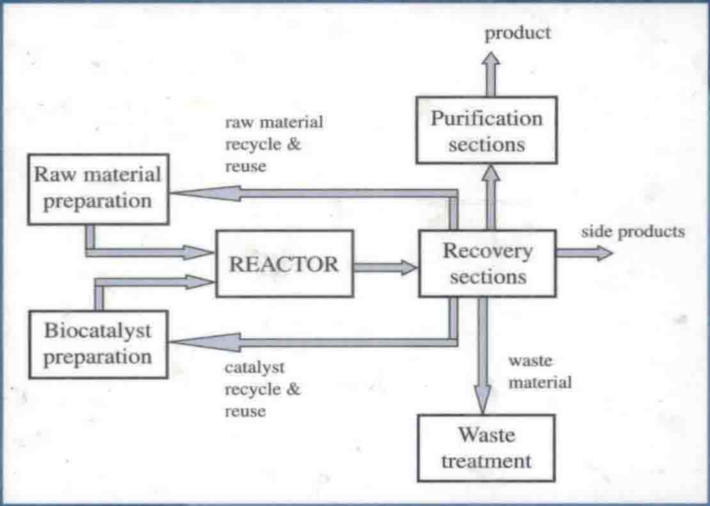
Biotransformations and Bioprocesses



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Biotransformations and Bioprocesses

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Series Introduction

Biotechnology encompasses all the basic and applied sciences as well as the engineering disciplines required to fully exploit our growing knowledge of living systems and bring new or better products to the marketplace. In the era of biotechnology that began with recombinant DNA and cell fusion techniques, methods and processes have developed mostly in service of protein production. That development is documented in this series, which was originally called Bioprocess Technology. Many protein products that are derived from the technology are already marketed and more are on the way.

With the rapid expansion of genomics, many new biological targets will likely be identified, paving the way for the development of an even wider array of products, mostly proteins. As knowledge of the targets develop, so will rational drug design, which in turn may lead to development of small molecules as healthcare products. Rational genetic manipulation of cells as factories for growing products is also developing. Other examples of the application of genomics in health care include the development of gene therapy by insertion of genes into cells and the blocking of gene expression with antisense nucleotides. In such new directions, nucleotides and other small molecules as well as protein products will evolve. Technologies will develop in parallel.

Transgenic technology, in which the genome of an organism is altered by inclusion of foreign genetic material, is also just beginning to develop. Recombinant protein products can already be made, for example, in the milk of transgenic animals, as an alternative to conventional bioreactors. Newer applications for transgenic technology in agriculture may take time to develop, however. Questions continue to be raised about the long-term environmental consequences of such manipulation.

As technology develops in newer as well as established areas, and as knowledge of it becomes available for publishing, it will be documented in this continuing series under the more general series name of Biotechnology and Bioprocessing.

W. Courtney McGregor

Preface

Biotransformation deals with the use of a biocatalyst for the mediation of a chemical reaction, for the synthesis of an organic chemical or destruction of an unwanted chemical. Bioprocess deals with the application of technology and engineering principles to design, develop, and analyze these processes. The tools of the chemical engineer will be essential to the successful exploitation of bioprocesses. Biotransformation is now playing a key role in many industries, including the arenas of food, chiral drugs and vitamins, specialty chemicals, and animal feed stock. The techniques are also finding their way in the manufacturing of bulk and commodity chemicals. The use of enzyme and microbes for chemical transformation and organic synthesis is expected to grow tremendously since the industries are being forced by the public and nongovernmental organizations (NGOs) to shift toward "green chemistry," which will produce less toxic effluents and also use safer and cleaner chemicals in their manufacturing processes. This interdisciplinary book is well suited to address some of these points.

This book is concise yet comprehensive, covering chemistry and engineering aspects of biotransformation and giving an overview of the various steps involved during the transition from a lab to the plant. Although chemical engineers and organic chemists have worked together during process scale-up related to chemical transformation, together they are entering a completely different field. This book will help them overcome some misconceptions. Organic chemists and chemical engineers differ in their approach to problem solving and this book helps each group see the other's point of view. Other topics covered include molecular structure property, enzyme and microbial kinetics, biotransformation, fermentation, reactors (an in-depth analysis of stirred and tower reactors), separation processes, scale-up issues, and waste treatment with industrial examples.

