

ENZYME CATALYSIS

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AT FIRST GLANCE it surprises enzymologists that enzymes are not utilized much more widely in batch chemical engineering processes. This attitude comes from the enzymologists' creed: "Enzymes will catalyze virtually any chemical reaction, and they will do it better than any other catalyst."

At second glance, however, the surprise is gone. The problems associated with using enzyme technology in chemical engineering processes are immense: They range from the lack of education about enzymes amongst chemical engineers and the lack of education and interest about ChE applications among enzymologists, to the severity of the problems well trained biochemical engineers must solve in order to keep enzymes happy in environments dictated by engineering considerations.

The apparently recent realization that the earth is a finite system has accelerated interest in waste treatment processes, solar energy utilization, clean-burning fuels, food technology, human population control, and environmental quality. Each of these important engineering problems is related in some way to biological (and therefore enzyme-catalyzed) processes. Biological degradation of waste is the oldest and still most widely-used process of waste disposal. The **sole** energy source in biology is the sun, so living systems are old hands at capturing and utilizing solar energy. Because micro-organisms have to co-exist with what they excrete, relatively clean burning fuels are the natural product of many biological fermentations. Food is a biological material so its manipulation and interconversion by enzyme-catalyzed processes is clearly involving obvious biological components.

For these and other reasons it became clear that the catalysis program in our department

should include a graduate level course about enzyme catalysis. We designed this course to provide engineers and students in the sciences with a basic understanding of how enzymes and multi-enzyme systems function as catalysts. Special emphasis is on enzyme specificity, efficiency and control, and how these characteristics relate to potential applications in biochemical engineering. The course is structured so that the student is exposed to: [1] basic concepts about enzymes and enzyme catalysis and [2] the methodologies of enzyme chemistry and enzyme kinetics.

The course is designed for first level graduate students in engineering, but senior-level undergraduate students in chemical engineering, chemistry, or the biological sciences, as well as graduate students in these sciences, and interested medical students are welcome to register. I would expect adequately prepared students in any of these categories to be able to do well in this course. Obviously, a knowledge of biochemistry

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and/or enzymology would be helpful, but it is not essential. The prerequisites listed for the course are calculus, physical chemistry, organic chemistry, and elementary computer programming. The principle reference material includes the following four books: *Enzyme Reactions and Enzyme Systems* [1], *Biochemical Regulatory Mechanisms in Eukaryotic Cells* [2], *Steady-state Applications in Enzyme Kinetics* [3], and *Immobilized Enzymes* [4].

DISCUSSION OF COURSE MATERIAL

I. The Chemical Structure of Enzymes

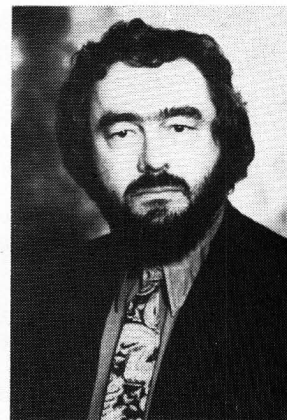
The purpose of this part of the course is to

introduce the student to basic concepts about enzyme structure and function.

The first section outlines the nomenclature recommended for enzymes in the 1961 Report of the Commission on Enzymes of the International Union of Biochemistry [5]. The second section provides the student with an understanding of the role in protein structure and enzyme activity of the peptide bond, other covalent bonds, hydrogen bonding, apolar associative forces, and other

TABLE I
Course Outline

- I. The Chemical Structure of Enzymes
 - A. Enzyme nomenclature
 - B. Primary, secondary, tertiary and quaternary structure
 - C. The concept of the "active site"
 - D. The concept of the "regulatory site"
 - E. Cofactors
 - F. Control properties implicit in chemical structure
- II. Kinetic Properties of Single Enzymes in Solution
 - A. The general theory of enzyme kinetics, the law of mass action
 - B. Initial transient kinetics
 - C. The quasi-steady-state approximation and its validity in enzyme kinetics
 - D. Quasi-steady-state models
 - E. Near-equilibrium techniques and their kinetic analysis
 - F. Near-equilibrium versus quasi-steady-state tracer distribution kinetics
 - G. Simulation of enzyme models on analog and digital computers
 - H. Computer methods for generating rate equations for enzyme models
 - I. The collection and analysis of enzyme kinetic data with on-line computational facilities.
- III. Kinetic Properties of Multi-enzyme Systems
 - A. The general theory of control; applications in biochemical engineering
 - B. The theory of far-from-equilibrium systems; applications to multi-enzyme systems
 - C. Kinetics of multi-component systems without feedback control
 - D. Kinetics of multi-component systems with feedback control
 - E. Integrated reaction kinetics of enzyme reactors
 - F. Simultaneous reactors and diffusion in enzyme reactors
- IV. Immobilized Enzymes and Enzyme Systems
 - A. Types of support
 - B. Covalent coupling methods
 - C. Effects due to coupling on enzyme activity and other enzyme properties
 - D. Theoretical effects of immobilization on enzyme kinetics
 - E. Consideration of physical and diffusional constraints imposed by the carrier on enzyme catalysis



