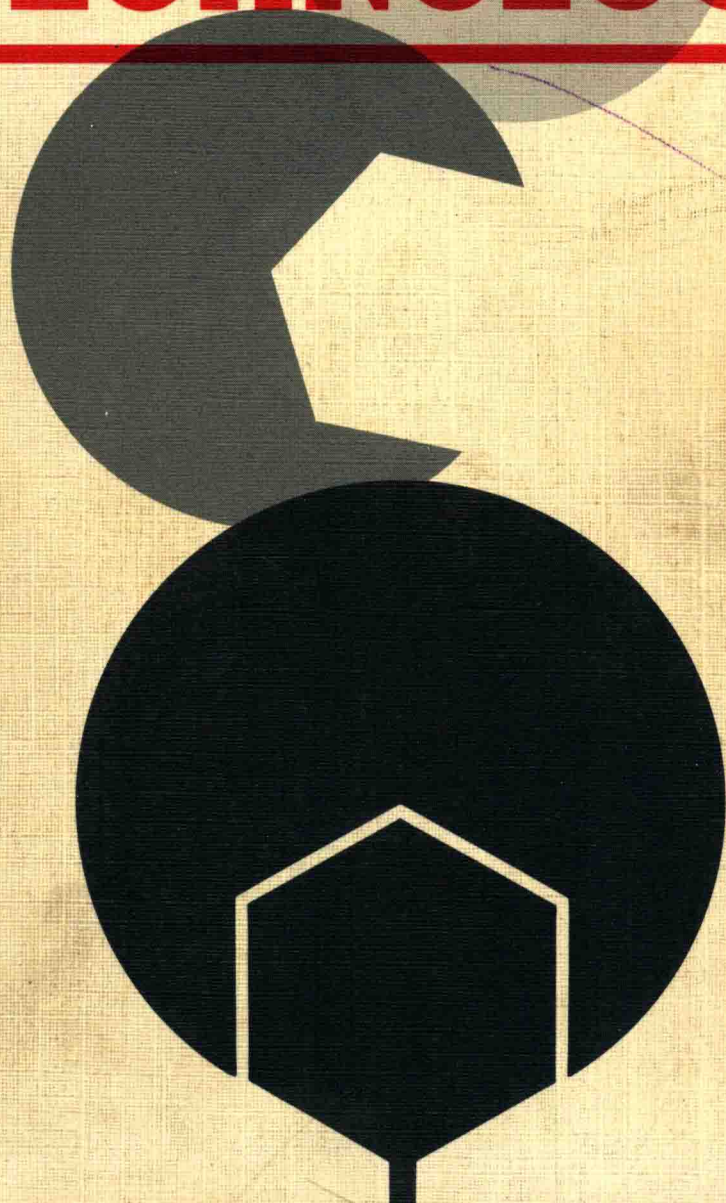


M.F. CHAPLIN AND C. BUCKE

ENZYME TECHNOLOGY



Enzyme technology

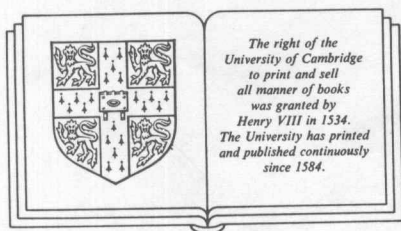
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Enzyme technology

To my parents June and Frank,
to my wife Philippa
and to my children Timothy, Helen and Katy.

M.F.C.

To my parents.

C.B.

Preface

Enzyme technology has made a major impact on society and is the central theme around which most biotechnological science revolves. In this volume, we have attempted to put it in its rightful place, to show why it is so important, and to show how it is being used at the moment and how it may develop in the future.

This book is intended to be a text for budding and established biotechnologists. It deals with the enzyme technology necessary for diploma, undergraduate and postgraduate students of biotechnology, applied and industrial biology, biochemical engineering, food science and allied subjects. We also hope that it will find favour with their teachers, offering them a fresh approach to the subject area. It is designed to take the reader rapidly through the necessary background and towards a full discussion of the uses of enzymes as industrial catalysts. Overall, we have attempted to give a critical view of current theory, the original references being checked wherever possible. During this process we have come across a number of often-quoted misconceptions, which we hope to dispel.

We attempt to give the kinetic background which we feel is necessary for a proper understanding of the activity of industrial enzymes. In this, we hope that we have achieved a balance between rigour and utility. In particular, we feel that students (and teachers) should, as far as possible, understand the derivation of the equations that are used. The emphasis is on the practical significance of enzyme kinetics. For this reason, enzymic denaturation and the kinetics of reversible reactions have both received significant attention. Throughout, we have tried to instil a feel for the economics of the processes and the reasoning behind practical decision-making.

For each chapter we give a brief summary, containing the 'take-home messages', and a recent bibliography that should enable the interested student (and teacher) rapidly to enter the relevant literature.

