

Biotechnology

**edited by
H.-J. Rehm and G. Reed**

Volume 6b

Special Microbial Processes

**Volume Editor:
H.-J. Rehm**



0045729

Biotechnology

edited by H.-J. Rehm and G. Reed

Volume 6b
Special Microbial Processes

Volume Editor:
Hans-Jürgen Rehm



Prof. Dr. H.-J. Rehm
Institut für Mikrobiologie
der Universität
Corrensstraße 3
D-4400 Münster
Federal Republic of Germany

Dr. G. Reed
Universal Foods Corp.
Technical Center
6143 N 60th Street
Milwaukee, WI 53218
USA

This book was carefully produced. Nevertheless, authors, editors and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Editorial Director: Dr. Hans F. Ebel
Editorial Manager: Christa Maria Schultz
Production Manager: Peter J. Biel

This book contains 298 figures and 136 tables

Deutsche Bibliothek Cataloguing-in Publication-Data

Biotechnology: a comprehensive treatise in 8 vol. / ed. by H.-J. Rehm and G. Reed. –
[Ausg. in 8 Bd.]. – Weinheim; Basel (Switzerland); Cambridge; New York, NY: VCH.
Teilw. mit d. Verl.-Angabe Verl. Chemie, Weinheim, Deerfield Beach, Florida, Basel. –
Teilw. mit d. Erscheinungsorten Weinheim, Deerfield Beach, FL. –
Teilw. mit d. Erscheinungsorten Weinheim, New York
NE: Rehm, Hans J. [Hrsg.]
[Ausg. in 8 Bd.]
Vol. 6b. Special microbial processes / Vol. ed.: Hans-Jürgen Rehm. – 1988
ISBN 3-527-26093-5 (Weinheim ...) Gewebe
ISBN 0-89573-413-3 (New York ...) Gewebe

© VCH Verlagsgesellschaft mbH, D-6940 Weinheim (Federal Republic of Germany), 1988
All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprint, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers.
Compositor and Printer: Zechnersche Buchdruckerei, D-6720 Speyer
Bookbinding: Klambt-Druck GmbH, D-6720 Speyer
Printed in the Federal Republic of Germany

Biotechnology

Volume 6b



Biotechnology

A Comprehensive Treatise in 8 Volumes

Volume 1

Microbial Fundamentals

Volume 2

Fundamentals of Biochemical Engineering

Volume 3

Biomass, Microorganisms for Special Applications,
Microbial Products I, Energy from Renewable Resources

Volume 4

Microbial Products II

Volume 5

Food and Feed Production with Microorganisms

Volume 6a

Biotransformations

Volume 6b

Special Microbial Processes

Volume 7a

Enzyme Technology

Volume 7b

Gene Technology

Volume 8

Microbial Degradations

© VCH Verlagsgesellschaft mbH, D-6940 Weinheim (Federal Republic of Germany), 1988

Distribution

VCH Verlagsgesellschaft, P.O. Box 101161, D-6940 Weinheim (Federal Republic of Germany)

Switzerland: VCH Verlags-AG, P.O. Box, CH-4020 Basel (Switzerland)

Great Britain and Ireland: VCH Publishers (UK) Ltd., 8 Wellington Court, Wellington Street,
Cambridge CB1 1HW (Great Britain)

USA and Canada: VCH Publishers, Suite 909, 220 East 23rd Street, New York, NY 10010-4606 (USA)

ISBN 3-527-26093-5 (VCH Verlagsgesellschaft)

ISBN 0-89573-413-3 (VCH Publishers)

Preface

This volume describes various fermentations and biotechnological processes which did not readily fit into the subject matter of preceding volumes.

The first three chapters deal with processes which have been developed in the past, and brings the reader up-to-date on newer developments. Plant cell and animal cell cultures have already been briefly described in Volume 1; the present volume covers the methodology in considerable more detail.

The chapter on poly-beta-hydroxybutyric acid is highly applications oriented, because PHB is a new microbial product with an excellent potential for successful commercial development.

The chapter "Coal in Biotechnology" also documents the opening of a new field in biotechnology.

The contribution on the microbial elimination of nitrogen and phosphorus supplements Volume 8. In the second edition of "Biotechnology" it will be included in the volume "Microbial Degradations".