Charles F. Walter received his B.S. (1957), M.S. (1959), and Ph.D. (1962) degrees in chemistry at Florida State University. He was appointed Assistant Professor (1964) and Associate Professor (1968) of Biochemistry at the University of Tennessee Medical School in Memphis. In 1970 he was appointed Associate Professor of Biomathematics and Associate Professor of Biochemistry at the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute in Houston. In 1974 Dr. Walter was appointed to his present position as Professor of Chemical Engineering at the University of Houston. His research and teaching interests include enzymology applied to biochemical engineering problems in hydrogen generation from cellulose, the chemistry of nucleotide-nucleotide.

non-covalent interactions. Section C, about the catalytic sites of enzymes, emphasizes current ideas about the indigenous nature of certain sites on enzyme surfaces, and how sites not indigenous on the enzyme surface can be induced by the proximity of a ligand (usually the substrate) to the site area. Similarly, Section D, which is about regulatory binding sites on enzymes, emphasizes current concepts about how these noncatalytic sites, when associated with specific ligand molecules, interact with catalytic sites and thereby alter catalytic activity. General models involving cooperative interactions between regulatory and catalytic sites and stimulation or inhibition of catalytic behavior are emphasized [6, 7]. Section E deals with the role of cofactors in the induction of non-indigenous substrate binding sites, and the last section is about the type of control of catalytic activity that is "built in" by the primary, second, tertiary, and quaternary structure of enzymes.

II. Kinetic Properties of Single Enzymes in Solution

The purpose of this part of the course is to provide the students with a fundamental understanding of the kinetics and control properties of single isolated enzymes in homogenous solutions.

The first section reviews the law of mass action and its applications to enzyme models. The overall chemical reaction, the development of empirical kinetic equations for enzyme-catalyzed reactions, the relation between these kinetic equations and initial rate equations, and a description

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of a general initial quasi-steady-state rate equation for multisubstrate enzyme models are included in Section A. The second section is about the kinetic behavior of enzyme-catalyzed reactions prior to the attainment of a quasi-steady-state. Section C includes a rigorous discussion of the concept of a quasi-steady-state in a closed system, and a derivation of the relationship between the error introduced by using the quasi-steady-state approximation, the magnitude of the kinetic constants for the enzyme, and the experimental initial conditions used. Section D is a review of quasi-steady-state enzyme models, the use of the King and Altman algorithm [8] to derive them, and the relationship between these approximate mathematical models and the real chemical mechanisms they approximate. Sections E and F are about the relaxation kinetics of enzyme reactions that have been perturbed slightly from thermodynamic equilibrium. Perturbation techniques that are discussed include stepwise temperature changes, periodic pressure variations, and the addition of small quantities of a radioactively labelled reactant. In section F the usefulness of kinetic experiments involving the addition of traces of labelled reactant to an enzyme and its reactants near thermodynamic equilibrium, and kinetic experiments wherein large quantities of labelled substrate are added to an enzyme and its reactants far from equilibrium, is compared. Section G is about the simulation of enzyme models on analog computers, and their numerical "simulation" on digital machines. For the digital simulations we have chosen the program by Chance, Shephard and Curtis, "The

University College London Enzyme Simulator" [9]. This program is especially easy to use because it "automatically" translates the individual chemical steps into mathematical relationships via a built-in equation translator routine. Section H explains the use of a computer program [10, 11] that uses the King and Altman [8] algorithm to derive quasi-steady-state rate equations for any enzyme model. The last section of this part is a description of how enzyme kinetic data is properly analyzed. This section includes a comparison of the graphical and "standard" statistical procedures usually employed in the analysis of enzyme rates, a discussion of one-line methods for the collection and analysis of data from enzyme-catalyzed reactions, a critical evaluation of integrated forms of quasi-steady-state rate equations, and the use of experimental data and digital computer programs like "The University College London Enzyme Simulator" [9] to estimate individual rate constants in assumed enzyme models.

III. Kinetic Properties of Multi-enzyme Systems

The purpose of this part of the course is to provide the students with a comprehensive understanding of the kinetics and control properties of a sequence of enzyme-catalyzed reactions in a homogeneous solution.

