OPEN UNIVERSITY PRESS BIOTECHNOLOGY SERIES

Enzyme Technology

P Gacesa J Hubble

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Open University Press
Milton Keynes


Taylor & Francis
New York • Philadelphia

Co-published by:
Open University Press
Open University Educational Enterprises Limited
12 Cofferidge Close
Stony Stratford
Milton Keynes MK11 1BY, England

Taylor and Francis
Publishing office
3 East 44th Street
New York NY 10017
USA

Sales office
242 Cherry Street
Philadelphia, PA 19106
USA

First Published 1987

British Library Cataloguing in Publication Data
Gacesa, Peter
Enzyme technology.—(Biotechnology series).
1. Enzymes—Industrial applications
I. Title II. Hubble, John III. Series
661′.8 TP248.E5

ISBN 0-335-15168-x

ISBN 0-335-15160-4 Pbk

Library of Congress Cataloging in Publication Data
Gacesa, Peter
Enzyme technology.
Includes bibliographies and index.
1. Enzymes—Industrial applications. 2. Biochemical engineering. I. Hubble, John. II. Title. [DNLM: 1. Biotechnology. 2. Enzymes. QU 135 G121e]
TP248.E5G33 1987 660′.63 87-10189

ISBN 0-8448-1515-2

ISBN 0-8448-1516-0 (pbk.)

Text design by Clarke Williams

Printed in Great Britain at the Alden Press, Oxford

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To Karen, Tom, Luke and Sue

Preface

Biotechnology has undoubtedly been one of the major growth areas in science and engineering over the last ten to fifteen years. The promise of new techniques with consequent development of novel processes has been publicized both in the scientific literature and in information that has been disseminated to a wider audience. Unfortunately, not all the claims for the potential of biotechnology have been based on sound analysis and the resultant over-selling of the topic has been a serious problem. However, the area of enzyme technology was not only well established before the current fervour for biotechnology but has grown successfully within it, providing a sound basis for a promising future.

In compiling this book we have aimed at producing a text that will be suitable for final year undergraduates, postgraduates, research workers and the technically-informed manager. The objective of the book is two-fold. We hope to give readers with an engineering background an appreciation of the subtleties of enzymes and the potential of the new techniques in molecular genetics for the tailoring of these catalysts to specific needs. For those with a biochemical/biological background who are more familiar with enzyme properties, we aim to provide an appreciation of biochemical engineering considerations. We do not claim to provide a comprehensive analysis of all the biochemical and engineering problems (there are several excellent texts already on the market) but rather to enable the interested reader to see an approach to a particular problem. Our philosophy has been to explain general principles, as far as this is possible, by using specific examples of enzyme applications. What we have tried to avoid is producing merely a catalogue of enzyme-catalysed processes.

It should be emphasized that from an engineering point of view enzymes are simply a special category of catalysts. They have several advantages in terms of specificity and mild reaction conditions but also disadvantages such as problems of

instability. In some circumstances enzymes have replaced traditional catalysts or opened up new applications where, for example, specificity is a major criterion. However, our understanding of certain enzyme mechanisms has done much to further the development of conventional catalysis and chemists are now able to synthesize novel, low molecular weight, non-enzymic catalysts. Clearly, enzymes will not replace the majority of chemical catalysts and it may be argued that this application of enzymology is only a transient phase in the evolution of applied catalysis. However, what is clear is that the case for enzymes is well established and that the number of products of enzyme technology continues to increase. Also, the recent advances centred on the use of enzymes in non-aqueous media has the potential of opening up large new markets. It is likely that the application of enzymes to the production of new products will be the area of greatest growth potential rather than trying to cost-cut existing processes.

We hope that the book will widen the general awareness of the next generation of scientists and engineers to the commercial potential of enzymes, and will in some small part contribute to the development of this challenging field of enzyme technology.

