INDUSTRIAL ENZYMOLOGY

THE APPLICATION OF ENZYMES IN INDUSTRY

Tony Godfrey
& Jon Reichelt

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FOREWORD

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Considerable literature has accumulated on the laboratory production, purification, and theoretical behaviour of enzymes. Little has been written on their practical application. *Industrial Enzymology* fills this void. This text is a unique collection of practical data covering sources, performance, and specific application of various enzymes in the industrial world. As such it will be of value to a wide variety of workers in industry as well as academia. Although written almost exclusively by Europeans, the appeal of the book should be worldwide. The reader will find in the text information on industrial applications of enzymes in Western Europe, USA, Japan, and Australia, plus tabulated details of enzyme suppliers throughout the world.

The book also contains comments on current legislative thinking regarding enzymes, plus toxicological consideration and test systems for using enzymes. Of particular interest to those working with enzymes should be the material in Chapter 3 giving guidance on safety consideration when working with enzymes including the approval status of various industrial enzymes in the major countries. For example, there is a tabulated list of permitted as well as recommended industrial enzymes for several countries, including the USA GRAS listings.

In Chapter 4 the use of enzymes in 17 different and varied industries is described. This includes such industries as wine, baking, brewing, leather, paper, textiles, etc. In addition to those established industries, application in newly developing industries such as fuel, flavours, colours, and analytical are described.

However, this book is not just a source book on enzymes. Chapter 2 is devoted to the development of the basic mathematical theory of enzyme behaviour. Hence, this text should have broad based value to all those persons interested in the practical applications of enzymes.

PREFACE

The current increased demand for better utilization of regenerable resources, and the pressure on industry to operate within environmentally compatible limits, has been a stimulus to the development of new concepts in biotechnology. A parallel increase in developments using industrial enzymes is maintaining the growth of the enzyme markets that have already enjoyed more than thirty years of steadily widening applications.

This book contains a collected account of current industrial practice in the use of enzymes, several summary indexes, and comparative descriptions of industrially used enzyme preparations that is intended to provide data for use by the natural product processing industries, research teams and university teaching departments for both current understanding and their future development. It is not claimed that all industrial applications have been described, in fact some cannot yet be mentioned until industrial confidentiality restrictions can be lifted. The editors invite readers to draw their attention to such omissions to enable them to ensure that future editions may continue to be valuable up-to-date statements of the practice of industrial enzymology.

The book contains many detailed and specific examples of actual enzyme use, using named trade products, in order that meaningful treatments can be described. It is not intended that these should be considered exclusive, and by the use of simple analytical procedures it is possible to make internally valid comparisons of samples of different sources of similar enzymes. However, since most enzyme producers use their own definitions of activity, only by named products can the examples be given worthwhile meaning.

Care has been taken to ensure that the factual information in the book is accurate, but the opinions expressed are always those of the contributor and not of their employer or company. The reader is reminded that nothing is stated in this book that forms the basis of warranty or guarantee for use without prior testing, and care should be taken that patents are not infringed or local regulations violated when making use of industrial enzymes.

In the preparation of this book the editors have endeavoured to assemble a diverse account of the many applications of enzymes so that the opportunities for technology transfer become more readily apparent. Individual industry contributions may be considered as 'state of the art' for that industry, but also as the source of information and concepts for application in other industrial sectors where similar solutions to the problems of natural product processing are required. The early chapters are intended to give guidance, in the value of the mathematical concepts of kinetics as they affect industrial operations, and in the current thinking on the legal and regulatory responsibilities of both producer and user of enzymes.

The application of enzymes is a well-established part of the biotechnical society, serving both the traditional and the new industries. It is the aim of this book to bring together a substantial part of that application data to form a useful reference source.

TG
JRR

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TG
JRR

My grateful thanks to my wife, Eirlys, and the family, who have had to take too much of the strain; to Novo Industri A/S for allowing me to undertake this project, together with much help from my colleagues; to all at Novo Enzyme Products, for encouragement and support; to Sally James for unstinting hours of typing; to Rosemary Foster of Macmillan, who insisted the book should be written, and to her team who performed the transformation of the manuscripts.

