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Handbook of Enzyme Biotechnology

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E7951643



ELLIS HORWOOD LIMITED

Publisher, Chichester

Halsted Press . a division of

JOHN WILEY & SONS Inc.

New York • London • Sydney • Toronto

First published 1975 by
ELLIS HORWOOD LIMITED
Coll House, Westergate, Chichester, Sussex, England

DISTRIBUTED IN

Australia, New Zealand, South-east Asia by
JOHN WILEY & SONS AUSTRALASIA PTY LIMITED
110 Alexander Street, Crow's Nest, N.S.W. Australia

Europe, Africa by
JOHN WILEY & SONS LIMITED
Baffins Lane, Chichester, Sussex, England

N. & S. America and the rest of the world by
Halsted Press a division of
JOHN WILEY & SONS INC.
605 Third Avenue, New York, N.Y. 10016, U.S.A.

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Library of Congress Cataloging in Publication Data

Wiseman, Alan, comp.
Handbook of enzyme biotechnology.

1. Enzymes—Industrial applications. 2. Biochemical engineering. I. Title. [DNLN: 1. Enzymes—Chemical synthesis. QU135 W814h]
TP248.E5W58 1975 661'.8 75-2466
ISBN 0-470-95617-8 (Halsted Press)
ISBN (Ellis Horwood) 85312 023 4

Printed in England by Butler & Tanner Ltd,
Frome, Somerset

Handbook of
ENZYME BIOTECHNOLOGY

Foreword

Professor D. V. W. Parke, Head of Biochemistry Department,
University of Surrey

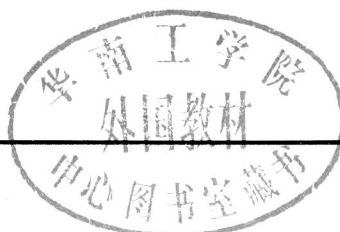
Paralleling the scientific advances of the 19th century in chemistry, with its consequent innovations for chemical industry in the production of dyestuffs, plastics, synthetic fibres and drugs, has been the rapid growth of biochemistry of the 20th century with the utilization of enzymes in the service 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.

Table of Contents



Foreword	PROFESSOR D. V. PARKE Department of Biochemistry, University of Surrey	v
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PART I: PRINCIPLES OF ENZYME PRODUCTION AND UTILIZATION

Chapter 1	INTRODUCTION TO PRINCIPLES DR ALAN WISEMAN Department of Biochemistry, University of Surrey	3
Chapter 2	PRINCIPLES OF INDUSTRIAL ENZYMOLOGY DR B. J. GOULD Department of Biochemistry, University of Surrey	5
	2.1 Introduction	5
	2.2 Enzyme Specificity	8
	2.3 Enzyme Stability	8
	2.3.1 Storage Stability	8
	2.3.2 Stability in Use	8
	2.4 Enzyme Activity	10
	2.4.1 Enzyme and Substrate Concentration	10
	2.4.2 Reaction Time	11
	2.4.3 Substrate Concentration	12
	2.4.4 Inhibitors	12
	2.4.5 Cofactors	15
	2.4.6 Allosteric Enzymes	16
	2.4.7 Ionic Strength	16
	2.4.8 pH, Temperature and Enzyme Stability	17
	2.5 Enzyme Assay	18
	2.6 Enzyme Reactors	20
	2.7 Enzymes for Analysis	22
	2.8 Summary	24
	References	24
Chapter 3	LARGE-SCALE PRODUCTION OF ENZYMES: TECHNIQUES IN FERMENTATION DR W. A. VINCENT and DR G. PRIESTLEY Western Biological Equipment Ltd, Sherborne, Dorset	27
	3.1 Sources of Enzymes	28
	3.2 Production of Enzymes	29
	3.3 Environmental Factors affecting Enzyme Production	30
	3.3.1 Mixing and Aeration	31
	3.3.2 Measurement of Fermentation Parameters	39
	3.3.3 Control of Fermentation Parameters	43
	3.3.4 Fermentation Equipment	49
	References	54