This book is not intended to be an encyclopedia, but covers the current and relevant matter in a succinct way, addressed to an interdisciplinary audience. The book has illustrations, homework problems, and innovative extensions. This approach will encourage students to obtain a more in-depth understanding of key scientific and engineering concepts. It is designed to be a textbook for undergraduate and graduate-level courses in biotechnology (including fermentation) and other interdisciplinary courses in pharmacy, biosciences, and organic synthesis.

A combination of biotransformations and chemical process engineering (such as kinetics, separations, scale-up) is discussed here, and hence the book will appeal to a diverse audience of chemists, biologists, and chemical technologists/engineers. It will be useful for biologists who would like an overview of chemical and engineering principles and to chemical engineers with no knowledge of biotransformations and biochemical engineering fundamentals. The book assumes that engineers have very little background in synthetic chemistry, and therefore builds up the knowledge from the basics. Similarly, the book assumes that organic chemists have very little knowledge in chemical reaction engineering.

The initial chapters start from the fundamentals of chemistry with an introduction to molecules, structures and their relationships, different types of reactions; from small to supra molecules and extended to enzymes and proteins. Later, an in-depth discussion of the mechanism of the reactions catalyzed by enzymes, whole cells, and microbes is presented. The various experimental and analytical techniques that a bio-organic chemist will employ in the lab are also presented. These techniques are very specific to biocatalytic reactions.

The thermodynamics and kinetics of biocatalytic reactions is dealt with in detail. Selection of a suitable reactor for carrying out the desired transformation from a plethora of reactors based on several criteria is discussed, followed by an in-depth design study of two of the most popular reactors (stirred and tower). Since the fermentor has become the workhorse of the biochemical industry, it cannot be ignored. Chapter 9 describes fermentation technology and design of process control strategies.

The underlying reaction engineering and scale-up principles are examined in a detailed discussion that can be viewed as a primer for organic chemists. A manufacturing plant also consists of downstream recovery and purification as well as waste treatment sections. Hence, for the sake of completeness an overview of traditional chemical engineering, special separation techniques, and waste treatment techniques is also included in this book. One chapter deals with a current biochemical industrial scenario with a few process flow sheets for the manufacture of pharmaceutical intermediates and specialty chemicals.

One chapter very briefly describes the frontier research areas in the area of biotransformation (which includes cross-linked enzymes, designer enzymes, abzymes, site selective modification of enzymes, etc.; all aimed toward improving their stability, activity, and specificity). The book does not cover molecular and cell biology, protein engineering, or metabolic pathways.

This book is based on the lecture courses that all three authors have given to undergraduate and postgraduate students of biotechnology, organic chemistry, and food technology over many years. Dr. Kumar is a conventional synthetic organic chemist and Dr. Gaikar is a solid chemical engineer, while Dr. Doble, who is also a chemical engineer, can be viewed as the bridge between the two disciplines, having worked with chemists for two decades.

Mukesh Doble
Anil Kumar
Vilas Gajanan Gaikar

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1

Introduction and Overview

Biotransformation deals with use of natural and recombinant microorganisms (e.g., yeast, fungi, bacteria), enzymes, whole cells, etc., as catalysts in organic synthesis. Biotransformation plays a key role in the area of foodstuff, chiral drug industry, vitamins, specialty chemicals, and animal feed stock (Fig. 1.1). Scaling up a bioprocess from the lab to a commercial scale is challenging and needs several innovations. Nevertheless, more and more industries are moving toward developing processes based on biocatalysis because of their inherent advantages. In the year 2000 biotechnology stocks traded in the Nasdaq exchange outperformed the overall index by 24% (outperformed the Internet stocks by 17%)! This observed general buoyancy is due to the successful applications of biotransformations in the field of pharmaceuticals, environmental bioremediation, textiles, plastics, and agriculture. Biopolymers made from dextrose and plastics made from corn sugar beet and other biomass compete with polymers made from hydrocarbons. Breakthroughs in the area of optimization, reactor design, separation techniques, and molecular modeling are a few of the underlying reasons for these successes.

