Handbook of
Enzyme Biotechnology

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Handbook of
ENZYME BIOTECHNOLOGY

Foreword

Professor D. V. W. Parke, Head of Biochemistry Department,
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Paralleling the scientific advances of the 19th century in chemistry, with its consequent innovations for chemical industry in the production of dyestuffs, plastics, synthetic fibres and drugs, has been the rapid growth of biochemistry of the 20th century with the utilization of enzymes in the service of industry, society and medicine. Incredible as it may seem to the biochemist or biology technologists of today, the very existence of enzymes, the 'ferments of Bunsen', were still being questioned at the start of this century and, with the exception of the enzymes of mammalian digestion, little was known of their mode of action even as recently as twenty-five years ago. The current explosive growth of knowledge in the various fields of biochemistry has profoundly changed this scene to place the enzyme at the very origin of all living processes, and the recent marriage of biology with chemical technology has yielded its first progeny, the esoterically creative, versatile and rapidly developing, enzyme biotechnology. The circumstances of the birth of this new industry were no doubt highly favoured. The fermentation industries, based on centuries of craft experience in brewing, cheesemaking and similar trades, were stimulated by the need in the 1940s to produce antibiotics for medical treatment and developed the bulk cultivation of micro-organisms, the major source of so many of the enzymes used industrially.

The result has been the large-scale production, purification and stabilization of enzymes from animal, plant, but increasingly, microbial sources, and their widespread usage in food production, detergents, medicines, textile- and paper-manufacture. The use of isolated enzymes instead of the intact micro-organisms and living tissues has the advantages of increased specificity of chemical reaction, giving the desired product with the maximum of control over quality and yields, and the minimum of undesirable side-products. Immobilization of these enzymes on inert matrix supports, and the use of enzyme reactors, has further advanced this technology to extend the life and activity of the enzyme and progress towards continuous processes. The uses of these enzyme preparations in brewing, bread-making, cheese-making, meat-tenderizing, in textile-desizing, leather- and paper-making, and in 'biological' detergents, are now commonplace. In the field of medicine, the use of enzymes, 'insolubilized' enzymes, and enzyme electrodes is enabling the analyst and clinical biochemist to keep pace with the ever-increasing number of analyses required by modern medical diagnosis and treatment. More recent developments include the use of enzymes in the treatment of disease, with lysozyme being prescribed as an antibacterial agent, asparaginase as an anticancer therapy, and most recently, hyaluronidase and β-glucuronidase for the treatment of cardiovascular disease.

In such a rapidly expanding and successful technology future developments will be legion and, although suggestions as to their nature must be speculative and biased by individual experience and preferences, future needs will include application to the production of new food materials, to the processing of sewage and effluents to degrade and remove toxic chemicals such as pesticide residues, drugs and detergents, and to uses in the wider treatment of disease, especially those metabolic errors due to genetic aberrations.

The need for a publication in this field to lay the foundation of the scientific principles involved and to review the present state of the art, has been widely acknowledged. Dr Wiseman's Handbook of *Enzyme Biotechnology* provides precisely these needs, since the first part of his book is devoted to the principles of enzyme production and usage, and the second part to a compilation of data on these industrial enzymes, the details of their production, purification and immobilization, and of industrial enzyme practices. The inspiration and incentive for the creation of this volume have arisen, at least in part, from the very successful series of short courses on Enzyme and Fermentation Biotechnology held at the University of Surrey over the past four years. The many expert contributors to the book have also participated in the lecturing and teaching of these courses and several of the industrial companies who have helped with the courses have also made detailed information available which is included in part two of the book. In such forward-looking ventures Dr A. Wiseman and Dr B. Gould are to be highly complimented for their organization of these interesting and valuable courses, which have attracted—and I am sure will continue to attract—so many enthusiastic participants both from the United Kingdom and overseas. Dr Wiseman is to be further congratulated on the production of this excellent treatise which, in addition to serving as a permanent record of the teaching of these courses, provides a valuable introduction and source of reference to the many who are employed in this technology and the many more, especially students, who may become interested in the industrial applications of biochemistry.

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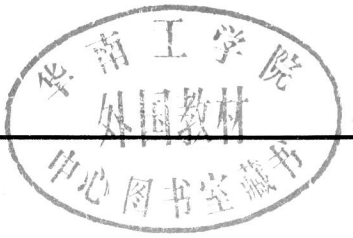

PART II:
DATA FOR INDUSTRIAL APPLICATION OF ENZYMES

Part I

PRINCIPLES OF ENZYME PRODUCTION AND UTILIZATION

PART I: CHAPTER 1

Introduction to Principles

Dr ALAN WISEMAN, Department of Biochemistry, University of Surrey

Part I of this book is intended to convey the scientific principles, chemical, biochemical, biological mainly microbiological, and engineering that constitute the theory of enzyme biotechnology. Each of these subjects contributes to the understanding of enzyme action, and practice as seen in the food industry, including brewing, and many other industries, including pharmaceutical, medical, textile, paper and pollution control. For enzyme utilization we need to consider substrate structures and enzyme mechanisms (chemistry), enzyme kinetics and stability (biochemistry), enzyme production (microbiological or biological along with engineering) and enzyme reactors (engineering).

