Klaus Buchholz, Volker Kasche,
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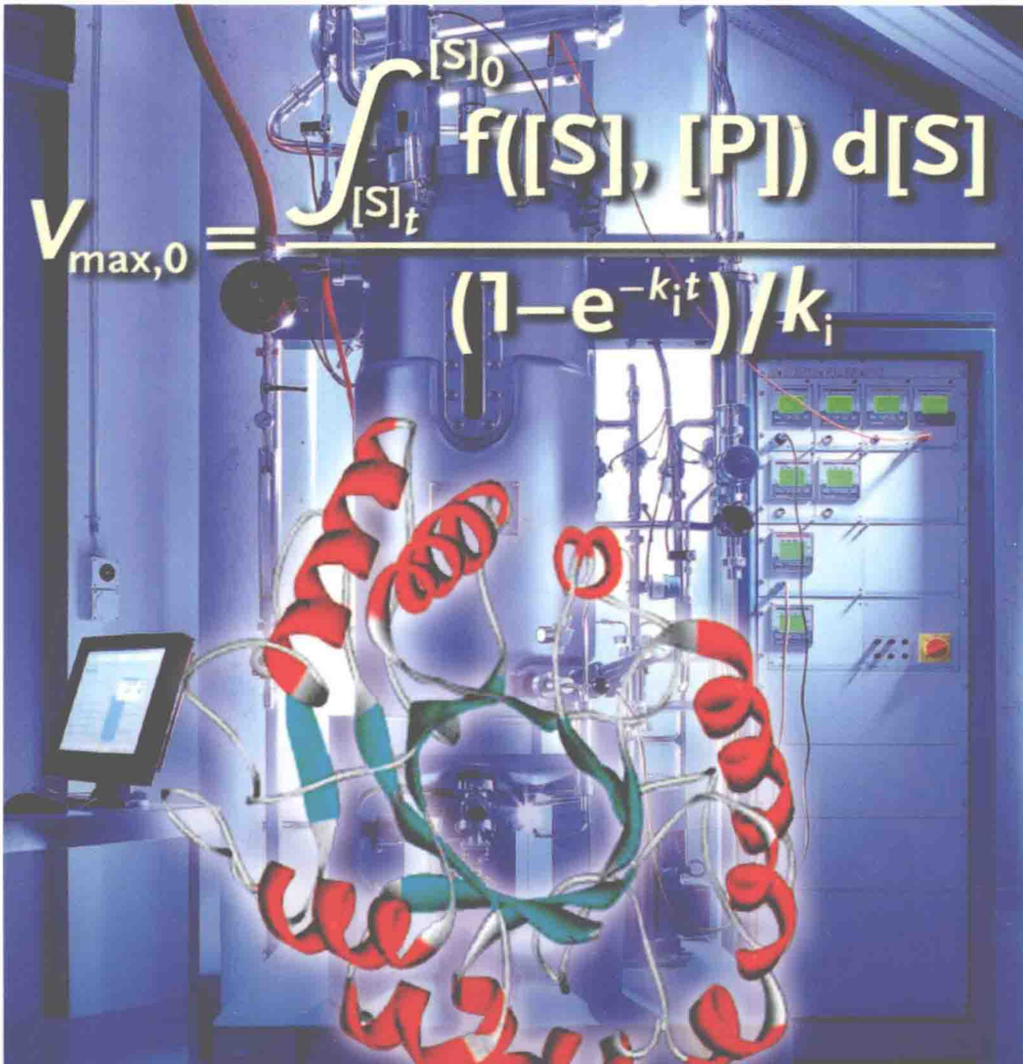
WILEY-VCH

Biocatalysts and Enzyme Technology



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WILEY-VCH Verlag GmbH & Co. KGaA

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Cover illustration:
Bioreactor with kind permission from Bioengineering, Switzerland.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnd.ddb.de>.