Chapter 4	LARGE-SCALE EXTRACTION AND PURIFICATION OF ENZYMES	58
	DR J. MELLING and B. W. PHILLIPS	
	Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire	
	4.1 Introduction	58
	4.2 Extraction by Chemical Methods	59
	4.2.1 Alkali	59
	4.2.2 Lysozyme and EDTA	59
	4.2.3 Detergents	59
	4.2.4 Cold Shock	60
	4.2.5 Osmotic Shock	60
	4.3 Extraction by Physical Methods	61
	4.3.1 Sonication	61
	4.3.2 Freezing and Thawing	61
	4.3.3 Solid Shear	62
	4.3.4 Grinding or Agitation with Abrasives	62
	4.3.5 Liquid Shear	63
	4.4 Isolation and Purification	64
	4.4.1 Nucleic Acid Removal	64
	4.4.2 Concentration by Precipitation	67
	4.4.3 Concentration by Ultrafiltration	68
	4.4.4 Concentration by Freeze-drying	71
	4.4.5 Gel Chromatography	72
	4.4.6 Ion Exchange Chromatography	75
	4.4.7 Affinity Chromatography	77
	4.4.8 Non-Specific Adsorbents	79
	4.4.9 Electrophoretic Techniques	81
	References	83
Chapter 5	PRINCIPLES OF IMMOBILIZED-ENZYME TECHNOLOGY	89
	PROFESSOR S. A. BARKER and Dr I. KAY	
	Department of Chemistry, University of Birmingham	
	5.1 Outline of Preparation Techniques	89
	5.1.1 Adsorption	89
	5.1.2 Occlusion	90
	5.1.3 Cross-linking	91
	5.1.4 Covalent Binding	92
	5.2 Choice of Immobilization Method	94
	5.3 Choice of Enzyme Reactor	96
	5.4 Properties of Immobilized Enzymes	99
	5.4.1 Stability	99
	5.4.2 Kinetic Properties	100
	5.5 Applications of Immobilized Enzymes	101
	5.5.1 Analysis	102
	5.5.2 Preparative Uses	104
	5.5.3 Food Industry	104
	5.5.4 Medical Uses	105
	5.5.5 Membrane Bound Enzymes as Model Systems	105
	References	106

Table of Contents

ix

Chapter 6	ENZYME UTILIZATION IN INDUSTRIAL PROCESSES	111
	DR ALAN WISEMAN	
	Department of Biochemistry, University of Surrey	
6.1	Theoretical Considerations	111
6.1.1	Advantages of Using Enzymes	111
6.1.2	Criteria for Enzymic Treatment	111
6.1.3	Choice of Enzyme, and its Control	112
	Brief Table of Classification	113
6.2	Utilization of Carbohydrases	114
6.2.1	α -Amylase (Fungal)	114
6.2.2	α -Amylase (Bacterial)	115
6.2.3	α -Amylase (Cereal)	116
6.2.4	Amyloglucosidase (Glucamylase)	116
6.2.5	Pectinases	116
6.2.6	Cellulases	117
6.2.7	Hemicellulases (including β -Glucanase)	118
6.2.8	Invertase	118
6.2.9	Lactase (β -Galactosidase)	118
6.2.10	Dextranase	119
6.2.11	Lysozyme	119
6.3	Utilization of Proteases	119
6.3.1	Papain	119
6.3.2	Trypsin (and Chymotrypsin)	119
6.3.3	Pepsin	120
6.3.4	Rennin	120
6.3.5	Fungal Proteases	120
6.3.6	Bacterial Proteases	121
6.4	Utilization of Other Hydrolases	121
6.4.1	Lipases	121
6.4.2	Penicillin Acylase (also called Amidase)	122
6.5	Utilization of Oxidoreductases	122
6.5.1	Glucose Oxidase	122
6.5.2	Catalase	122
6.5.3	Lipoxidase	123
6.6	Utilization of other Enzymes	123
6.6.1	Glucose Isomerase	123
	References	123

PART II:

DATA FOR INDUSTRIAL APPLICATION OF ENZYMES

Chapter 1	INTRODUCTION TO DATA	127
	DR ALAN WISEMAN	
	Department of Biochemistry, University of Surrey	
Chapter 2	ENZYME DATA	128
	DR B. J. GOULD	
	Department of Biochemistry, University of Surrey	
2.1	Collection of Data	128
2.2	Comments on Industrial Enzymes Data	128
2.3	Comments on Research and Analytical Enzymes Data	131
	References	160