We are indebted to a number of our colleagues for their useful discussions and suggestions. In particular we thank Drs T.P. Coultate, E.G. Killick, N. Morgan and M.D. Trevan, Mr S.A. Roulston and Ms H. Shukla.

We apologise for any errors we have inadvertently allowed into print and welcome comments and criticisms from readers.

December 1988

M.F.C.
C.B.

Symbols

$[\]$	concentration of the material within the brackets (M) ¹
$[\]_0$	concentration at zero time
$[\]_o$	bulk concentration
α	(alpha), relative volume of organic phase
β	(beta), dimensionless substrate concentration ($= [S]/K_m$)
δ	(delta), effective thickness of the unstirred layer surrounding an immobilised enzyme
ϵ	(epsilon), porosity
η	(eta), (1) effectiveness factor; (2) dynamic viscosity ($\text{g m}^{-1} \text{s}^{-1}$)
θ	(theta), shape factor
Λ	(lambda), electrostatic partition coefficient
μ	(mu), external substrate modulus
μ_H	proton modulus
μ_i	external substrate modulus in the presence of inhibitor
γ	(nu), kinematic viscosity ($\text{m}^2 \text{s}^{-1}$)
ρ	(rho), (1) the ratio of radial distance to particle radius (r/R); (2) density (kg l^{-1})
Σ	(sigma), sigma factor (m^2)
τ	(tau), (1) tortuosity; (2) average residence time in reactor
T	(tau), ionic strength (M)
ϕ	(psi), (1) substrate modulus for internal diffusion; (2) volumetric throughput (s^{-1})
ω	(omega) angular velocity (s^{-1})
A	(1) average specific activity; (2) area
C_b	concentration in bottom phase (M)
C_t	concentration in top phase (M)
d	particle diameter
D	(1) diffusivity ($\text{m}^2 \text{s}^{-1}$); (2) sedimentation distance
D_F	filter cake thickness

¹ Where appropriate, the most commonly encountered units are given (g, gram; h, hour; J, joule; kg, kilogram; l, litre (dm^3); M, mol l^{-1} ; m, metre; min, minute; mol, mole; s, second).

xiv *List of symbols*

D_S	diffusivity of a substrate ($\text{m}^2 \text{s}^{-1}$)
D_P	diffusivity of a product ($\text{m}^2 \text{s}^{-1}$)
DE	dextrose equivalent
DH	degree of hydrolysis
E	enzyme
ES	enzyme-substrate complex
EP	enzyme-product complex
f	(1) fluid velocity (m s^{-1}); (2) frequency of oscillation
f_m	mass flow rate ($\text{kg m}^{-2} \text{s}^{-1}$)
F	(1) flow rate (l s^{-1}); (2) Faraday (= 96487 coulombs molecular equivalent $^{-1}$)
g	gravitational acceleration (= 981 cm s^{-2})
ΔG^*	standard free energy of activation (kJ mol^{-1})
ΔH	change in enthalpy (kJ mol^{-1})
$[\text{H}_o^+]$	bulk hydrogen ion concentration (M)
i	degree of backmixing
k	(1) rate constants (units vary); (2) proportionality constant
k_{cat}	catalytic rate constant (s^{-1})
k_d	first-order inactivation constant (s^{-1})
k_L	mass transfer coefficient (m s^{-1})
k_L^{HB}	mass transfer coefficient of the conjugate acid HB (m s^{-1})
k_L^{H}	mass transfer coefficient of the hydrogen ion (m s^{-1})
k_L^{S}	mass transfer coefficient of the substrate (m s^{-1})
k_L^{P}	mass transfer coefficient of the product (m s^{-1})
K	kinetic constant in reversible reactions (M)
K_a	acid dissociation constant
K_{biphasic}	apparent equilibrium constant of biphasic system
K_{eq}	equilibrium constant
K_m	Michaelis constant (M)
K_m^{app}	apparent Michaelis constant (M)
K_{org}	equilibrium constant in an organic solvent
K_S	substrate inhibition constant (M)
K_P	product inhibition constant (M)
K_W	equilibrium constant in water
L	characteristic length of a system
Lf	Le Goff number
$\text{Log } P$	solvent polarity
Re	Reynolds number
pI	Isoelectric point
$\text{p}K_a$	$-\log_{10}$ (acid dissociation constant)