Many other new fields – models for biotechnological photosynthesis, accumulation

and leaching of metals, future perspectives of biotechnology in space and more earth-bound matters such as the microbial processing of tobacco, flax, and leather, for example – have been treated in this volume because of their potential for future developments. It is hoped that this will stimulate the interest of readers in these new areas of biotechnological research.

The authors of this volume have worked diligently to make their wide knowledge and expertise available to the reader. Their contributions in Volume 6b will supplement the subject matter of preceding volumes so that the reader will get a clearer understanding of the entire field of biotechnology.

I wish to thank Dr. G. Reed for his invaluable editorship, Dr. H. F. Ebel of the VCH Verlagsgesellschaft for his always helpful advice and Mrs. Christa Schultz, also of the VCH Verlagsgesellschaft, for her untiring and always friendly and constructive help in getting this volume into print.

Münster, October 1988

Hans-Jürgen Rehm

0552890

Editorial Advisory Board

of "Biotechnology"

Prof. Dr. S. Aiba

Osaka University
Osaka, Japan

Prof. Dr. N. Blakebrough

Department of Chemical Engineering
University of Malaya
Kuala Lumpur 22-11
Malaysia

Prof. Dr. H. Brauer

Technische Universität Berlin
Berlin (West), Germany

Prof. Dr. H. Dellweg

Institut für Gärungsgewerbe
und Biotechnologie
Berlin (West), Germany

Prof. Dr. A. L. Demain

Massachusetts Institute of Technology
Cambridge, Mass., USA

Prof. Dr. G. Durand

Institut National des Sciences Appliquées
Toulouse, France

Prof. Dr. K. Esser

Ruhr-Universität Bochum
Bochum, Federal Republic of Germany

Prof. Dr. A. Fiechter

ETH Zürich
Zurich, Switzerland

Prof. Dr. S. Fukui

Kyoto University
Kyoto, Japan

Prof. Dr. Vera Johanides

University of Zagreb
Zagreb, Yugoslavia

Prof. Dr. K. Kieslich

Gesellschaft für Biotechnologische Forschung
Braunschweig-Stöckheim
Federal Republic of Germany

Prof. Dr. H. Klaushofer

Universität für Bodenkultur
Vienna, Austria

Prof. Dr. A. Kocková-Kratochvilová

Slovak Academy of Sciences
Bratislava, Czechoslovakia

Prof. Dr. R. M. Lafferty

Technische Universität Graz
Graz, Austria

Prof. Dr. M. D. Lilly

University College London
London, United Kingdom

Prof. Dr. R. I. Mateles

Stauffer Chemical Company
Westport, Conn., USA

Prof. Dr. K. Mosbach

ETH Zürich
Zurich, Switzerland

Prof. Dr. G. Shelef

Technion - Israel Institute of Technology
Haifa, Israel

Prof. Dr. G. K. Skryabin

USSR Academy of Sciences
Moscow, USSR

List of Contributors

of Volume 6b

Prof. Dr. Reinhard Bachofen

Institut für Pflanzenbiologie
Universität Zürich
CH-8008 Zürich
Switzerland

Dr. Linda D. Birch

Institut für Pflanzenbiologie
Universität Zürich
CH-8008 Zürich
Switzerland

Dr. Hubert Bahl

Institut für Mikrobiologie
Universität Göttingen
D-3400 Göttingen
Federal Republic of Germany

Dr. Michael Butler

Department of Biological Sciences
Manchester Polytechnic
Manchester M1 5GD
United Kingdom

Prof. Dr. T. E. Base

Department of Mechanical Engineering
Faculty of Engineering Science
The University of Western Ontario
London N6A 5B9
Canada

Prof. Dr. Alden Emery

Department of Medical Chemistry
and Pharmacognosy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, Indiana 47907
U.S.A.

Dr. Michael Beyer

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Priv.-Doz. Dr. Ulrich Fischer

Fachbereich Biologie
AG Geomikrobiologie
Universität Oldenburg
D-2900 Oldenburg
Federal Republic of Germany

Dr. Geoffrey M. Gadd

Department of Biological Sciences
University of Dundee DD1 4HN
United Kingdom

Prof. Dr. Gerhard Gottschalk

Institut für Mikrobiologie
Universität Göttingen
D-3400 Göttingen
Federal Republic of Germany

Alfred M. Gottscho

Vice President
Helme Tobacco Co.
Lancaster, Pennsylvania 17604
U.S.A.

