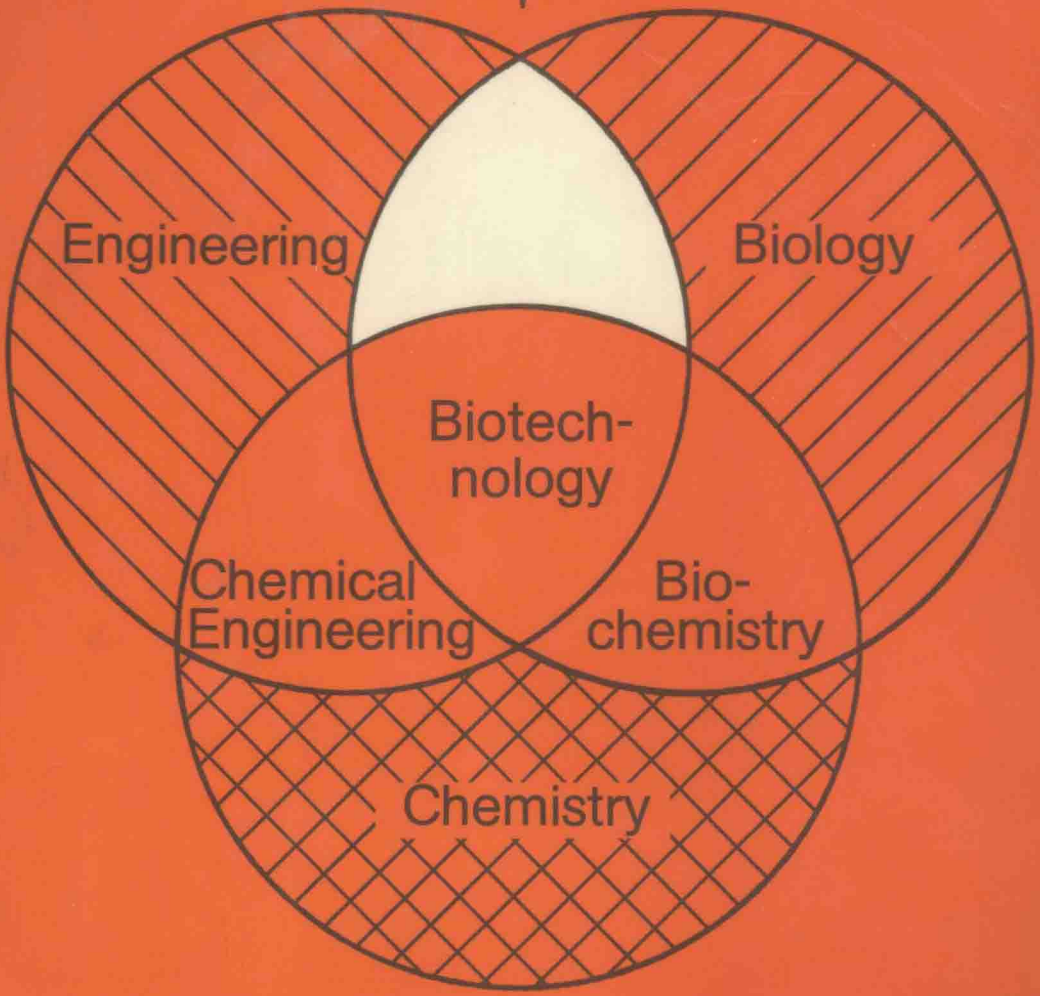
Chmiel · Hammes · Bailey (eds.)

Biochemical Engineering



Gustav Fischer

Biochemical Engineering

A Challenge for Interdisciplinary Cooperation

Edited by

H. Chmiel, W.P. Hammes and J.E. Bailey

334 Figures and 70 Tables

Gustav Fischer Verlag · Stuttgart · New York
1987

Addresses of Editors:

Prof. Dr. H. Chmiel
Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik
Nobelstraße 12
D-7000 Stuttgart 80

Prof. Dr. W. P. Hammes
Institut für Lebensmitteltechnologie
Garbenstraße 25
D-7000 Stuttgart 70

Prof. Dr. J. E. Bailey
Department of Chemical Engineering
Caltech
University of California
Pasadena CA 91125
U.S.A.

This publication was supported by the Ministerium für Wissenschaft und Kunst, Baden-Württemberg in Stuttgart/West Germany.