Acknowledgements

We would like to express our thanks and appreciation for the invaluable advice received from a number of our colleagues including Dr R.A. John, Dr A.J. Knights, Professor J.A. Howell, and in particular to Dr Robert Eisenthal who was responsible for fostering our interest in enzymes during our period of study at Bath University.

We would also like to record our gratitude to Professor J.F. Richardson and the late Professor K.S. Dodgson who encouraged this venture and who supported us through the critical initial stages.

Chapter 1

Fig. 1.1 Lilly, M.D. (1977) *Biotechnological Applications of Proteins and Enzymes* (Bohak, Z. and Sharon, N. eds.) New York, Academic Press. (Original figure number 3, p. 135.)

Fig. 1.2 Solomons, G. (1977) *Biotechnological Applications of Proteins and Enzymes* (Bohak, Z. and Sharon, N. eds.) New York, Academic Press. (Original figure number 1, p. 52.)

Table 1.1 Lilly, M.D. (1977) *Biotechnological Applications of Proteins and Enzymes* (Bohak, Z. and Sharon, N. eds.) New York, Academic Press. (Original table number 4.)

Table 1.2 Reichelt, J. (1983) *Industrial Enzymology* (Godfrey, A. and Reichelt, J. eds.) Byfleet, The Nature Press. (Original table number 3.2.3., p. 149.)

Table 1.3 Reichelt, J. (1983) *Industrial Enzymology* (Godfrey, A. and Reichelt, J. eds.) Byfleet, The Nature Press. (Original table number 3.2.2., p. 148.)

Table 1.4 Aunstrup, K. (1977) *Biotechnological Application of Proteins and Enzymes*

(Bohak, K., and Sharon, N. eds.) New York, Academic Press. (Original table number 1, p. 40.)
Table 1.5 Poulsen, P.B. (1984) *Proceedings of the Third European Congress on Biotechnology*, Weinheim, V.C.H. (Original table number 4, p. IV–344.)

Chapter 2

Fig. 2.2 de Duve, C. (1985) *A Guided Tour of the Living Cell*, New York, Scientific American Books. (Diagram on page 93.)

Chapter 3

Fig. 3.1 Amicon Corporation, Lexington, MA. Copyright 1980.

Chapter 6

Fig. 6.3 Goldstein, L. (1976) *Methods in Enzymology* **44** (Mosbach, K. ed.) New York, Academic Press. (Original figure number 1, p. 403.)
Fig. 6.4 Horvath, C. and Engasser, J-M. (1974) *Biotechnology and Bioengineering* **16,** New York, John Wiley. (Original figure number 7, p. 919.)
Fig. 6.5 Engasser, J-M. and Horvath, C. (1976) *Applied Biochemistry and Bioengineering* **1,** New York, Academic Press. (Original figure number 6, p. 140.)

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Fig. 7.1 Antrium, R.L., Kolilla, W. and Schnyder, B.J. (1979) *Applied Biochemistry and Bioengineering* **2,** New York, Academic Press. (Original figure number 1, p. 126.)
Table 7.1 Godfrey, A. (1983) *Industrial Enzymology* (Godfrey, A. and Reichelt, J. eds.) Byfleet, The Nature Press. (Original table number 4.5.3, p. 227.)
Table 7.2 Godfrey, A. (1983) *Industrial Enzymology* (Godfrey, A. and Reichelt, J. eds.) Byfleet, The Nature Press. (Original table number 4.8.1, p. 295.)

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Fig. 8.5b Mosbach, K. and Danielsson, B. (1981) *Analytical Chemistry* **53,** 83A–94A. (Original figure number 2.)
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Table 8.1 Bowers, L.D. and Carr, P.W. (1980) *Advances in Biochemical Engineering* **15,** New York, Springer-Verlag. (Original table numbers 6 and 7, p. 106–107.)
Table 8.2 Mosbach, K. and Danielsson, B. (1981) *Analytical Chemistry* **53,** 83A-94A. (Original table number 1.)
Table 8.3 Lowe, C.R., Goldfinch, M.J. and Lias, R.J. (1984) *Biotech 83,* Northwood, Online Publications Ltd. (Original table number 1.)