The design and operation of industrial reactors for bioprocesses are inherently different from the conventional reactors. This book deals not only with how a biocatalyst could be used for synthesis of an organic molecule but also with the steps involved in the scale-up of a process from the bench scale to

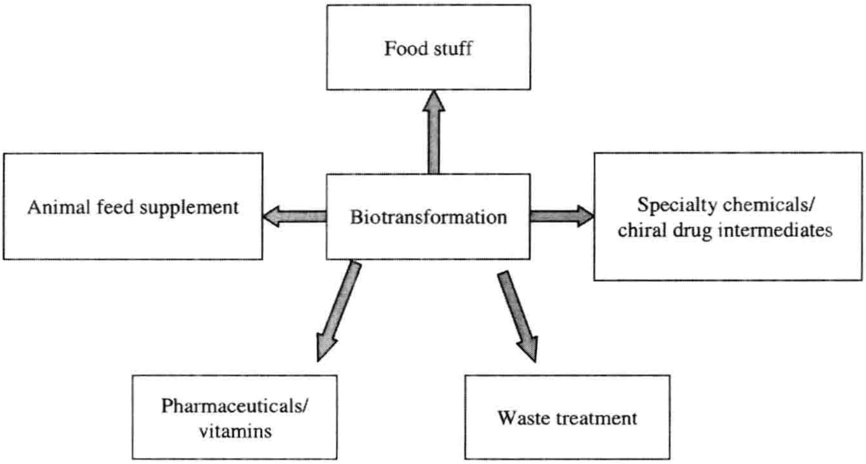

FIGURE 1.1 Role of biotransformation.

the full commercial scale and the reaction engineering aspects of the manufacturing technology, with an in-depth analysis of bioreactor design.

As shown in Fig. 1.2 the field of biotransformation and bioprocess is interdisciplinary in nature. As the process moves from the lab scale to full-scale commercial production, it requires the expertise of biochemist, molecular biologist, synthetic chemist, physical chemist, biotechnologist, and chemical and instrument engineers. All the aspects listed in Fig. 1.2 are dealt in the various chapters of this book. At times the process as it is scaled up may have to go back to the lab because of issues not foreseen earlier.

A biochemical process generally consists of five sections; they are catalyst and raw material preparation, reaction, biocell recovery for reuse or destruction, product recovery and purification, and waste disposal (Fig. 1.3). The book is divided into four parts. The first part deals with the fundamentals, namely chemistry of biotransformation and the associated areas such as synthetic chemistry, enzyme chemistry, frontiers in biotransformations, and enzyme and biocell kinetics. The second part deals with bioreactors selection, types of bioreactors and their design including fermentors, and aspects of biochemical engineering. The third part touches on the downstream separation techniques, and the fourth part, on industrial examples of biotransformations, waste treatment, and scale-up of bioreactions.

The book is written for practicing biochemists and pharmacists who would like to understand the reaction engineering aspects and to chemical engineers who wish to understand the synthetic techniques and organic chem-