CIP-Titelaufnahme der Deutschen Bibliothek
»Biochemical engineering«: a challenge for interdisciplinary cooperation / H. Chmiel ... (eds.). – Stuttgart; New York: Fischer, 1987
ISBN 3-437-30574-3 (Stuttgart) kart.
ISBN 0-89574-259-4 (New York) kart.
NE: Chmiel, Horst [Hrsg.]

Total production: Graph. Großbetrieb Friedrich Pustet, Regensburg
Printed in Germany

ISBN 3-437-30574-3
US-ISBN 0-89574-259-4

Addresses of Authors

Dr. I. Adler, Sandoz AG, Verfahrenstechnik, Bau 240/126, 4002 Basel, Switzerland
Dr. N. Ahlmann, Institut für Technische Chemie, Rehbockstraße 38, 3000 Hannover 1, FRG
Dr. K. Akai, Snow Brand Milk Products, 13 Honshio-Cho, Shinyuku, Tokio, Japan
Dr. A.W. Alfermann, Institut für Entwicklungs- und Molekularbiologie der Pflanzen, Universität Düsseldorf, 4000 Düsseldorf, FRG
Dr. H. Allgaier, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Prof. Dr. J.E. Bailey, Dept. of Chem. Engineering, Caltech University of California, Pasadena CA 91125, USA
Dr. U. Bartz, GH Essen, Fachbereich 13/Verfahrenstechnik, 4300 Essen,
Prof. Dr. W. Bauer, Fraunhofer-Institut, Schragenhofstraße 35, 8000 München, FRG
Dr. W.-M. Becker, Forschungsinstitut Borstel, Parkallee 35, 2061 Borstel, FRG
Prof. Dr. L.A. Behie, Department of Chemical Engineering, 2500 University Drive N.W., University of Calgary, Calgary, Alberta, T2N 1N4, Canada
Prof. Dr. G. Belfort, Dept. of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA
Dr. W. Bessler, Institut für Immunbiologie der Universität Tübingen, 7400 Tübingen, FRG
Dr. D. Billig, Pharmacia AB, Pharmacia Biotechnology, Postbox 175, 75104 Uppsala, Sweden
Prof. Dr. H. Blenke, Institut für Chem. Verfahrenstechnik, Universität Stuttgart, Böblingerstraße 72, 7000 Stuttgart, FRG
Dr. P. Bond, Wolfson Unit of Plant Cell Biotechnology, University of Sheffield, Sheffield, UK
Dr. A. Borchert, Dechema Frankfurt, Postfach 970146, 6000 Frankfurt 97, FRG
Dr. M. Breuling, Pharmazeutisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. K.H. Brunner, Westfalia Separator AG, 4740 Oelde, Westfalen, FRG
Dr. H. Brückner, Institut für Lebensmitteltechnologie, Universität Hohenheim, Garbenstraße 25, 7000 Stuttgart 70, FRG
Dr. K. Buchholz, Institut für Landwirtschaftliche Technologie und Zuckerindustrie an der TU Braunschweig, 3300 Braunschweig, FRG
Dr. F. Buck, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Dr. H.-J. Bühring, Medizinische Universitätsklinik Tübingen, 7400 Tübingen, FRG
Dr. H.P. Call, Institut für Biologie IV (Mikrobiologie), Worringer Weg, 5100 Aachen, FRG
Dr. H. Chiba, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto, Japan
Prof. Dr. H. Chmiel, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. S. Cizmek, Faculty of Technology, Dept. of Reaction Engineering, 41000 Zagreb, Yugoslavia
Prof. Dr. C.L. Cooney, MIT, Dept. Chem. Engineering, Room 66-468, Cambridge, Ma 02139, USA
Dr. R.H. Cumming, Teesside Polytechnic, Cleveland TS1 3BA, Middlesbrough, UK
Dr. A.B. Czuppon, Biotec Diagnostica International GmbH, 3000 Hannover, FRG

