A Course in

ENZYME AND BIOCHEMICAL ENGINEERING

L. L. TAVLARIDES
Illinois Institute of Technology
Chicago, Illinois 60616

THE CURRENT INTENSE interest in novel methods of enzyme applications in the food, pharmaceutical, biomedical and waste treatment processes obviated the need to augment the food technology program in our department with a graduate level course in Enzyme and Biochemical Engineering. The title implies all engineering aspects; however, the essence of the course focused upon kinetics and reactor design with emphasis on immobilized enzyme systems. The course was structured to expose the graduate student and researcher to basic concepts, methodologies, and techniques in enzyme technology which would permit rational design and analysis of immobilized enzyme reactor systems and fermentor reactor design.

I. Enzyme Structure, Kinetic Action, Preparation and Immobilization and II. Enzyme and Biological Reactor Design. An attempt is made to develop an appreciation of how enzymes function, the sensitive and specific nature of enzymes and the immobilization methods recently developed which promise to make enzyme utilization in large scale process feasible. (see Table I).

The course is presented towards a first level graduate chemical engineering student with undergraduate transport phenomena, reaction engineering and mathematics through partial differential equations desirable. Preferably the student should have a background in biology and/or biochemistry. Advanced level biology and biochemistry students fare reasonably well but deficiencies in chemical engineering and mathematics courses made aspects of the second part of the course disconcerting.

Several problem assignments and a term paper with an oral presentation were the student requirements. Readings in the various topics were encouraged.

Lawrence L. Tavlarides received his B.S. (1963), M.S. (1964) and Ph.D. (1968) degrees in Chemical Engineering at the University of Pittsburgh. Several years of industrial experience were gained with Gulf Research and Development Company. He pursued postdoctoral research studies at the Technische Hogeschool in Delft Holland for one year prior to joining the Chemical Engineering Department at Illinois Institute of Technology in 1969 as an Assistant Professor. His research and teaching interests include enzyme kinetics, reactor analysis and transport phenomena and mixing effects in dispersions.

DISCUSSION OF COURSE MATERIAL

Enzyme Structure, Kinetic Action, Preparation and Immobilization

THE FIRST THREE sections of Part I introduces the student to the biochemistry of enzymes, the classes of reactions which enzymes catalyze and the kinetic mechanism postulated to describe the enzyme action. The biochemistry of proteins is discussed starting with the amino acids and how enzyme specificity is determined by

TABLE I

Enzyme and Biochemical Reaction Engineering Course Outline

Part I.	Enzyme Structure, Kinetic Action, Preparation and Immobilization
A.	Structure of Enzymes
B.	Classes of Enzyme Reactions
C.	Enzyme Kinetics
D.	Enzyme Production
E.	Enzyme Isolation and Purification
F.	Enzyme Immobilization Methods
Part II.	Enzyme and Biological Reactor Design
A.	Ideal Batch, Tubular and CSTR Reactors
B.	Ideal Reactor Concepts with Enzyme Kinetics
C.	Fermentation Kinetics and Reactor Design
D.	Physical and Chemical Rate Processes in Heterogenous Immobilized Enzyme Systems
E.	Diffusional Influences in Hollow Fiber Catalysts
F.	Immobilized Enzyme Deactivation and Parameter Determination
G.	Design of Immobilized Enzyme Reactors
Part III.	Student Presentation of Term Papers.

its particular sequence of amino acid residues and higher order structure. The primary, secondary, tertiary and quaternary structures of proteins are discussed with some detail given to the geometry of the peptide bond, α-helix and pleated sheet structures, and the various types of bonds which determine higher order structures.

Classes of enzyme reactions such as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases are then presented. Appropriate time is devoted to enzyme kinetics. Michaelis-Menten theory of enzyme substrates complex is presented and then applied to derive the reaction velocities for competitive, noncompetitive, substrate and product inhibition kinetics. Temperature, pH effects and enzyme inactivation effects are delineated. Methods of the determination of rate coefficients are illustrated. Examples of starch hydrolysis, glucose isomerization, and lypase glycerolysis are employed to indicate enzyme kinetics of current interest. Various references (1-8) were helpful in the preparation of the material.

Methods of enzyme production, isolation and purification then followed. Examples of the various plant, animal and micro-organism sources of enzymes were presented with specific attention given to the last source. Specific examples (9-11) illustrated how optimum yields were obtained in these fermentations. Isolation and purification was presented in three stages of (a) cell removal, disruption or extraction, (b) initial fractionation techniques, and (c) high resolution techniques (see Table II). Adequate references exist (12-30) which delineate specific aspects and entire enzyme production schemes.

Immobilized enzymes was the last section discussed in Part I. Excellent reviews are available (31-36). The methods discussed were covalent attachment to water insoluble supports, covalent intermolecular crosslinking, adsorption, containment within devices and entrapment with crosslinking polymers.

Enzyme and Biological Reactor Design

Material and energy balances for ideal homogeneous batch, CSTR and plug flow reactors with enzyme and fermentation kinetics are presented in the first three sections. Michaelis-Menten kinetics with and without substrate inhibition are employed. Effects of nonideal flow and possibilities of multiple steady states for substrate inhibition kinetics are introduced. The Monad model for fermentation kinetics is presented. Batch and continuous fermentations are discussed with some attention to washout phenomena, multistaged reactors, nonideal flow and micro-mixing effects. Models of hydrocarbon fermentation are presented which consider microbial sorption to/from droplets, growth on the droplet surface and within broth, droplet size distribution and mixing, and oxygen absorption. Chemical reaction engineering texts and various other references were employed (15, 37-42).

TABLE II

Enzyme and Biochemical Reaction Engineering
Enzyme Isolation and Purification

(Subsection E of Part I).
Introductory Comments, Enrichment, Yields, Lab. Results.
Solid-Liquid Separation
- Centrifugation
- Filtration

Disruption of Microorganisms
- Nonmechanical
- Mechanical

Initial Fractionation Procedures
- Salt Precipitation
- Solvent Precipitation

High Resolution Techniques
- Electrophoresis
- Ultrafiltration
- Gel Filtration—Gel Chromatography
- Affinity Chromatography

The course introduces the student to the biochemistry of enzymes and merges the techniques of chemical reactor engineering with immobilized enzyme and biochemical kinetics and exposes methods of reactor design for these systems.

The interaction of chemical and physical rate processes are presented for the single particle. Various limiting cases such as external mass transfer with surface reaction, diffusional resistances and reaction within the particle are discussed and isothermal effectiveness factors are introduced. Diffusional influences in membrane catalysts for planar, cylindrical (hollow fiber) or spherical geometry are also formulated for Michaelis-Menten kinetics. Overall rate expressions for single particles and membranes are formulated. Various references employed are (43-45). To complete the discussion, a formulation of enzyme kinetics with inactivation is presented for various modes of deactivation. Deactivation parameter estimations for various fluid-solid reactor configurations as discussed in Levenspiel (37) are extended to Michaelis-Menten kinetics and examples are presented.

The performance equations are employed with the rate expressions developed to predict conversion for fixed

bed immobilized enzyme reactors, slurry reactors with dispersed immobilized enzyme, and tubular membrane reactors. Modes of reactor operation for deactivating immobilized enzymes to maximize production are discussed for the glucose isomerase reaction. A fixed bed reactor with plug flow of fluids is considered and varying temperature policy (44) or substrate flow rate is employed to maximize yields and/or maintain constant product quality. □

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