There is not the usual emphasis on classical enzyme kinetics once the borderline into industrial utilization of enzymes has been crossed. In processing, as distinct from research and development, the main interest is clearly in obtaining an enzyme cheap enough for the job required (counting also the cost of cofactor)—and not impure enough to ruin the process. Indeed it may be that the contaminating activity is useful, such as with the β-glucanase in *B. Subtilis* mixed amylase and protease preparations. β-glucanase, to lower the viscosity of wort or beer, is an added advantage of using such bacterial exoenzymes.

There is a very definite interest in the heat stability, in the presence of substrate preferably, of the activity required of the commercial enzyme product. This is usually so that it may be used at a reasonable temperature where its action is fast, but is not rapidly lost. Also, it is usually necessary to destroy the enzymes present by heating after the desired conversion has occurred. In dough processing however, it is essential that heat labile α-amylase (fungal) is used and not heat stable α-amylase (bacterial)—otherwise the action continues too long during baking and spoils the product.

All applications depend on using a suitable pH for the enzyme employed, and using a suitable quantity of the enzyme purchased. This can sometimes be decided from the activity quoted by the manufacturers. Quick assay or accurate assay methods may both be quite suitable to decide this—but often the assay results are quoted in non-standard ways. Kinetics under working conditions are of most interest, even if usually meaningless for academic studies (Chapter 2)

Large-scale production of enzymes is usually achieved by fermentation techniques so that appropriate micro-organisms may produce the desired exoenzyme, most efficiently. Some enzymes must still be extracted from animal tissues (rennin, trypsin, chymotrypsin, pepsin) and plants (papain, bromelain, ficin). Microbial substitutes are being sought for most of these. Selections of micro-

organisms by genetic manipulation has improved enzyme yields and sometimes produced more useful enzymes. The amount of enzyme produced by a particular strain of micro-organisms can of course be increased by growth conditions and also by induction, e.g. maltase, by maltose, in yeast or by lifting of catabolite repression in this case by excluding glucose from media. Derepression of enzymes involved in synthetic pathways, e.g. for amino acid production, can also be achieved by excluding the end product (amino acid) of the particular pathway (Chapter 3). Microbial strains may also be selected that are constituitive for the required enzymes and are not affected by repression.

Purification of enzymes, sometimes with particular application in mind, e.g. clinical for asparaginase, is of great importance. Nevertheless, most commercial enzymes are very impure indeed when judged upon recognition of contaminating activities. Large-scale applications involve many scale-up problems however, e.g. in column chromatography (Chapter 4).

Most recent interest has centred around the preparation and application of immobilized forms of enzymes (Chapter 5), and many of the present industrial applications of enzymes (Chapter 6) may prove amenable to immobilized enzyme techniques, involving stirred tanks or fixed beds, e.g. columns. Enzymes for therapeutic use will be used in gelatin microcapsulated form for replacement and other therapy.

PART I: CHAPTER 2

Principles of Industrial Enzymology

Dr B. J. GOULD, Department of Biochemistry, University of Surrey

2.1 Introduction

Enzymes can be used in several industries (Reed, 1966). These uses are determined by specificity, stability, activity, availability, and cost. Later sections of this chapter consider the first three of these factors. The fourth, together with relevant information on enzymic properties, comprises the major section of Chapter 2 in Part II. The cost of using enzymes is not considered directly; however, the use of appropriate enzyme assays to avoid the use of excess enzyme in industrial applications is discussed.

Relatively small quantities of enzyme are normally required for an industrial process because of the tremendous efficiency of enzymes, e.g. for a typical enzyme, each molecule catalyses between 100 and 1,000 reactions every second. It is known that enzymes achieve this great increase in reaction rate by reducing the energy of activation required before a molecule of substrate can react. But the way in which this is achieved is not understood for any particular enzyme. However, several possibilities are being considered with the hope that, sometime in the future chemical catalysts having the desirable properties of enzymes, i.e. efficiency and specificity, will be made, which are also considerably more stable than enzymes.

2.2 Enzyme Specificity

This term refers to the fact that enzymes only catalyse the reaction of a limited range of compounds, i.e. an enzyme catalyses the conversion of substrates to products. This characteristic property of enzymes is the basis of the classification scheme of enzymes *Recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry* on Enzyme Nomenclature. These recommendations are followed in Part II, Chapter 2 of this book for classifying the enzymes, but a brief summary is appropriate here.

There are three general principles. Firstly, the suffix -ase implies an enzyme. Secondly, the enzymes are classified and named according to the reaction they catalyse, since this is the characteristic property which distinguishes one enzyme from another. And thirdly, enzymes are divided into groups on the basis of the type of reaction catalysed.

This system works well for the vast majority of the 1,770 purified enzymes and for some industrially important enzymes such as glucose oxidase and