intermediates, the intrinsic kinetics can be found.

Historically, it was found that the kinetics of enzyme catalyzed reactions exhibit features which indicate that a simple single step reaction is not occurring. Invertase, which catalyzes the hydrolysis of sucrose to glucose and fructose, was shown in 1902 by Brown to exhibit kinetics which were first order in the reactant sucrose at low sucrose concentrations, but zeroth order at high concentrations. These results were in contrast to the first order dependence on sucrose concentration found with acid-catalyzed hydrolysis. This hyperbolic dependence of reaction rate on substrate concentration was found to be common for all enzyme-catalyzed reactions. Typically, enzyme rate data are reported as *initial rate* data, where the rate of substrate consumption or product formation is determined over a short period of time following the initiation of the reaction. An example of this is shown in Figure 1.2.

When the initial concentration of substrate is varied, the hyperbolic dependence of the initial reaction rate can be seen (Figure 1.3). This behavior is sometimes referred to as "saturation kinetics". The dependence of the initial rate on enzyme concentration is generally first order.

Specific Activity

Enzyme concentrations are often given in terms of "units" rather than in mole or mass concentration. We rarely know the exact mass of the enzyme in a sample, since it is generally prepared via isolation of the enzyme from microorganisms, or animal or plant tissues and often contains a great deal of non-catalytic protein, the amount of which may vary from sample to sample. Hence a different approach must be adopted, and enzyme concentration is reported in units of *specific activity*. A "unit" is defined as the amount of enzyme (e.g.,

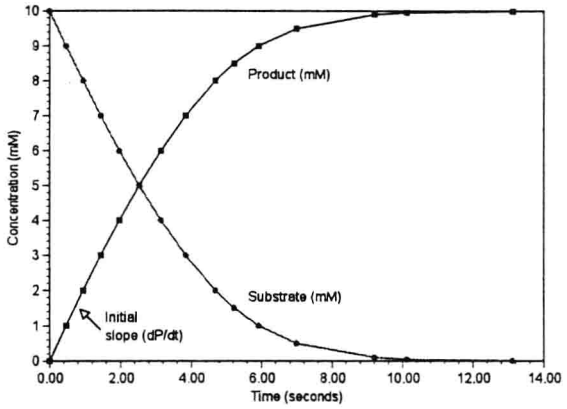

Figure 1.2. *An example of the time course of urea hydrolysis, catalyzed by urease, illustrating the determination of the initial rate of reaction (v_0 in millimoles/liter-sec). The initial rate is the slope of the tangent passing through the origin. The values of the kinetic parameters are k_2 = 30,800 sec^{-1}, K_M = 4.0 mM at pH 8.0 and 20.8°C, $[E_0]$ = 0.1 μM. The reaction catalyzed is*

$$NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

microgram) which gives a certain amount of catalytic activity under specified conditions (e.g., producing 1.0 micromole of product per minute in a solution containing a susbstrate concentration sufficiently high to be in the "saturation" region, as shown in Figure 1.3).

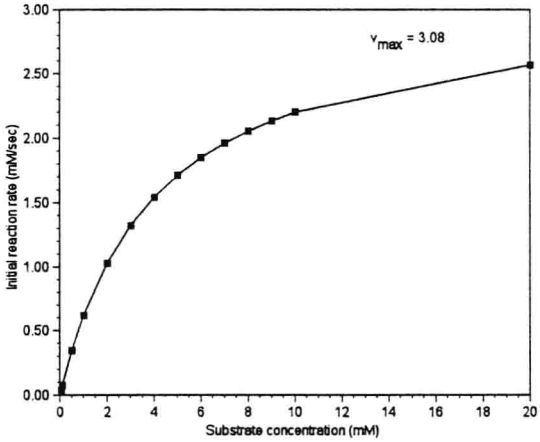

Figure 1.3. *An example of the initial rate of reaction as a function of substrate and enzyme concentrations for urease; reaction parameters are the same as given in Figure 1.2.*