TG

My thanks to all my colleagues at Miles Kali-Chemie, especially to Dr H. U. Geyer and Dr G. Richter, for their help and encouragement. Thanks also to Mr J. T. Brady, Dr W. Goldstein and Dr J. Marshall of Miles Laboratories Inc., Elkhart, Indiana. Special appreciation and thanks to my wife, Lesley, for her help, encouragement and typing.

JRR

CONTENTS

* Editors' note: The concluding seven sections of this chapter of industrial applications for enzymes have been separated from the preceding materials. They serve to illustrate the diversity and versatility that can be extracted from enzyme technology and translated by lateral thinking into applications representing the commercial and industrial development of biotechnology that is already taking place.

Chapter 1

INTRODUCTION TO INDUSTRIAL ENZYMOLOGY

T. Godfrey and J. R. Reichelt

This book is about the industrial application of commercially available enzymes, and while no section is given to their production, some discussion of the nature of the commercial products is appropriate to introduce them.

Estimates of the 1981 world market for industrial enzymes put the sales at around 65,000 tonnes of commercial product valued at $400 million. Predictions of growth of the market forecast a rise to 75,000 tonnes, valued at $600 million by the end of 1985.

There are only a few enzyme producing companies, probably numbering about 25, of which 6 are clearly dominant in both quantity and value. Among the Western nations, almost half of all enzyme production is in Denmark, with Holland producing a further 20 per cent. American production is responsible for about 12 per cent, but a large proportion of this production is by companies who have their own 'inhouse' uses for the limited range of enzymes produced, with none of this material generally available on the free market. Japan, West Germany, France, Switzerland and the UK together with Ireland account for the remainder of enzyme production. Production in the USSR and China is significant but not readily quantified, since virtually none of the products is made available to Western industry.

1. Enzyme types and sources

More than 80 per cent of all industrial enzymes are hydrolytic in action and they are used for the depolymerization of natural substances. Almost 60 per cent of these are proteolytic, for use by the detergent, dairy and leather industries. The carbohydrases, used in baking, brewing, distilling, starch and textile industries, now represent almost 30 per cent of the total enzyme usage, leaving the lipases and highly specialized enzymes with the remainder (*see* Figure 1.1).

There are some 12 categories of enzymes used in industrial processing, with 30 different activity types in common use. When it is considered that several thousand different enzymes have been identified and characterized, the future for new processing aids is attractive. However, much new knowledge will be needed if the energy-dependent and cofactor-using enzymes are to be made

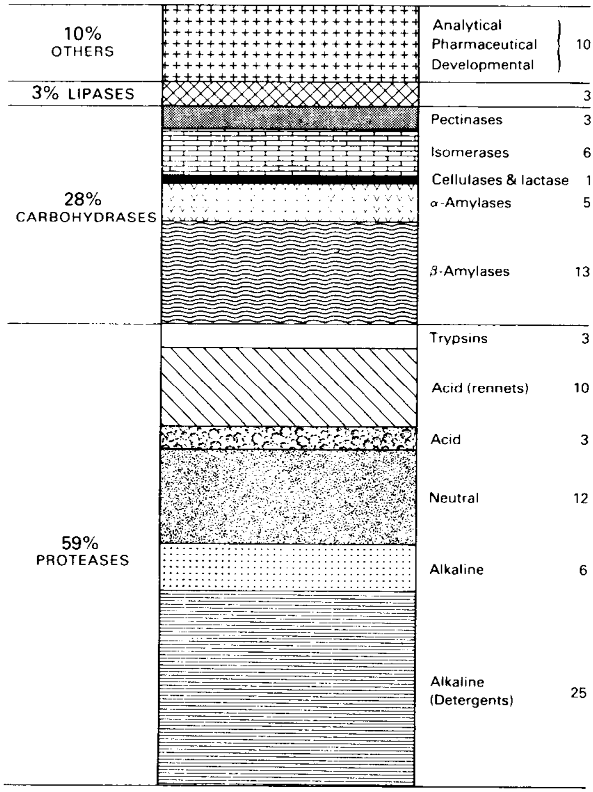

Figure 1.1 Distribution of industrial enzymes.

commercially effective. When they are available, a range of polymerizing processes will provide a completely new range of products. Some even hold out expectations that substitutes for petrochemical products will be derived by enzymic processing.

