Enzyme Technology

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Springer

ENZYME TECHNOLOGY

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Foreword

The generic subject of enzyme technology has a largely industrial and commercial purpose. Consequently it behoves researchers to operate within a culture of awareness of the constraints implicit on industrial practice. These are often reflected in allowable costs leading to the primacy of yield and limitations on the use of recovery, separation and purification processes to achieve saleable product. Because of the nature of the products, the process conditions and the raw materials, industrial safety is a significant feature. Also, as many of the products are used in food and food related applications, product safety is often paramount. Appreciation is also required of the product targets such as formulation, purity, activity, dispense, handling, stability, storage, specificity, safety, uses and conditions of use.

In contrast, the industrial practitioner needs an acute awareness of the underpinning science and the range of practical choices that can be explored to create a process and product, which both exploits the available science and meets the industrial and commercial constraints. Such 'process synthesis' represents one of the most elegant, intellectually challenging and ultimately satisfying achievements of any technology, and together with the desirable product characteristics is often referred to as 'product engineering' to emphasise the primacy of saleable product material as its objective.

In 'Enzyme Technology' the Editors have set out to provide a snap-shot of current practice and research which will assist both the researcher and the industrial technologist meet their respective goals. This has been achieved by providing an extensive basis of industrial enzymology, examples of industrial success covering the production and uses of many industrial enzyme products, and by illustrations of evolving practice. These 'developed areas' are complemented by descriptions of speciality process practices and descriptions of pioneering work concerned with improving the enzymes themselves, using protein engineering and other techniques, for use in industrial conditions with commercial objectives, and in the creation of new diagnostic and therapeutic products.

Enzyme technology is applied by companies, large and small, on a worldwide basis as part of their industrial production, often a very small part. The Editors have brought together this widespread custom and practice through a catholic range of authors, to give a worldview of a fascinating subject. To achieve coherence they have guided the contributors towards a presentational 'template' to provide a balance of information, style and format – and in this they are to be congratulated.

Overall, you will find a text which not only benefits researchers and practitioners but also provides a kaleidoscope of images of enzyme technology for those entering the area.

Bernard Atkinson

Preface

Enzymes are now a material well established in the field of biotechnology. This book covers different aspects related with the enzymes such as the producing microorganisms, their mode of cultivation, downstream processing, industrial production, properties and applications, with a special emphasis on industrial enzymes.

The book comprises 36 chapters written by internationally reputed authors in the field, which are classified and presented in four parts. Part I deals with general and fundamental aspects of enzyme technology and has nine chapters in it. Chapter one gives an overview of subject matter, beginning with the historical developments on the applications of enzymes since ancient times even before when their functions and properties were understood. The chapter provides brief details on the demand and business of enzymes, giving the names of the companies involved in the business, classification and nomenclature system of enzymes, and enzyme action, safety and therapy. Chapter two describes in details the general properties of enzymes such as chemical nature, structure, cofactors, enzyme specificity, measurement and expression of enzyme activity, stability and denaturation and factors affecting enzyme activity such as reaction time, amount of enzyme, substrate concentration, temperature, pH, ionic strength, pressure, inducers and inhibitors. Third chapter in this part is on enzyme kinetics and modeling of enzymatic systems. It provides theories of enzyme kinetics- free and immobilized. It describes alternative formulations of enzyme kinetics such as fractal and virial approaches, reversible enzyme inhibitors and substrate inhibition, and also deals with the mathematical models in enzyme kinetics, citing examples of modeling of enzyme reactions such as dipeptide synthesis, computation analysis of substrate binding, diagnostic tests, etc. The next chapter, i.e. fourth one is also on fundamental aspects dealing with the thermodynamics of enzyme catalyzed reactions. It first provides information on general principles such as thermodynamics of reactions involving specific chemical species and sum of chemical species- biochemical reactions. Then it gives details on what information that thermodynamics provides could be useful for industrial applications. The chapter also discusses experimental methods, physiological tables and cycles and estimation methods and quantum chemical calculations. Chapters fifth and sixth of the book are on biocatalysis; former one is on regeneration of cofactors for enzyme biocatalysis in which authors have discussed the importance and regeneration of various cofactors such as NAD^-, NADH, $NADP^-$, NADPH, ATP, sugar nucleotides, CoA, PAPS, etc for effective biocatalysis. The sixth chapter deals on biocatalysis in organic media. Although enzymes when added in organic solvents may lose their intrinsic activity because of denaturation, dehydration, inhibition, or chemical modification, they have been successfully used in biphasic media containing water and organic solvents. It discusses several related issues with the subject matter and also the potential application of using enzymes in organic solvents such as for kinetic resolution of enantiomers, asymmetric synthesis, peptide synthesis, glycoside synthesis and esterification, etc. Chapter seventh of the book describes the application of knowledge generated as described in the chapters as above for the biotransformation (biocatalysis) using crude enzymes and

whole cells. It discusses information about existing biocatalysts, their genetic modification and screening for novel biocatalysts. It also provides information on general procedures for isolation and selection of microorganisms, biocatalysts treatment and operation, and biotransformation such as asymmetric synthesis and steroids and terpenes biotransformation. The chapter finally discusses specific case studies dealing with biotransformation for flavour compounds such as methyl ketones, alkylpyrazines, etc. Chapter eighth of the book is again on application describing enzymes as tools for the stereospecific carbon-carbon bonds formation in monosaccharides and analog synthesis. It deals with DHAP and pyruvate aldolases, thiamine pyrophosphate dependent enzymes, transketolase, etc. In this chapter a glimpse has also been put on exploring the biodiversity to find new catalysts. The ninth and last chapter of this part describes enzymes engineered for new reactions, aiming at novel catalysis for organic synthesis. Enzyme-catalyzed synthesis in a non-aqueous reaction medium is a standard synthetic tool in chemistry. Biotechnological opportunities offer unique opportunity in redesigning and modifying enzymes for a targeted application. The chapter discusses protein engineering strategies to attain these.

