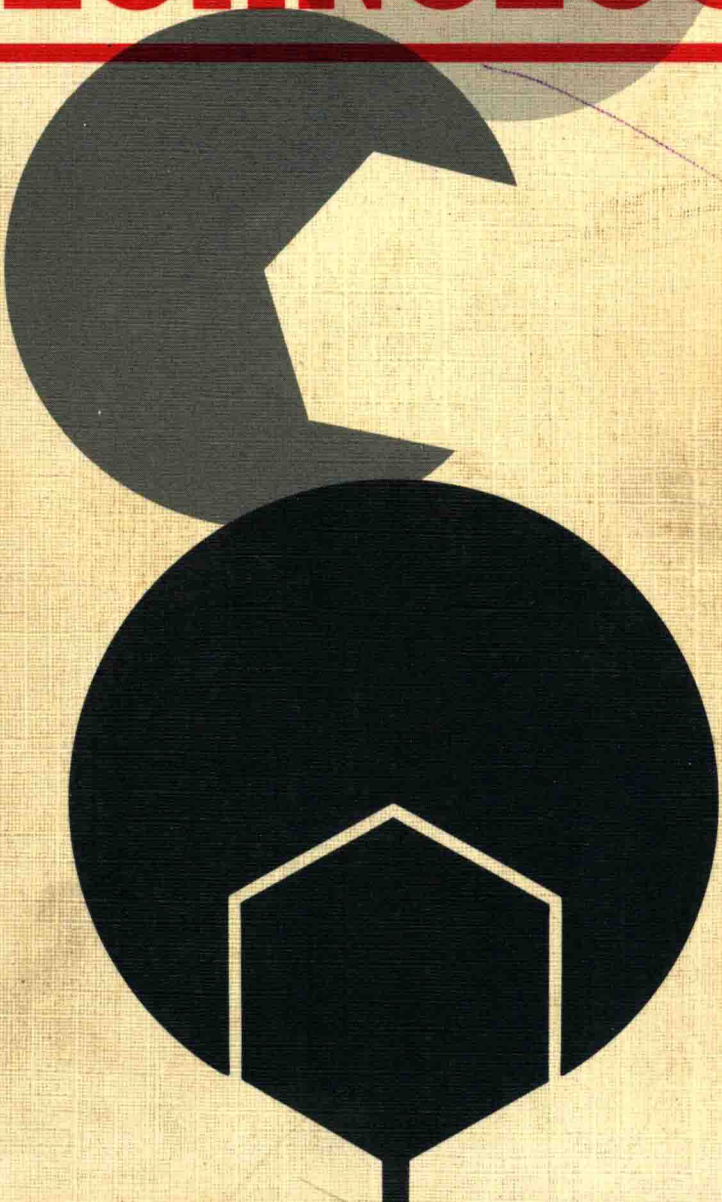
M.F. CHAPLIN AND C. BUCKE

ENZYME TECHNOLOGY



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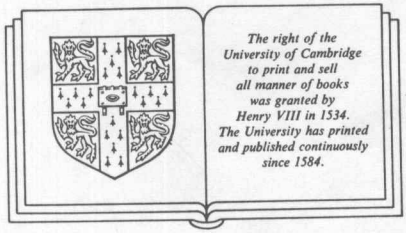

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Enzyme technology

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 (g m^{-1} 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 (m^2 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 (m^2 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).

D_S	diffusivity of a substrate ($m^2\ s^{-1}$)
D_P	diffusivity of a product ($m^2\ s^{-1}$)
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 ($kg\ m^{-2}\ s^{-1}$)
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 ($kJ\ mol^{-1}$)
$[H_o^+]$	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
Log P	solvent polarity
Re	Reynolds number
pI	Isoelectric point
pK_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 ($= 8.314$ J K^{-1} 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 (kg cm^{-1} 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_{max}^{app}	apparent maximum rate of reaction (mol s^{-1})

V_{org}	volume of the organic phase
V^{r}	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

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 classses 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):