While almost any living organism can be considered a potential source of useful enzymes, in practice a limited number of plant and animal tissues are economic sources and the greatest diversity comes from microorganisms.

Plant enzymes. These include the well-known proteases papain, bromelain and ficin and the amylolytic enzymes of the cereals, soya bean lipoxygenase and specialized enzymes from the citrus fruits. Most plant enzymes are available as comparatively unpurified powder extracts, although papain is notable for being recently available as a stabilized and purified liquid. Prospects for increased supply of plant enzymes, in response to greater use in traditional applications or for new processes, depend on several factors. The influence of cultivation conditions, growth cycle and climatic requirements make new supplies comparatively long-term projects, and the influence of agricultural economics competing for cultivated land must be coupled with national and international political forces which control agricultural activities.

Animal enzymes. These include the pancreatic trypsins and lipases and the rennets, which are produced in both ultrapure and industrial bulk qualities. Again, the prospects for large increases in supply depend on the political and agricultural policies that control the production of livestock for slaughter. Currently, these enzymes cannot meet demand on a world basis adequately, with the result that the more price sensitive user industries are increasing their interest in microbial enzymes.

Microbial enzymes. As will be seen by reference to Data Index 4 of this book, there are very few species of microorganism in use for industrial enzyme production. This is largely a limitation imposed by the desire of producing companies to have the widest possible market range for their products, which includes food processing, and the consequent high cost of obtaining approvals from legislative authorities. This cost is substantially increased if a product is produced by an organism without a recognized history of satisfactory approvals, and if it must be evaluated for toxicity and safety. Most industrial microbial enzymes are produced from no more than 11 fungi, 8 bacteria and 4 yeasts; producers generally seek new target enzymes from among these same species.

In response to demand fluctuations, it is relatively simple to increase or decrease the fermentation capacity devoted to the production of a particular enzyme. Within three to four months, an inventory policy change can be implemented and product availability brought into line. A full range of product qualities can be produced by the implementation of different sections of downstream processing of fermentation biomass or liquors. Applications vary widely in the purity standards required of processing enzymes and producers generally offer two grades of product, and sometimes three or four. Food grades tend to be the dominant quality, since they can be produced in great bulk to a common standard and then function satisfactorily in most applications where lower quality would suffice. The overall economy of bulk production by well-proven fermentations, including continuous culture, provides cost effective products for most applications. It is certain that the six or so of the most frequently used enzymes now trade as commodities in the marketplace and have shown unit cost rises far below the general inflationary trends of recent years. In several cases it is now cheaper to use an enzyme than it was five years ago. This has greatly assisted the expansion of natural product processing by enzymes, especially in the alcohol producing, brewing, dairy, detergent, starch and wine industries.

Composition and stability. With such a huge variation in potency and physical presentation, it is not sensible to present specific statements about enzyme product composition. Those produced by extraction from plant and animal tissues will contain different substances, in addition to the active enzyme, than those from microbial fermentations. The table below shows the typical composition of many industrial enzyme preparations based on percentage of dry solids:

	Range of content (% dry solids)
Proteins and amino acids	10–15
Active enzyme protein	2–5
Complex carbohydrates	5–12
Sugars	2–40
Inorganic salts	3–40
Preservatives	0–0.3

Apart from noting the small proportion that is actually active enzyme protein, it should be said that sugars and inorganic salts are used as alternatives when establishing the stability of the finished product for storage and distribution, and are selected according to acceptability in the intended application. Salts and sometimes carbohydrates such as starch are used to dilute extracted enzymes to standard activity. Preservatives are generally restricted to liquid enzyme preparations and conform to regulatory information relating to the intended use and the country of destination.

Industrial enzymes are commonly used at levels of 0.1–0.5 per cent of the substrate being processed, with rare exceptions above these levels. Therefore, when the actual amount of any constituent of the preparation is evaluated as a constituent of the final processed product, it is unlikely that it will make a significant contribution in relation to other similar materials, present or introduced, in the total process.