Printed in the Federal Republic of Germany
Printed on acid-free and low chlorine paper

Cover Design Grafig-Design SCHULZ, Fußgönheim
Typesetting Fotosatz Detzner, Speyer
Printing Strauss GmbH, Mörlenbach
Binding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN-13: 978-3-527-30497-4
ISBN-10: 3-527-30497-5

Preface

To the First German Edition

Biotechnology is the technical application of biological systems or parts thereof to provide products and services to meet human needs. It can, besides other techniques, contribute towards doing this in a sustainable manner. Since, in the majority of cases, renewable raw materials and biological systems are used in biotechnological processes, these processes can – and should – be performed practically without waste, as all of the byproducts can be recycled.

The development of natural and engineering science fundamentals for the design of such processes remains a challenge to biotechnology – a field that originated from the overlapping areas of biology, chemistry, and process engineering.

The requisite education for a career in biotechnology consists, in addition to a basic knowledge of each of these fields, of further biotechnological aspects which must provide an overview over the entire field and a deeper insight into different areas of biotechnology. The biotechnological production of various materials is performed either in fermenters using living cells (technical microbiology), or with enzymes – either in an isolated form or contained in cells – as biocatalysts. Indeed, the latter aspect has developed during recent years to form that area of biotechnology known as enzyme technology, or applied biocatalysis.

The aim of the present textbook is to provide a deeper insight into the fundamentals of enzyme technology and applied biocatalysis. It especially stresses the following inter-relationships: A thorough understanding of enzymes as biocatalysts and the integration of knowledge of the natural sciences of biology (especially biochemistry), cell and molecular biology; physico-chemical aspects of catalysis and molecular interactions in solutions; heterogeneous systems and interphase boundaries; and the physics of mass transfer processes. The same applies to the inter-relations between enzyme technology and chemical and process engineering, which are based on the above natural sciences.

In less than a century since the start of industrial enzyme production, enzyme technology and its products have steadily gained increasing importance. In the industrial production of materials to meet the demands of everyday life, enzymes play an important role – and one which is often barely recognized. Their application ranges from the production of processed foods such as bread, cheese, juice and beer,

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to pharmaceuticals and fine chemicals, to the processing of leather and textiles, as process aids in detergents, and also in environmental engineering.

Meeting the demand for these new products – which increasingly include newly developed and/or sterically pure pharmaceuticals and fine chemicals – has become an important incentive for the further development of biocatalysts and enzyme technology. Of similar importance is the development of new sustainable production processes for existing products, and this is detailed in Chapter 1, which forms an Introduction.

Enzymes as catalysts are of key importance in biotechnology, similar to the role of nucleic acids as carriers of genetic information. Their application as isolated catalysts justifies detailed examination of the fundamentals of enzymes as biocatalysts, and this topic is covered in Chapter 2. Enzymes can also be analyzed on a molecular level, and their kinetics described mathematically. This is essential for an analytical description and the rational design of enzyme processes. Enzymes can also catalyze a reaction in both directions – a property which may be applied in enzyme technology to achieve a reaction end-point both rapidly and with a high product yield. The thermodynamics of the catalyzed reaction must also be considered, as well as the properties of the enzyme. The amount of enzyme required for a given conversion of substrate per unit time must be calculated in order to estimate enzyme costs, and in turn the economic feasibility of a process. Thus, the quantitative treatment of biocatalysis is also highlighted in Chapter 2.

When the enzyme costs are too high, they can be reduced by improving the production of enzymes, and this subject is reviewed in Chapter 3 (*Chapter 4 in the present book*).

In Chapter 4 (*here Chapter 5*), applied biocatalysis with free enzymes is described, together with examples of relevant enzyme processes. When single enzyme use is economically unfavorable, the enzymes can be either reused or used for continuous processes in membrane reactors (Chapter 4; *here Chapter 5*) or by immobilization (Chapters 5 and 6; *here Chapters 6 and 7*). The immobilization of isolated enzymes is described in detail in Chapter 5, while the immobilization of microorganisms and cells, with special reference to environmental technology, is detailed in Chapter 6.