Dr. Helmut Günther

Lehrstuhl für Organische Chemie
und Biochemie
Technische Universität München
D-8046 Garching
Federal Republic of Germany

Prof. Dr. Kurt Hannig

Abteilung Biochemie
Max-Planck-Institut
D-8033 Martinsried
Federal Republic of Germany

Prof. Dr. Peter F. Heinstein

Department of Medical Chemistry
and Pharmacognosy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, Indiana 47907
U.S.A.

Dr. Werner Hodek

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Prof. Dr. Ion I. Inculet

Department of Electrical Engineering
Faculty of Engineering Science
The University of Western Ontario
London N6A 5B9
Canada

Prof. Dr. Harald Jüntgen

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Prof. Dr. Isao Karube

Research Center
for Advanced Science and Technology
The University of Tokyo
Japan

Dr. Joseph J. Katz

Chemistry Division
Argonne National Laboratory
Argonne, Illinois 60439
U.S.A.

Dr. Jürgen Klein

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Prof. Dr. Helmut König

Abteilung für Angewandte Mikrobiologie
Universität Ulm
D-7900 Ulm
Federal Republic of Germany

Dr. Brigitta Korsatko

Institut für Pharmazeutische Chemie
Karl-Franzens Universität Graz
A-8010 Graz
Austria

Dr. Werner Korsatko

Institut für Pharmazeutische Chemie
Karl-Franzens Universität Graz
A-8010 Graz
Austria

Prof. Dr. Naim Kosaric

Faculty of Engineering Science
Department of Chemical
and Biochemical Engineering
The University of Western Ontario
London N6A 5B9
Canada

Dr. Sabine Kunst

Institut für Siedlungswasserwirtschaft
und Abfalltechnik
Universität Hannover
D-3000 Hannover
Federal Republic of Germany

Prof. Dr. Robert M. Lafferty

Institut für Biotechnologie,
Mikrobiologie und Abfalltechnologie
Technische Universität Graz
A-8010 Graz
Austria

Prof. Dr. N. M. Lefcoe

Faculty of Medicine
The University of Western Ontario
London N6A 5B9
Canada

Dr. Jeen-Lee Lin

Helme Tobacco Co.
Lancaster, Pennsylvania 17604
U.S.A.

Robert P. Lyng

R. P. Lyng and Associates
London N5Y 3B9
Canada

Dr. Ian S. Maddox

Department of Biotechnology
Massey University
Palmerston North
New Zealand

Prof. Dr. W. E. McKeen

Department of Plant Sciences
Faculty of Science
The University of Western Ontario
London N6A 5B9
Canada

Prof. Dr. Klaus Mudrack

Institut für Siedlungswasserwirtschaft
und Abfalltechnik
Universität Hannover
D-3000 Hannover
Federal Republic of Germany

Dipl.-Biol. Frank Pfeifer

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Dipl.-Ing. Ernst Pfeleiderer

Sparte Enzyme
Abteilung Ledertechnologie
Röhm GmbH
D-6100 Darmstadt
Federal Republic of Germany

Prof. Dr. Hans-Jürgen Rehm

Institut für Mikrobiologie
Universität Münster
D-4400 Münster
Federal Republic of Germany

Priv.-Doz. Dr. Hans Reichenbach

GBF Gesellschaft für Biotechnologische
Forschung
Arbeitsgruppe Mikrobielle Sekundärstoffe
D-3300 Braunschweig
Federal Republic of Germany

Dr. Roland Reiner

Sparte Enzyme
Abteilung Ledertechnologie
Röhm GmbH
D-6100 Darmstadt
Federal Republic of Germany

Dr. Margit Sára

Zentrum für Ultrastrukturforschung
und Ludwig Boltzmann Institut
für Ultrastrukturforschung
Universität für Bodenkultur
A-1180 Wien
Austria

Prof. Dr. Rolf D. Schmid

GBF Gesellschaft für Biotechnologische
Forschung
Bereich Enzymtechnologie
und Naturstoffchemie
D-3300 Braunschweig
Federal Republic of Germany

Dr. Reiner Schnettler

Lehrstuhl für Biotechnologie
Universität Würzburg
D-8700 Würzburg
Federal Republic of Germany