Naturally, consultation with individual suppliers is advised when storage life of enzyme products is considered, but generally, dry products have longer shelflife than liquids, and cool conditions extend the time still further. Thus a general expectation would be

Dry products	— ambient to 25 °C (max.)	6 months
	— cooled 0–4 °C	12 months
Liquid products	— ambient to 25 °C (max.)	3 months
	— cooled 0–4 °C	6 months

These storage times would be satisfactory for most industrial stock rotations to give full performance after such storage times, providing the product remained factory-sealed and was stored according to the producer's recommendations.

Selection of processing enzymes. When the decision to investigate the use of an enzyme for a particular process has been taken, it becomes necessary to make a selection from the available enzymes with activities that appear appropriate. Many factors are involved in the selection (*see* Table 1.1).

TABLE 1.1
Key factors influencing choice of enzyme

Specificity	Sledgehammer or surgical knife?
pH	Limits to be imposed
Temperature	Rate vs inactivation vs hygiene and time
Activators and inhibitors	Control facility
Analysis method	Control/detection
Availability	Actual
	Safety
	Approved
Technical service support	Guidance on handling, dosing performance factors – new data and superceding products
Cost	Per unit of conversion
	Per cent of process

Specificity. The generally claimed specific action of enzymes is not as sharply defined as is often expected. Proteases are broad in the range of amino acid bonds they hydrolyse and exhibit only a degree of specificity. Careful investigation of the range of bonds attacked, as exemplified in Chapter 5, Table 5.1, and testing for comparable action on the actual protein target, will enable an enzyme to be chosen that has suitable performance. Some proteases are extremely narrow in their action, for example the various cheese rennets.

Among the carbohydrases, there are both highly specific enzymes, such as the components of the pectinolytic group or the lactases, and also broad acting amylases and β-glucosidases. Within any group there are small differences of action depending upon the source and type of enzyme, and these can be used to advantage in obtaining precise reaction products. Thus, the first question must be to determine what degree of specificity is required in the reaction.

pH considerations. Both the optimum operating value determined under analytical conditions, and the actual ability of the proposed industrial system to adjust away from the possibly unsuitable pH (imposed by both upstream and downstream operations) in relation to the enzyme stage, must be considered. Some limits will be dictated by the practicalities of the overall process, and these may influence the choice of a particular enzyme among alternatives. Again, the operation of an enzyme outside the anticipated best performance range may modify its specificity or sensitivity to heat in both beneficial or adverse ways.

Temperature considerations. When considering enzyme processes, the general rule is that the temperature quotient is between 1.8–2.0. (The reaction rate will increase or decrease by this order for each shift of 10°C.) By using high temperatures, the reaction may be of short duration and hygienic conditions may be maintained more easily. Conversely, the use of much greater heat than for thermolabile enzymes will be necessary to inactivate the enzyme at the end of the process. Alternatively, a significant shift of pH may be necessary to inactivate without a rise in temperature. Not all processes require the inactivation of the enzyme at the conclusion of the desired reaction, but it is important to consider this point when designing food product applications, or to prevent subsequent further modification by residual enzyme activity.

Activators and inhibitors. These are usually well-defined for specific enzymes and should be taken into account if they create expensive additional costs, or costly treatments to ensure their elimination from the reaction. Under some circumstances, the deliberate omission of a known activator will alter the pH or temperature sensitivity of an enzyme to an extent that limits the reaction, or simplifies the end process inactivation conditions.

Analysis method. This is an essential tool in process control and the detection of low levels of activity when monitoring for residual action after an inactivation treatment. Many industrial users of enzymes also maintain routine activity checks on stock enzymes to ensure good stock rotation. Therefore, when selecting an enzyme, it is helpful to have one with an established analytical method readily available (*see* Data Index 5).

Availability. Not only from the point of view of supply but also with the long term use in mind, the operator of enzyme processes will need to choose enzymes that can be supplied in consistent quality and activity. This is especially important for continuously metered enzyme dosing systems and immobilized reactor enzymes whose investment costs demand reliable and steady performance. In many processes the choice of enzyme will also relate to the safety record of industrial use, and the regulatory approval for its use, both in the process and the products made, in the countries where the