The first section reviews the general theory of control for linear and nonlinear systems. Application of the Lyapunov direct method to biological control systems of interest in biochemical engineering is illustrated, and the existence and significance of limit cycles in biochemical systems is discussed. The next section is about the theory of far-from-equilibrium chemical systems; dissipative structures and the spatial and temporal organization of such systems are discussed from the point of view of the rich organizational behavior implicit in the nonlinear partial differential equations that describe them. Sections C and D are about the dynamics, stability, and control properties of nonlinear multi-chemical systems with or without feedback control; emphasis in these sections is on multi-enzyme systems with negative feedback of the Yates and Pardee [12] type, or positive feedback of the type thought to be responsible for the limit cycle concentration oscillations of the components in the glycolytic pathway [13, 14]. Analysis of the stability properties of these control systems is carried out with the aid of: [1] the usual analysis for

linearized systems [15, 16]; [2] perturbation theory; [3] the Aisermann conjecture [17]; [4] the Lur's transformation and algorithm for obtaining a global Lyapunov function [18]; and [5] computer simulations of the nonlinear differential equations [19, 20]. The analysis of the control properties is carried out by comparing the sensitivity of metabolic levels of the components in the models to parameter variations. The last two sections are about applications of the theory of enzyme kinetics and control to chemical reactor processes. Section E deals with situations where diffusion is not important, and Section F with examples where the effects of diffusion must be included in the differential equations describing the enzyme-catalyzed reactions in the reactor.

IV. Immobilized Enzymes and Enzyme Systems

The purpose of the last part of the course is to acquaint students with heterogeneous enzyme catalysis and its role in problems in biochemical engineering.

The first two sections review the various types of supports and covalent coupling methods used to bind enzymes. Section C is about the effects of immobilization methods on enzyme structure, and especially on those aspects of structure changes which effect enzyme activity via modification of the active site and/or control sites. This section introduces the student to possible effects of these structural modifications on the overall kinetics of the immobilized enzymes. The next section compares the kinetics of homogeneous and heterogeneous enzymes or enzyme systems. The last section examines the physical and diffusional constraints imposed on enzymes or multi-enzyme systems by immobilization. □

REFERENCES

1. C. Walter, *Enzyme Reactions and Enzyme Systems*, Marcel Dekker, Inc., Publishers, New York, 1975.
2. C. Walter, Ch. 11 in *Biochemical Regulatory Mechanisms in Eukaryotic Cells*, Edited by E. Kun and S. Grisolia, John Wiley and Sons, Inc., Interscience Publishers, New York 1972.
3. C. Walter, *Steady-state Applications in Enzyme Kinetics*, Ronald Press Publishers, New York, 1965.
4. O. Zaborisky, *Immobilized Enzymes*, Edited by R. Weast, Chemical Rubber Company Press Publishers, Cleveland, 1973.
5. Report of the Commission on Enzymes of the International Union of Biochemistry, Pergamon Press Publishers, London, 1961.

6. J. Botts, *Trans. Faraday Soc.* 54, 593 (1958).
7. C. Walter, *Proc Biophys. Soc.* 14, 120a (1970).
8. E. King and C. Altman, *J. Phys. Chem.* 60, 1375 (1956).
9. For a description of this program, see reference 1, Chapter 4.
10. D. Fisher and A. Schultz, *Math. Biosciences* 4, 189 (1969).
11. A. Schultz and D. Fisher, *Canadian J. Biol. Chem.* 47, 889 (1968).
12. P. Yates and A. Pardee, *J. Biol. Chem.*, 221 (1956).
13. A. Ghosh and B. Chance, *Biochem. Biophys. Res. Commun.* 16, 174 (1964).
14. J. Higgins, *Proc. Natl. Acad. Sci.* 51, 989 (1964).
15. C. Walter, *Biophys. J.* 9, 863 (1969).
16. G. Viniestra-Gonzalez and H. Martinez, *Proc. Biophys. Soc.* 13, 210A (1969).
17. C. Walter, *J. Theor. Biol.* 23, 23 (1969).
18. C. Walter, *J. Theor. Biol.* 23, 39 (1969).
19. C. Walter, *J. Theor. Biol.* 27, 259 (1970).
20. C. Walter, *J. Theor. Biol.* 44, 219 (1974).

BOOK REVIEW

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processes and the treatment is thorough and complete and certainly not elementary. The fundamentals are covered in 5 chapters in Section I and then in Section II two chapters are devoted to modeling and rate process fundamentals followed by 5 chapters on adsorption, distillation and extraction. In these chapters several different models are proposed and then selected ones are used to model actual field results for industrial columns. For example a packed distillation column 34 ft. high and containing 9260 lbs. of Pall Rings is modeled in detail as is a packed extractor 72 ft. high and 5 ft. in diameter handling 12,000 barrels of kerosene per day. There are other examples for plate towers. The results appear to be uniformly good. Calculated and experimental product compositions agree well over wide variations in the input parameters.

The book should be useful in a senior or graduate level design course. There are numerous problems and plenty of references. If very much use was to be made of the techniques, access to a computer would be needed for solving sets of equations for separations involving many components and many plates. The book should also be useful to industrial designers although I would think that most would already be familiar with the methods in this book since the techniques have been published in various journals and theses. □