Dr. Heinrich Seewald

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Dr. H. S. Shekhar Sharma

Department of Agriculture
for Northern Ireland
Plant Pathology Research Division
Belfast BT9 5PX
United Kingdom

Prof. Dr. Helmut Simon

Lehrstuhl für Organische Chemie
und Biochemie
Technische Universität München
D-8046 Garching
Federal Republic of Germany

Prof. Dr. Uwe B. Sleytr

Zentrum für Ultrastrukturforschung
und Ludwig Boltzmann Institut
für Ultrastrukturforschung
Universität für Bodenkultur
A-1180 Wien
Austria

Prof. Dr. Arpad E. Torma

Department of Materials and
Metallurgical Engineering
The Sullivan Center for In-Situ Research
New Mexico Institute of Mining
and Technology
Socorro, New Mexico 87801
U.S.A.

Dr. Evamaria Wolff-Fischer

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Dipl.-Biol. Manfred van Afferden

Bergbau-Forschung GmbH
D-4300 Essen
Federal Republic of Germany

Prof. Dr. Ulrich Zimmermann

Lehrstuhl für Biotechnologie
Universität Würzburg
D-8700 Würzburg
Federal Republic of Germany

Prof. Dr. Gerhard Wenzel

Biologische Bundesanstalt für Land-
und Forstwirtschaft
Institut für Resistenzgenetik
D-8059 Grünbach
Federal Republic of Germany

Contents

Chapter 1

Microbial Production of Butanol/Acetone 1

by *Hubert Bahl*
and *Gerhard Gottschalk*

Chapter 2

Microbial Production of 2,3-Butanediol 31

by *Ian S. Maddox*

Chapter 3

Microbial Production of Glycerol and Other Polyols 51

by *Hans-Jürgen Rehm*

Chapter 4

Microbial Production of Hydrocarbons 71

by *Linda D. Birch*
and *Reinhard Bachofen*

Chapter 5

Microbial Production of Hydrogen 101

by *Naim Kosaric*
and *Robert P. Lyng*

Chapter 6

Microbial Production of Poly- β -hydroxybutyric Acid 135

by *Robert M. Lafferty*,
Robert Korsatko,
and *Brigitta Korsatko*

Chapter 7

Microbial and Enzymic Production of Labeled Compounds 177

by *Helmut Simon*
and *Helmut Günther*

Chapter 8

Photosynthesis in Biotechnology – Biomimetic Models 193

by *Joseph J. Katz*

XIV *Contents*

Chapter 9

Processes with Plant Cell Cultures 213

by *Peter F. Heinstei*
and *Alden Emery*

Chapter 10

Processes with Animal Cell and Tissue Cultures 249

by *Michael Butler*

Chapter 11

Biosensors and "Bioelectronics" 317

by *Rolf D. Schmid*
and *Isao Karube*

Chapter 12

Leaching of Metals 367

by *Arpad E. Torma*

Chapter 13

Accumulation of Metals by Microorganisms and Algae 401

by *Geoffrey M. Gadd*

Chapter 14

Microbial Elimination of Nitrogen and Phosphorus 435

by *Sabine Kunst*
and *Klaus Mudrack*

Chapter 15

Sulfur in Biotechnology 463

by *Ulrich Fischer*

Chapter 16

Coal in Biotechnology 497

by *Jürgen Klein, Michael Beyer, Manfred van Afferden, Werner Hodeck, Frank Pfeifer, Heinrich Seewald, Evamaria Wolff-Fischer, and Harald Jüntgen*

Chapter 17

Aerosols and Transportation of Microorganisms in Air 569

by *Ion I. Inculet, T. E. Base, N. M. Lefcoe, and W. E. McKeen*

Chapter 18

Membrane Biotechnology: Two-dimensional Protein Crystals for Ultrafiltration Purposes 615

by *Margit Sára*
and *Uwe B. Sleytr*

Chapter 19

*Biotechnology in Space:
Potentials and Perspectives* 637

by Ulrich Zimmermann,
Reiner Schnettler,
and Kurt Hannig

Chapter 20

*Gliding Bacteria
in Biotechnology* 673

by Hans Reichenbach

Chapter 21

Archaeobacteria 697

by Helmut König

Chapter 22

*Microorganisms in Processing
of Leather* 729

by Ernst Pfeleiderer
and Roland Reiner

Chapter 23

*Microorganisms in Processing
of Flax* 745

by H. S. Shekhar Sharma

Chapter 24

*Microorganisms in Processing
of Tobacco* 757

by Alfred M. Gottscho
and Jeen-Lee Lin

Chapter 25

*Biotechnology in Agriculture –
an Overview* 771

by Gerhard Wenzel

Index 797

Chapter 1

Microbial Production of Butanol/Acetone

Hubert Bahl
Gerhard Gottschalk

Institut für Mikrobiologie
der Georg-August-Universität
Göttingen, Federal Republic of Germany