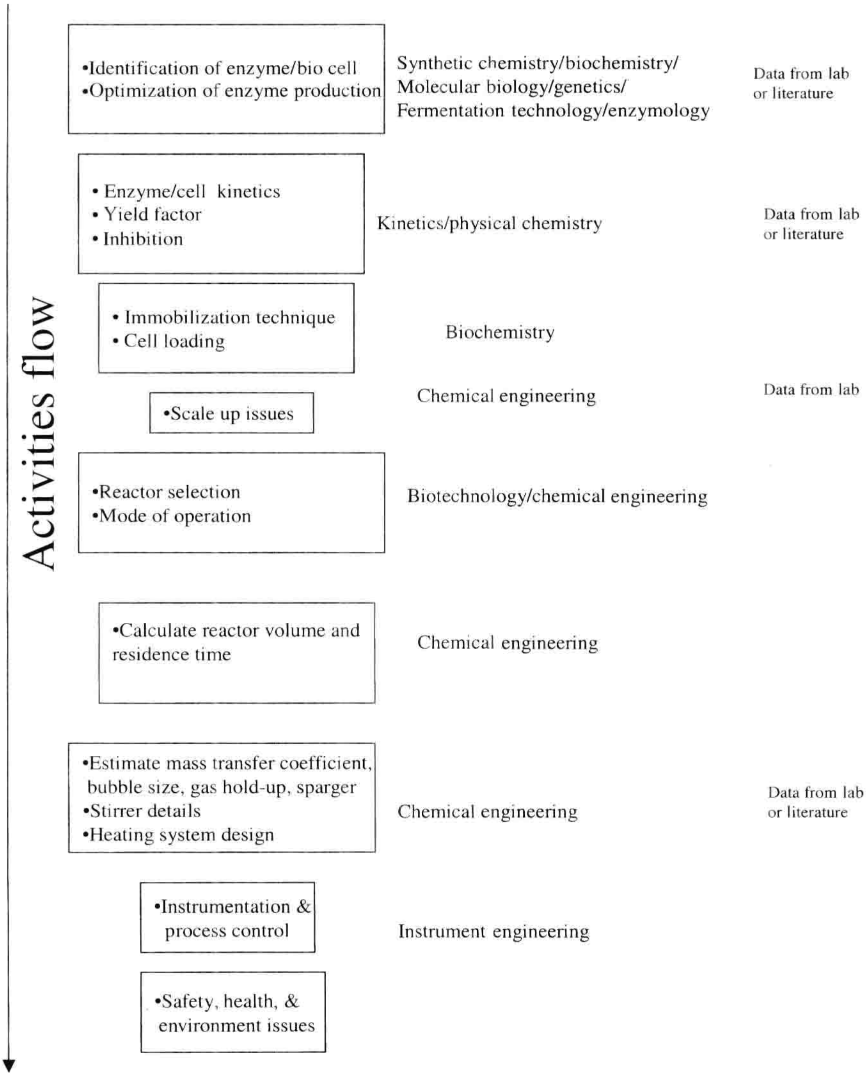

FIGURE 1.2 Biocatalysis and bioprocess: an interdisciplinary field.

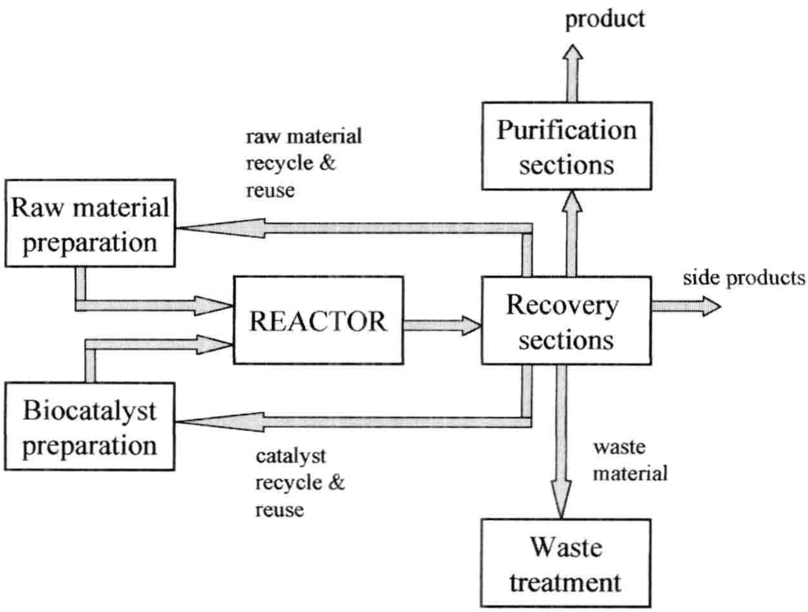

FIGURE 1.3 General flow sheet of a biocatalytic manufacturing process.

istry aspects of this vast field. A large number of problems are given in the end of many of the chapters for students to sharpen their knowledge they would have acquired. This book is not intended to be an encyclopedia for biotransformations or bioreactors, but a ready reference to the practioners relating the science and engineering.