Chapter 9

Fig. 9.2 Kaiser, E.T. and Lawrence, D.S. (1984) *Science* **226,** 505–511, Copyright 1984 by the AAAS. (Original figure 1.)
Table 9.2 Danno, G. (1970) *Agricultural and Biological Chemistry* **34,** 1805–1814. (Original table 1.)
Table 9.3 Jacobsen, H., Klenow, H. and Overgaard-Hansen, K. (1974). *European Journal of Biochemistry* **45,** 623–627. (Original table 1.)
Table 9.4 Kaiser, E.T. and Lawrence, D.S. (1984) *Science* **226,** 505–511, Copyright 1984 by the AAAS. (Original table 3.)

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Fig. 10.4 Zaks, A. and Klibanov, A.M. (1984) *Science* **224,** 1249–1251, Copyright 1984 by the AAAS. (Original figure 2A.)
Fig. 10.6 Bender, M.L., D'Souza, V.T. and Lu, X. (1986) *Trends in Biotechnology* **4,** 132–135, Copyright 1986 Elsevier Science Publishers. (Original figure 1.)

Symbols and units

A problem encountered in texts covering material which spans two or more traditional subject areas is that of constancy in the use of symbols and units.

Enzyme technology as presented in this text draws upon fundamental science arising from the study of enzymology, fluid dynamics and electronics. Each of these disciplines has its own well-established convention for use of symbols, making some overlap unavoidable. Rather than attempt to redefine all symbols to a common basis (and risk offending purists), we have defined them on the basis of the chapter in which they appear. For example, the symbol V denotes reactor volume in Chapter 4 whereas in Chapter 8 it denotes voltage.

In some disciplines it has been common practice to use various symbols to denote the same variable, e.g. the maximum rate for an enzyme reaction may be seen as V or V_m or V_{max}. While this diversity is usually frowned upon, it does allow us some scope to minimize conflict and to maintain the use of familiar symbols; to this end we must apologize for occasionally departing from use of the officially approved symbol.

In order to maintain consistency all dimensions are given in SI units or derived SI units of common usage. For a complete breakdown of derived SI units, the reader is referred to a specialist data book (Perry, 1984).

Although a rigid adherence to the SI nomenclature leads to problems with the magnitude of some terms (e.g. rate, kg mol m^{-3} s^{-1}, may lead to very small numbers in certain contexts), we feel that dimensional consistency must be stressed, especially for those readers from a biological background.

Chapter 4

Symbol	*Interpretation*	*Units*
A	Arrhenius constant	depends on reaction order
D	Dilution rate	s^{-1}
ε	Packed bed voidage	—
E	Activation energy	$kJ\,kg\,mol^{-1}$
$[E]$	Active enzyme concentration	$kg\,mol\,m^{-3}$
$[ER]$	Concentration of enzyme–reactant complex	$kg\,mol\,m^{-3}$
$[E_0]$	Total active enzyme concentration	$kg\,mol\,m^{-3}$
$[E^t]$	Active enzyme concentration after time t	$kg\,mol\,m^{-3}$
$[ERR]$	Concentration of inactive enzyme–reactant complex	$kg\,mol\,m^{-3}$
$[EP]$	Concentration of inactive enzyme–product complex	$kg\,mol\,m^{-3}$
k_1	Second-order rate constant	$kg\,mol\,m^{-3}\,s^{-1}$
k_{-1}	First-order rate constant	s^{-1}
k_{-2}	Second-order rate constant	$kg\,mol\,m^{-3}\,s^{-1}$
k_2	First-order rate constant	s^{-1}
k_d	First-order decay constant	s^{-1}
K_m	Michaelis constant	$kg\,mol\,m^{-3}$
$K_{\acute{m}}$	Apparent Michaelis constant	$kg\,mol\,m^{-3}$
K_i	Inhibition constant	$kg\,mol\,m^{-3}$
K_{eq}	Equilibrium constant	—
$[P]$	Product concentration	$kg\,mol\,m^{-3}$
Q	Volumetric flow rate	$m^3\,s^{-1}$
R	Gas constant	$kJ\,K^{-1}\,kg\,mol^{-1}$
$[R]$	Reactant (i.e. substrate) concentration	$kg\,mol\,m^{-3}$
T	Temperature	K
v	Observed rate of reaction	$kg\,mol\,m^{-3}\,s^{-1}$
V	Reactor volume	m^3
V_l	Liquid volume	m^3
V_{tot}	Total volume	m^3
V_{max}	Maximum theoretical rate of reaction	$kg\,mol\,m^{-3}\,s^{-1}$
$V_{\acute{max}}$	Apparent V_{max}	$kg\,mol\,m^{-3}\,s^{-1}$
X	Fractional conversion	—