In order to describe analytically the processes associated with immobilized biocatalysts that are required for rational process design, the coupling of reaction and diffusion in these systems must be considered. To characterize immobilized biocatalysts, methods which were developed previously for analogous biological and process engineering (heterogeneous catalysis) systems can be used (Chapter 7; *here Chapter 8*).

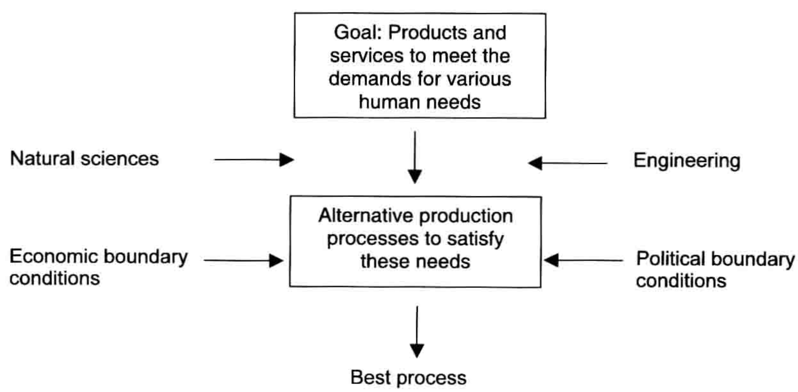
Details of reactors and process engineering techniques in enzyme technology are provided in Chapter 8 (*here Chapter 9*), while the analytical applications of free and immobilized enzymes is treated in Chapter 10 (*not covered in the present book*).

Within each chapter an introductory survey is provided, together with exercises and references to more general literature and original papers citing or relating the content of that chapter.

This textbook is designed to address both advanced and graduate students in biology, chemistry and biochemical, chemical and process engineering, as well as scientists in industry, research institutes and universities. It should provide a solid foundation that covers all relevant aspects of research and development in applied biocatalysis/enzyme technology. It should be remembered that these topics are not of equal importance in all cases, and therefore selective use of the book – depending on the individual reader's requirements – might be the best approach to its use.

In addition to a balanced methodological basis, we have also tried to present extensive data and examples of new processes, in order to stress the relevance of these in industrial practice.

From our point of view it is also important to stress the interactions, which exist beyond the scientific and engineering context within our society and environment. The importance and necessity of these interactions for a sustainable development has been realized during the past two decades, and this has resulted in new economic and political boundary conditions for scientific and engineering development. Problems such as allergic responses to enzymes in detergents and, more recently, to enzymes produced in recombinant organisms, have direct influences on enzyme technology/applied biocatalysis. Therefore, an integrated process design must also consider its environmental impact, from the supply and efficient use of the raw materials to the minimization and recycling of the byproducts and waste. Political boundary conditions derived from the concept of sustainability, when expressed in laws and other regulations, necessitates due consideration in research and development. The design of sustainable processes is therefore an important challenge for applied biocatalysis/enzyme technology. Ethical aspects must also be considered when gene technology is applied, and this is an increasing consideration in the production of technical and pharmaceutical enzymes. The many interactions between research and development and economic and political boundary conditions must be considered for all applications of natural and engineering sciences. Most importantly, this must be appreciated during the early phases of any development, with subsequent evaluation and selection of the best alternative production processes to meet a variety of human needs, as is illustrated in the following scheme:



This book has been developed from our lecture notes and materials, and we also thank all those who provided valuable help and recommendations for the book's production. In particular, we thank Dipl.-Ing. Klaus Gollembiewsky, Dr. Lieker, Dr. Noll-Borchers, and Dipl. Chem. André Rieks.

Klaus Buchholz
Volker Kasche

To the First English Edition

The basic philosophy of the previous German edition is retained, but the contents have been revised and updated to account for the considerable development in enzyme technology/applied biocatalysis since the German edition was prepared some 10 years ago. Hence, a new chapter (Chapter 3) has been added to account for the increasing importance of enzymes as biocatalysts in organic chemistry. Recent progress in protein design (by rational means and directed evolution) has been considerably expanded in Chapter 2. The final chapter has been amended with more detailed case studies to illustrate the problems that must be solved in the design of enzyme processes. An appendix on information retrieval using library and internet resources has also been added, and we thank Thomas Hapke (Subject Librarian for Chemical Engineering at the Library of the Technical University Hamburg-Harburg) for help in the preparation of this material. The chapter on enzymes for analytical purposes has been removed in this English edition as it now is beyond the scope of this textbook.