P	product
P	(1) protein content; (2) partition coefficient; (3) pressure through a filter
P_{∞}	product present at equilibrium
P_0	product, at time zero
P_m	maximum protein releasable
P_r	protein released
P_t	protein remaining at time t
P_t	total product present
P_P	partition coefficient of the product
P_S	partition coefficient of the substrate
Q_{10}	increase in the rate of a reaction per 10 deg. C rise in temperature
r	(1) radial distance within a porous biocatalyst; (2) radius of rotation
R	(1) radius of a porous biocatalyst (m); (2) gas constant ($= 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$); (3) resistance of thermistor
S	substrate
S	solubility (g l^{-1})
$S_{1/2}$	substrate present when an enzyme-catalysed reaction is at half the maximal rate
$S^{\#}$	substrate that can react in a reversible reaction
S_0	substrate, at zero time
S_r	substrate present at radius r within an enzyme particle
S_R	substrate present at the surface of an enzyme particle of radius R
S_t	substrate present at time t
S_t	total substrate present
S_{∞}	substrate present at equilibrium
t	time
$t_{1/2}$	enzyme half life
T	absolute temperature (K)
U	enzyme activity unit
v	(1) rate of reaction (M s^{-1}); (2) rate of sedimentation ($\text{kg cm}^{-1} \text{ s}^{-1}$)
v_{free}	rate of reaction of a non-immobilised enzyme (mol s^{-1})
V	(1) kinetic constant in reversible reactions (mol s^{-1}); (2) volume of centrifuge (l); (3) volume of reactor
V^*	kinetic constant, maximum (hypothetical) rate of reaction with respect to the pH (mol s^{-1})
V^f	rate of forward reaction (mol s^{-1})
V_b	volume in bottom phase
V_{max}	maximum rate of reaction (mol s^{-1})
$V_{\text{max}}^{\text{app}}$	apparent maximum rate of reaction (mol s^{-1})

V_{org}	volume of the organic phase
V^{τ}	rate of backward reaction (mol s^{-1})
V_{t}	volume in top phase
V_{w}	volume of aqueous phase
Vol_{s}	volume of a batch reactor
X	fractional conversion
X_{o}	initial fractional conversion
X_t	fractional conversion at time t

Contents

<i>Preface</i>	xi
<i>List of symbols</i>	xiii
1 Fundamentals of enzyme kinetics	1
Why enzymes?	1
Enzyme nomenclature	2
Enzyme units	5
The mechanism of enzyme catalysis	7
Simple kinetics of enzyme action	8
Effect of pH and ionic strength on enzyme catalysis	12
Effect of temperature and pressure	18
Reversible reactions	23
Enzyme inhibition	27
Determination of V_{\max} and K_m	36
Summary	38
Bibliography	38
2 Enzyme preparation and use	40
Sources of enzymes	40
Screening for novel enzymes	41
Media for enzyme production	46
Preparation of enzymes	46
Centrifugation	49
Filtration	53
Aqueous biphasic systems	55
Cell breakage	57
Ultrasonic cell disruption	59
High-pressure homogenisers	60
Use of bead mills	62
Use of freeze-presses	63
Use of lytic methods	64
Preparation of enzymes from clarified solution	65
Nucleic acid removal	66

Concentration by precipitation	66
Heat treatment	68
Chromatography	68
Ion-exchange chromatography	69
Affinity chromatography	70
Gel exclusion chromatography	72
Preparation of enzymes for sale	72
Customer service	75
Safety and regulatory aspects of enzyme use	75
Summary	78
Bibliography	78
3 The preparation and kinetics of immobilised enzymes	80
The economic argument for immobilisation	80
Methods of immobilisation	81
Kinetics of immobilised enzymes	90
Effect of solute partition on the kinetics of immobilised enzymes	94
Effects of solute diffusion on the kinetics of immobilised enzymes	100
Analysis of diffusional effects in porous supports	119
Summary	135
Bibliography	136
4 The large-scale use of enzymes in solution	138
The use of enzymes in detergents	139
Applications of proteases in the food industry	142
The use of proteases in the leather and wool industries	146
The use of enzymes in starch hydrolysis	146
Production of glucose syrup	151
Production of syrups containing maltose	154
Enzymes in the sucrose industry	155
Glucose from cellulose	157
The use of lactases in the dairy industry	159
Enzymes in the fruit juice, wine, brewing and distilling industries	160
Glucose oxidase and catalase in the food industry	162
Medical applications of enzymes	162
Summary	165
Bibliography	165
5 Immobilised enzymes and their use	167
Enzyme reactors	167