Chapter 6

Symbol	*Interpretation*	*Units*
$a,b,c,$	Constants	—
C_b	Bulk concentration	$kg\,mol\,m^{-3}$
C_s	Surface concentration	$kg\,mol\,m^{-3}$
d_i	Diameter of impeller	m

d_p	Diameter of particle	m
D_c	Diffusivity of solute through the immobilization matrix	$m^2 s^{-1}$
D_s	Diffusivity of solute in solution	$m^2 s^{-1}$
e	Electronic charge	—
E	Active enzyme concentration	$kg\,mol\,m^{-3}$
E_o	Active enzyme concentration at time zero	$kg\,mol\,m^{-3}$
E^t	Active enzyme concentration after time t	$kg\,mol\,m^{-3}$
H_b^+	Hydrogen ion concentration in bulk solution	$kg\,mol\,m^{-3}$
H_s^+	Hydrogen ion concentration at the surface	$kg\,mol\,m^{-3}$
k	Boltzmann constant	$J\,K^{-1}$
k_d	Enzyme decay rate	s^{-1}
K_m	Apparent Michaelis constant	$kg\,mol\,m^{-3}$
K_s	Mass transfer coefficient	$m\,s^{-1}$
L	Thickness of particle	m
n	Stirrer speed	$revolutions\,s^{-1}$
P	Partition coefficient	—
$[R]$	Reactant concentration	$kg\,mol\,m^{-3}$
Re	Reynolds number	—
Re_i	Reynolds number for stirred system	—
Sh	Sherwood number	—
Sc	Schmidt number	—
t	Time	s
T	Absolute temperature	K
V_{max}	Maximum theoretical rate of enzyme-catalysed reaction	$kg\,mol\,m^{-3}\,s^{-1}$
V_{max}	Apparent V_{max}	$kg\,mol\,m^{-3}\,s^{-1}$
δ	Boundary layer thickness	m
ψ	Electrical potential	volts
ρ	Density	$kg\,m^{-3}$
ν	Dynamic viscosity	$m^2\,s^{-1}$
ϕ	Thiele modulus	—
χ	Porosity	—
τ	Tortuosity	m
μ	Liquid velocity	$m\,s^{-1}$

Chapter 8

Symbol	*Interpretation*	*Units*
B	Temperature constant for thermistor	K

E_G	Standard probe potential	volts
E_G'	Observed probe potential	volts
E_{ref}	Internal reference probe potential	volts
E_{asym}	Asymetric potential	volts
F	Faraday constant	C mol^{-1}
$[R]$	Reactant concentration	kg mol m^{-3}
R_1	Resistance of thermistor 1	ohms
R_2	Resistance of thermistor 2	ohms
δR	Change in resistance of thermistor	ohms
T	Absolute temperature	K
V	Bridge excitation voltage	volts
v	Bridge output voltage	volts

Contents