We thank Prof. Dr. L. Jaenicke and Prof. Dr. J.K.P. Weder for their very constructive suggestions for corrections and improvements of the German edition.

The authors of this edition thank Prof. Dr. Andreas Bommarius, Dr. Aurelio Hidalgo, Dr. Janne Kerovuo, Dr. Tanja Kummer, Dr. Dieter Krämer, Dr. Brian Morgan, Sven Pedersen, Poul Poulsen, Prof. Dr. Peter Reilly, Dr. Klaus Sauber, Dr. Wilhelm Tischer, and Dr. David Weiner for valuable discussions, revisions and suggestions while preparing this book.

January 2005

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1 Introduction to Enzyme Technology

1.1 Introduction

Biotechnology offers an increasing potential for the production of goods to meet various human needs. In enzyme technology – a sub-field of biotechnology – new processes have been and are being developed to manufacture both bulk and high added-value products utilizing enzymes as biocatalysts, in order to meet needs such as food (e.g., bread, cheese, beer, vinegar), fine chemicals (e.g., amino acids, vitamins), and pharmaceuticals. Enzymes are also used to provide services, as in washing and environmental processes, or for analytical and diagnostic purposes. The driving force in the development of enzyme technology, both in academia and industry, has been and will continue to be:

- the development of new and better products, processes and services to meet these needs; and/or
- the improvement of processes to produce existing products from new raw materials as biomass.

The goal of these approaches is to design innovative products and processes that are not only competitive but also meet criteria of sustainability. The concept of sustainability was introduced by the World Commission on Environment and Development (WCED, 1987) with the aim to promote a necessary "... development that meets the needs of the present without compromising the ability of future generations to meet their own needs". To determine the sustainability of a process, criteria that evaluate its economic, environmental and social impact must be used (Gram et al., 2001; Raven, 2002; Clark and Dickson, 2003). A positive effect in all these three fields is required for a sustainable process. Criteria for the quantitative evaluation of the economic and environmental impact are in contrast with the criteria for the social impact, easy to formulate. In order to be economically and environmentally more sustainable than an existing processes, a new process must be designed to reduce not only the consumption of resources (e.g., raw materials, energy, air, water), waste production and environmental impact, but also to increase the recycling of waste per kilogram of product.

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1.1.1
What are *Biocatalysts*?

Biocatalysts are either proteins (*enzymes*) or, in a few cases, they may be nucleic acids (*ribozymes*; some RNA molecules can catalyze the hydrolysis of RNA. These ribozymes were detected in the 1980s and will not be dealt with here; Cech, 1993). Today, we know that enzymes are necessary in all living systems, to catalyze all chemical reactions required for their survival and reproduction – rapidly, selectively and efficiently. Isolated enzymes can also catalyze these reactions. In the case of enzymes however, the question whether they can also act as catalysts outside living systems had been a point of controversy among biochemists in the beginning of the twentieth century. It was shown at an early stage however that enzymes could indeed be used as catalysts outside living cells, and several processes in which they were applied as biocatalysts have been patented (see Section 1.3).

These excellent properties of enzymes are utilized in enzyme technology. For example, they can be used as biocatalysts to catalyze chemical reactions on an industrial scale in a sustainable manner. Their application covers the production of desired products for all human material needs (e.g., food, animal feed, pharmaceuticals, fine and bulk chemicals, fibers, hygiene, and environmental technology), as well as in a wide range of analytical purposes, especially in diagnostics. In fact, during the past 50 years the rapid increase in our knowledge of enzymes – as well as their biosynthesis and molecular biology – now allows their rational use as biocatalysts in many processes, and in addition their modification and optimization for new synthetic schemes and the solution of analytical problems.