- I. Introduction
- II. History
- III. Biology and Biochemistry of Solvent-producing Clostridia
 - A. Microorganisms
 - B. Biochemistry of Solvent Production
 - 1. Degradation of polymers
 - 2. Utilization of substrates
 - 3. Breakdown of substrates to pyruvate
 - 4. Formation of acetate, butyrate, and lactate
 - 5. Formation of ethanol, butanol, and acetone
 - 6. Shift from acidogenesis to solventogenesis
- IV. Industrial Fermentation Process
 - A. Culture Maintenance
 - B. Batch Culture Fermentation
 - 1. Inoculum development
 - 2. Medium preparation
 - 3. Main fermentation process
 - 4. Downstream processing
 - C. Limitations
- V. Future Process Development
 - A. Alternative Fermentation Substrates
 - B. Continuous Culture
 - C. Cell Immobilization and Recycling
 - D. Product Recovery
 - E. Strain Improvement
- VI. Conclusions
- VII. References

I. Introduction

It is fascinating that a few anaerobic microorganisms are able to ferment sugars to solvents such as *n*-butanol and acetone. This ability was already noticed by PASTEUR (1861), its industrial significance was recognized by FERNBACH and STRANGE (1911) and by WEIZMANN (1915), and from 1915 until about 1950 the acetone-butanol fermentation was the basis of an industrial process operated on a very large scale. With respect to fermenter volume it was only exceeded by the ethanol-producing industries. After 1950, the importance of this process declined rapidly, because the production of *n*-butanol and acetone from oil became economically more favorable. Only recently, a revival of the interest in this fermentation occurred. The acetone-butanol fermentation – as a result of the oil crisis – became again an appealing industrial process. So it was challenging to apply the achievements of modern biotechnology to it and to study in detail the reactions leading to acetone and butanol formation and their regulation. As a result, we have now a much better understanding of this fermentation as compared to the time when it was industrially operated. Following an excursion into the history of the process, focus of this chapter will be on these new developments.

A number of excellent reviews on the acetone-butanol fermentation have appeared in recent years (BARKER, 1956; BEESCH, 1953; ROOS, 1961; HASTINGS, 1978; SPIVEY, 1978; MOREIRA, 1983; EN-

NIS et al., 1986a; JONES and WOODS, 1986; MC NEIL and KRISTIANSEN, 1986; ROGERS, 1986); they should be consulted for additional information.

Some of the physical properties of acetone and butanol are summarized in Table 1. Acetone is an important intermediate in the manufacture of methacrylates and methyl isobutyl ketone and a solvent for resins, paints, varnishes, lacquers, and cellulose acetate; it is miscible in all proportions with water. *n*-Butanol is a precursor of butyl acetate and dibutyl phthalate and like acetone a good solvent. Its solubility in water is 8% (w/w).

II. History

The formation of butanol by fermentation was first noticed by PASTEUR in connection with his discovery of the butyrate fermentation (PASTEUR, 1861). The causative organism was named “vibrion butyrique”, and the term “anaerobic” was coined, when it was found that the bacteria responsible for this fermentation were killed in air. Pure cultures of butyrate producers additionally forming small amounts of *n*-butanol were obtained by WINOGRADSKY in 1895, and a strain (*Granulobacter butylicum*) yielding appreciable quantities of butanol was first described by BEIJERINCK (1896).

Table 1. Physical Properties of Acetone and Butanol

Property	Acetone	<i>n</i> -Butanol
Melting point at 101.3 kPa	−94.6°C	−90.2°C
Boiling point at 101.3 kPa	56.1°C	117.7°C
Specific gravity	0.7899	0.8098
Heat of vaporation	502.7 J/g	593.5 J/g
Heat of combustion	5587 J/g	36296 J/g
Vapor pressure at 20°C	24.62 kPa	0.61 kPa