Part II of the book has fifteen chapters, which provide information about specific industrial enzymes such as alpha amylase, glucoamylase, glucose isomerase, cellulase, pectinase, lipase, protease, xylanase, inulinase, phytase, tannase, peroxidases, chitinase, invertases and mannanases. Each of the chapter provides state-of-art information for specific enzyme, its sources such as plant, animal and microbial and then discusses details about microbial sources, production methods and strategies, purification and characterization. Each chapter also provides details on how to assay the enzymes using different methods.

Part III of the book is on bioreactors, downstream processing and applications of enzymes and has five chapters. First chapter in part, i.e. 25th chapter of the book describes bioreactor analysis and application for enzyme production and enzymatic processes. It discusses major types of the bioreactors (fermenters) used in submerged fermentation and solid-state fermentation. It also discusses operating parameters such as power consumption, mixing, shear stress, equilibrium phases, mass transfer, etc. Next chapter in this part is on isolation and purification of the enzymes suing any kind of bioreactor or fermentation mode/system. It describes processes of filtration and centrifugation for the removal of insolubles, extraction and purification for the solubles by ultrafiltration, liquid-liquid extraction, and recovery and purification of intracellular products. It provides great deals of chromatographic techniques for the purification of enzymes. The next chapter, *viz.* Chapter 27 is on industrial applications of enzymes, which provides details on enzymes production and markets such as in food industry, feed industry, paper and pulp industry, textiles and leather industry, detergents, personal care, energy (fuel alcohol), industrial waste treatment, etc. Chapter 28 of the book is on the immobilized enzymes for different purposes. It describes the properties of enzymes influenced by immobilization, principles, methods and examples of immobilization and chemical coupling of enzymes. The last chapter in this part is on protein engineering of industrial enzymes. It describes several industrial enzymes, ration design methods such as site-directed mutagenesis, and other random methods for their improvements.

The fourth and last part of the book deals with specific enzymes and their applications and has seven chapters, out of which five chapters are on thermozymes, cold-adapted enzymes, ribozymes, hybrid enzymes, diagnostic enzymes and therapeutic enzymes. Each of these chapters provides details on the microbial sources and application of the specific enzyme, their production and properties. The last chapter of the part and book, i.e. Chapter 36 is on inteins: enzyme generating protein splicing. It provides details on protein splicing pathway, control and applications, and inteins function and evolution.

We thank authors of all the articles for their cooperation and also for their preparedness in revising the manuscripts in a time-framed manner. We also acknowledge the help from the reviewers, who in spite of their busy professional activities, helped us by evaluating the manuscripts and gave their critical inputs to refine and improve the articles. We warmly thank Mr. NK Muraleedharan and the team of Asiatech Publishers, Inc. for their cooperation and strong efforts in producing this book.

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Contents

General Introduction

Ashok Pandey and Sumitra Ramachandran

1. INTRODUCTION

Enzymes are natural catalysts, which permit endogenous biological reactions to occur rapidly through well-defined pathways. They accelerate the rate of reactions, without being lost in the process. They occur in almost all creatures of the nature, from a minute microorganism to well-advanced human beings. They are located in the cells, cytoplasm, mitochondria, tissues, body fluids, etc. Enzymes are composed of one or more polypeptides organized in a specific three-dimensional structure. The efficiency of an enzyme's activity is often measured by the turnover rate, which measures the number of molecules of compound upon which the enzyme works per molecule of enzyme per second. Carbonic anhydrase, which removes carbon dioxide from the blood by binding it to water, has a turnover rate of 10^6. That means that one molecule of the enzyme can cause a million molecules of carbon dioxide to react in one second (Bell & Bell, 1988). Some of the outstanding features of the enzyme include high substrate specificity, specificity in promoting only one biochemical reaction with their substrate ensuring synthesis of a specific biomolecular product without the concomitant production of byproducts, stereospecificity and regiospecificity, which they express in catalysis. Enzymatic reactions occur within a narrow temperature range and an optimal pH. Effective catalysis also depends crucially upon maintenance of the molecule's elaborate three-dimensional structure. Any change occurring in the crucial factors such as pH or temperature may result in loss of structural integrity, which could lead to a loss of enzymatic activity.

Many enzymes require the presence of certain non-protein compounds for their action which helps in accelerating the enzyme action. These non-protein components tightly bound to the protein are called prosthetic groups. On the other hand, if the non-protein compounds are not firmly attached to the enzyme protein, but exist in free state in the solution contacting the enzyme protein only at the instant of enzyme action, they are called co-enzymes. Prosthetic groups usually are located in the active site of an enzyme molecule where catalytic events take place. It is also the place where the substrate and coenzymes bind just before reaction takes place. The entire enzyme system consisting of the enzyme protein and the coenzymes or prosthetic group is called the holoenzyme and the protein portion is sometimes called apoenzyme.

2. HISTORICAL DEVELOPMENTS

Enzymes have been exploited since ancient period, long before their functions and properties were understood. Only at the beginning of 19th century, the potential of enzymes in the process of fermentation