Chapter 3	FERMENTATION DATA AND EQUIPMENT (AND THEIR MANUFACTURERS)	163
	DR W. A. VINCENT and DR G. PRIESTLEY Western Biological Equipment Ltd, Sherborne, Dorset	
3.1	Fermenters	163
3.1.1	Construction of a Laboratory Fermenter	164
	Manufacturers of Fermenters	164
3.2	Large-Scale Processes for the Production of Enzymes	167
3.3	Dissolved Oxygen Measurement and Control	167
3.3.1	Electrodes	167
3.3.2	Meters and Controllers	167
3.4	pH Measurement and Control	168
3.4.1	Electrodes	168
3.4.2	Meters and Controllers	168
3.5	Temperature Measurement and Control	169
3.5.1	Thermistors	169
3.5.2	Resistance Bulbs	169
3.5.3	Thermocouples	169
3.5.4	Meters and Controllers	169
3.5.5	Recorders and Data Printers	170
3.6	Other Equipment	170
3.6.1	Indicators and Indicating Controllers (general)	170
3.6.2	Air Filters	170
3.6.3	Connectors	171
3.6.4	Couplings (Stirrer Shaft)	171
3.6.5	Flowmeters	171
3.6.6	Metering Pumps	171
3.6.7	Mixers	171
3.6.8	Motors and Tachogenerators	171
3.6.9	Motor Speed Controllers	172
3.6.10	Oxygen (gas) Analysers	172
3.6.11	Plastics	172
3.6.12	Pressure Gauges	172
3.6.13	Redox Systems	172
3.6.14	Seals	175
3.6.15	Timers (excluding Solid-State Timers)	176
Chapter 4	PRACTICAL ASPECTS OF LARGE-SCALE ENZYME PURIFICATION	181
	DR J. MELLING and B. W. PHILLIPS Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire	
4.1	Introduction	181
4.2	Enzyme Inactivation	181
4.3	Containers and Ancillary Equipment	182
4.3.1	Glass Vessels	182
4.3.2	Metal Vessels	182
4.3.3	Plastic Vessels	183
4.4	Liquid Transfer	184
4.4.1	Couplings	184
4.4.2	Pumps	184

Table of Contents

xi

4.5 Bacterial Disruption	185
4.5.1 Resuspension	185
4.5.2 Liquid Shear	185
4.5.3 Grinding	186
4.6 Centrifugation	187
4.6.1 Batch Centrifuges	187
4.6.2 Continuous Flow Centrifuges	187
4.7 Concentration	190
4.7.1 Ultrafiltration	190
4.7.2 Dialysis	192
4.8 Chromatography	192
4.8.1 Columns	192
4.8.2 Gel Chromatography	194
4.8.3 Ion Exchange Chromatography	197
4.8.4 Affinity Chromatography	201
References	202

Chapter 5 DATA ON TECHNIQUES FOR ENZYME IMMOBILIZATION	203
PROFESSOR S. A. BARKER and DR J. F. KENNEDY	
Department of Chemistry, University of Birmingham	
5.1 Covalent Bond Formation	203
5.2-5.19 Enzyme immobilization, each <i>via</i> a particular reactive group on the solid support	203
5.20 Activation of supports by the Ugi Reaction	230
5.21 Activation of supports by transition metal salts	234
References	235

Chapter 6 INDUSTRIAL PRACTICE WITH ENZYMES	243
DR ALAN WISEMAN	
Department of Biochemistry, University of Surrey	
6.1 Introduction	243
6.2 Carbohydrases	243
6.2.1 α -Amylase (Fungal)	243
6.2.2 α -Amylase (Bacterial)	244
6.2.3 α -Amylase (Cereal)	246
6.2.4 Amyloglucosidase (Glucamylase) also Pullulanase	246
6.2.5 Pectinases	247
6.2.6 Cellulases	248
6.2.7 Hemicellulases	248
6.2.8 Invertase	248
6.2.9 Lactase (β -Galactosidase)	248
6.2.10 Dextranase	249
6.2.11 Lysozyme	249
6.3 Proteases	249
6.3.1 Papain	249
6.3.2 Trypsin (Chymotrypsin)	250
6.3.3 Pepsin	250
6.3.4 Rennin (and substitutes)	250
6.3.5 Fungal Proteases	251
6.3.6 Bacterial Proteases	252

Table of Contents

6.4 Other Hydrolases	252
6.4.1 Lipases	252
6.4.2 Penicillin Acylases	253
6.5 Oxidoreductases	253
6.5.1 Glucose Oxidase	253
6.5.2 Catalase	253
6.5.3 Lipoxidase	253
6.6 Other Enzymes	272
6.6.1 Glucose Isomerase	272
References	272
Index	273

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 co-factor)—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 constitutive 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