This introductory chapter outlines the technical and economic potential of enzyme technology as part of biotechnology. Briefly, it describes the historical background of enzymes, as well as their advantages and disadvantages, and compares these to alternative production processes. In addition, the current and potential importance – and the problems to consider in the rational design of enzyme processes – are also outlined.

1.2
Goals and Potential of Biotechnological Production Processes

Biomass – that is, renewable raw materials – has been and will continue to be a sustainable resource which is required to meet a variety of human material needs. In developed countries such as Germany, biomass covers ≈30 % of the raw material need – equivalent to ~7000 kg per person per year. The distribution of biomass across different human demands is shown schematically in Figure 1.1. This distribution of the consumption is representative for a developed country in the regions that have a high energy consumption during the winter. However, the consumption of energy (expressed as tons of coal equivalent per capita in 1999) showed a wide range, from 11.4 in the United States, to 5.5 in Germany and the UK, 0.8 in China, and 0.43 in India (United Nations, 2002). This is mainly due to differences in energy use for housing,

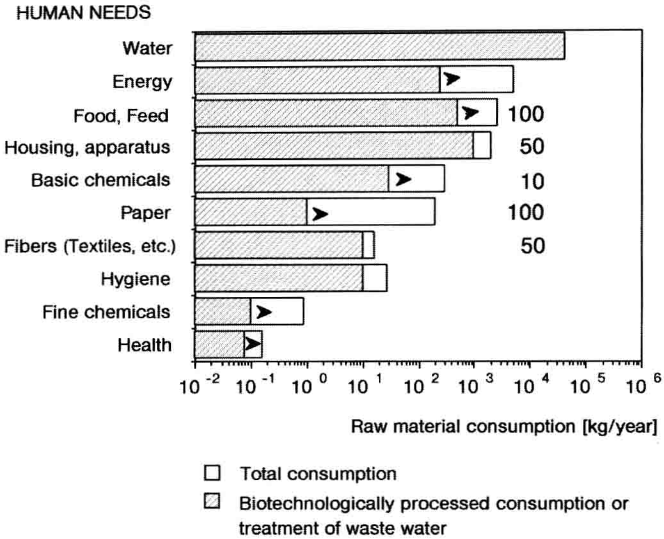

Fig. 1.1 Consumption of raw materials for various human needs per person and year in Germany 1992. The water consumption is only for household use. The arrowheads indicate the current increase in biotechnological processing of the products for different demands. For food and animal feed, only renewable raw materials (biomass) can be used, the figures to the right give the percentage biomass of the raw materials used for the production. For hygiene, fine chemicals and health products, 0–100 % of the raw materials can be biomass, depending on the product. After the use of the products the unavoidable waste must be recycled in a sustainable manner. Besides waste water, this results in about 1000 kg of solid waste (soil, building materials, plastics, sludge, etc.) that must be recycled in suitable environmental biotechnology processes. Energy is measured in coal equivalents.

transport and the production of other material needs. In less-developed countries, although the fraction of biomass as raw material to meet human demands is higher than in the developed countries, the total consumption is smaller.

Biomass – in contrast to non-renewable raw materials such as metals, coal, and oil – is renewable in a sustainable manner when the following criteria are fulfilled:

- the C-, N-, O-, and salt-cycles in the biosphere are conserved; and
- the conditions for a sustainable biomass production through photosynthesis and biological turnover of biomass in soil and aqueous systems are conserved.

Currently, these criteria are not fulfilled on a global level, one example being the imbalance between the CO_2 production to meet energy requirements and its consumption by photosynthesis in the presently decreasing areas of rain forests. This leads to global warming and other consequences that further violate these criteria. International treaties – for example, the Kyoto convention – have been introduced in an attempt to counteract these developments and to reach a goal that fulfills the above criteria.

When the above sustainability criteria are fulfilled, biomass can be used as raw material to meet the human demands illustrated in Figure 1.1. The needs for hu-