Chapter 2 gives an introduction to molecules, structures and their relationships, quantum mechanical approach, and different types of reactions starting from small to supra molecules. This explanation is then extended to enzymes and proteins.

Chapter 3 describes the structure and activity of enzymes and proteins, differences between enzymes and conventional heterogeneous catalysts and the thermodynamic aspects of the biocatalytic reaction.

Chapter 4 deals in detail with the reactions catalyzed by enzymes, whole cells, and microbes. Mechanistic aspects of these reactions are also discussed.

Chapter 5 deals with various experimental techniques and analytical techniques a bioorganic chemist will employ in the lab. These techniques are very specific to biocatalytic reactions.

Chapter 6 briefly describes the frontier research areas in the area of biotransformation that includes cross-linked enzymes, designer enzymes, abzymes, site-selective modification of enzymes, etc., all aimed toward improving their stability, activity, and specificity.

Chapter 7 deals with enzyme kinetics, inhibition, Michaleis-Menten approach to modeling biocatalytic reactions, and cell growth. Rate equation for different types of reactions is listed.

Chapter 8 deals with biochemical reactor selection, different types of reactor and their salient features. Basic design equations for various types of reactors and Monod equation are also described here.

Chapter 9 deals with fermentation, namely fermentation classification, issues in fermentation, modeling of molds, and four stages of biocell growth. Details of reactor and process control design are also described here.

Chapter 10 gives an overview of reaction engineering principles such as mass and heat transfers and how they are estimated for design purposes.

Chapter 11 deals with stirred bioreactors in detail, since they are used in general because of simple construction and ease of operation. Several correlations for gas-liquid mass transfer that are needed for design are described here.

Chapter 12 gives a detailed analysis of tower bioreactors including the gas, solid, and liquid mixing and heat transfer issues.

Chapter 13 is an introduction to biochemical separation and downstream processing and purification. The various traditional chemical engineering separation processes such as distillation, extraction, filtration, etc., and separations that are very specific to biochemical processes, namely, chromatography, membrane, electrophoresis, etc. are discussed here.

Chapter 14 deals with industrial examples of where biocatalyst is used successfully including chiral synthesis, pharmaceuticals, specialty chemicals, etc. This chapter is an eye opener to chemists and biologists, giving the industrial world scenario.

Chapter 15 deals with in situ and ex situ waste treatment procedures for solid, liquid, and gas. Different types of reactors used in waste treatment are also discussed. The current chemical methods available in waste treatment are also listed with their advantages and disadvantages over the biochemical approach.

Chapter 16 deals with a large number of scale-up rules which need to be followed for successfully translating a process from bench to commercial scale. They relate to mixing, heat transfer, solid suspension, etc. Several scale-up rules are listed depending upon the criteria one would like to select. A list of innovative techniques reported in literature for scaling up biochemical processes is also tabulated.

2

Chemical Bonding, Structure, and Reaction Dynamics

2.1 INTRODUCTION TO CHEMISTRY

Our unquenchable curiosity about our environs and ourselves is at the root of all the scientific endeavors. We marvel at the way the wings of the butterfly are colored, at the fine smell of a jasmine flower, at the gigantic colossus of the Sal tree trunks, and the list goes on endless. All of this myriad variety, we call *nature*, and in fact our own body is an eternal source of wonder and joy, involving intricate mechanisms (transformations) at the atomic, molecular, supramolecular, and the gross levels. Our journey begins, when we try to understand and imitate this infinite variety. How does all this happen? What are its components? Why is it happening only this way and not by any other way? These are some of the queries, that drive us to explore, analyze, understand, and know.

2.1.1 Origins of Organic Chemistry

As one of the tools that fostered an increased understanding of our world, the science of Chemistry—"the study of matter and the changes they undergo at a molecular level"—developed until near the end of eighteenth century. Initially, there was a single branch, but later with the continued studies of Lavoiser, Berzelius, Wohler, and others the three major branches were recognized. One branch was concerned with the matter obtained from natural