Membrane reactors	169
Continuous flow reactors	171
Packed-bed reactors	173
Continuous flow stirred tank reactors	175
Fluidised bed reactors	182
Immobilised-enzyme processes	183
High-fructose corn syrups (HFCS)	184
Use of immobilised raffinase	189
Use of immobilised invertase	190
Production of amino acids	190
Use of immobilised lactase	192
Production of antibiotics	193
Preparation of acrylamide	194
Summary	195
Bibliography	195
6 Biosensors	197
The use of enzymes in analysis	197
What are biosensors?	198
Calorimetric biosensors	203
Potentiometric biosensors	206
Amperometric biosensors	209
Optical biosensors	213
Piezo-electric biosensors	216
Immunosensors	216
Summary	219
Bibliography	219
7 Recent advances in enzyme technology	220
Enzymic reactions in biphasic liquid systems	220
The stabilisation of enzymes in biphasic aqueous–organic systems	222
Equilibria in biphasic aqueous–organic systems	225
Enzyme kinetics in biphasic aqueous–organic systems	233
Use of aqueous two-phase systems	235
Practical examples of the use of enzymes ‘in reverse’	236
Glycosidases used in synthetic reactions	240
Interesterification of lipids	241
Summary	243
Bibliography	243
8 Future prospects for enzyme technology	245
Whither enzyme technology?	245

Use of 'unnatural' substrates	245
Enzyme engineering	247
Artificial enzymes	252
Coenzyme-regenerating systems	253
Conclusions	255
Summary	256
Bibliography	256
<i>Index</i>	257

1 Fundamentals of enzyme kinetics

Why enzymes?

Catalysts increase the rate of otherwise slow or imperceptible reactions, without undergoing any net change in their structure. The early development of the concept of catalysis in the nineteenth century went hand in hand with the discovery of powerful catalysts from biological sources. These were called enzymes and were later found to be proteins. They mediate all synthetic and degradative reactions carried out by living organisms. They are very efficient catalysts, often far superior to conventional chemical catalysts, for which reason they are being employed increasingly in today's high-technological society, as a highly significant part of the biotechnological expansion. Their utilisation has created a billion dollar business, including a wide diversity of industrial processes, consumer products, and the burgeoning field of biosensors. Further applications are being discovered constantly.

Enzymes have a number of distinct advantages over conventional chemical catalysts. Foremost amongst these are their specificity and selectivity not only for particular reactions but also in their discrimination between similar parts of molecules (*regiospecificity*) or between optical isomers (*stereospecificity*). They catalyse only the reactions of very narrow ranges of reactants (*substrates*), which may consist of a small number of closely related classes of compounds (e.g. trypsin catalyses the hydrolysis of some peptides and esters in addition to most proteins), a single class of compounds (e.g. hexokinase catalyses the transfer of a phosphate group from ATP to several hexoses), or a single compound (e.g. glucose oxidase oxidises only D-glucose amongst the naturally occurring sugars). This means that the chosen reaction can be catalysed to the exclusion of side-reactions, eliminating undesirable by-products. Thus, higher productivities may be achieved, reducing material costs. As a bonus, the product is generated in an uncontaminated state, so reducing purification costs and the downstream environmental burden. Often a smaller number of steps may be required to produce the desired end-product. In addition, certain stereospecific reactions (e.g. the conversion of glucose into fructose) cannot be achieved by classical chemical methods without a large expenditure of time and effort. Enzymes work under

generally mild processing conditions of temperature, pressure and pH. This decreases the energy requirements, reduces the capital costs resulting from corrosion-resistant process equipment and further reduces unwanted side-reactions. The high reaction velocities and straightforward catalytic regulation achieved in enzyme-catalysed reactions allow an increase in productivity, with reduced manufacturing costs due to wages and overheads.

There are some disadvantages in the use of enzymes which cannot be ignored but which are currently being addressed and overcome. In particular, the high cost of enzyme isolation and purification still discourages their use, especially in areas which currently have an established alternative procedure. The generally unstable nature of enzymes, when removed from their natural environment, is also a major drawback to their more extensive use.

Enzyme nomenclature

All enzymes contain a protein backbone. In some enzymes this is the only component in the structure. However there are additional non-protein moieties usually present which may or may not participate in the catalytic activity of the enzyme. Covalently attached carbohydrate groups are commonly encountered structural features which often have no direct bearing on the catalytic activity, although they may well affect an enzyme's stability and solubility. Other factors often found are metal ions (*cofactors*) and low molecular weight organic molecules (*coenzymes*). These may be loosely or tightly bound by non-covalent or covalent forces. They are often important constituents contributing to both the activity and stability of the enzymes. This requirement for cofactors and coenzymes must be recognised if the enzymes are to be used efficiently and is particularly relevant in continuous processes where there may be a tendency for them to become separated from an enzyme's protein moiety.

Enzymes are classified according to the report of the Nomenclature Committee appointed by the International Union of Biochemistry (1984). This enzyme commission assigned each enzyme a recommended name and a four-part distinguishing number. It should be appreciated that some alternative names remain in such common usage that they will be used, where appropriate, in this text. The Enzyme Commission (EC) numbers divide enzymes into six main groups according to the type of reaction catalysed:

(1) *Oxidoreductases*, which involve redox reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules. This extensive class includes the dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide). For example: glucose oxidase (EC 1.1.3.4, systematic name, β -D-glucose : oxygen 1-oxidoreductase):