Dr. C. Dauner-Schütze, Dechema Frankfurt, Postfach 970146, 6000 Frankfurt 97, FRG
Dr. T. Döppner, RWTH Aachen, Worringer Weg 1, 5100 Aachen, FRG
Dr. M. Drazic, Faculty of Technology, Dept. of Reaction Engineering, 41000 Zagreb, Yugoslavia
Prof. Dr. C.C. Emeis, Institut für Biologie IV (Mikrobiologie), Worringer Weg, 5100 Aachen, FRG
Dr. L. Evans, MIT, Dept. Chem. Engineering, Room 66-468, Cambridge, Ma 02139, USA
Dr. B. Fera, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Prof. Dr. M.W. Fowler, Wolfson Unit of Plant Cell Biotechnology, University of Sheffield, Sheffield, UK
Dr. G.M. Gaucher, Department of Biological Sciences, University of Calgary, Calgary, Alberta, T2N 1N4, Canada
Dr. B. Gehrlein, Institut für Lebensmitteltechnologie, Universität Hohenheim, Garbenstraße 25, 7000 Stuttgart 70, FRG
Dr. E. Gibert, Vogelbusch GmbH, Blechturmgasse 11, 1050 Wien, Austria
Prof. Dr. E.D. Gilles, Institut für Regelungstechnik und Systemdynamik, Pfaffenwaldring 9, 7000 Stuttgart 80, FRG
Dr. I.S. Gosling, BIOSEP, Harwell Laboratory, Oxfordshire OXX110RA, UK
Dipl. Ing. W. Gudernatsch, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Prof. Dr. W.P. Hammes, Institut für Lebensmitteltechnologie, Universität Hohenheim, Garbenstraße 25, 7000 Stuttgart 70, FRG
Dipl. Ing. E. Hanssmann, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Prof. Dr. W. Hartmeier, RWTH Aachen, Worringer Weg 1, 5100 Aachen, FRG
Dr. C. Heath, Dept. of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180-3590, USA
Dr. L.M.G. Heilmeyer jr., Institut für Physiologische Chemie, Ruhr-Universität, 4630 Bochum, FRG
Dr. A. Heinrichs, RWTH Aachen, Worringer Weg 1, 5100 Aachen, FRG
Dr. F. Hill, Chem. Werke Hüls AG, Postfach 2840, Holsterhauserstraße 160, 4690 Herne 2, FRG
Dr. O. Holst, Department of Biotechnology, University of Lund, P.O. Box 124, 22100 Lund, Sweden
Dipl. Ing. M. Howaldt, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. M. Höckel, Frauenklinik der Universität Mainz, 6500 Mainz, FRG
Dr. Th. Hörner, Institut für Mikrobiologie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. W. Hummel, Institut für Enzymtechnologie der Universität Düsseldorf, KFA Jülich, Postfach 2050, 5170 Jülich, FRG
Dr. W. Hunger, Mühlwang 9, 2260 Bosbüll, FRG
Dr. B. Husadzics, Faculty of Technology, Dept. of Reaction Engineering, 41000 Zagreb, Yugoslavia
Dr. Y. Imada, Mitsubishi Chem. Ind. Research, 227 Yokohama- Shi, Japan
Prof. Dr. J.-C. Janson, Pharmacia AB, Bioteknik Gruppen, 751 82 Uppsala, Sweden
Prof. Dr. G. Jung, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG

Dr. U. Kabatek, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. N. Kalogerakis, Department of Chemical Engineering,University of Calgary, Calgary, Alberta, T2N 1N4, Canada
Dr. S. Katayama, Snow Brand Milk Products, 13 Honshio-Cho, Shinyuku, Tokio, Japan
Dr. R. Kaul, Department of Biotechnology, University of Lund, P.O. Box 124, 22100 Lund, Sweden
Dr. G. Kawanisi, Snow Brand Milk Products, 13 Honshio-Cho, Shinyuku, Tokio, Japan
Dr. R. Keller, Hoechst AG, Hauptlabor 3, Postfach 800320, 600 Frankfurt 80, FRG
Dr. R. Kellner, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. A. Kern, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Prof. Dr. H.G. Kessler Institut für Lebensmittelverfahrenstechnik und Molkereitechnik, Wüttingerstraße 45, 8050 Freising, FRG
Dr. M. Kieß, Institut für Lebensmitteltechnologie, Universität Hohenheim, Garbenstraße 25, 7000 Stuttgart 70, FRG
Dipl. Ing. K. Kimmerle, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. M. Kolvenbach, Institut für Biologie IV (Mikrobiologie), Worringer Weg, 5100 Aachen, FRG
Dr. B. Krützfeld, Mineralogisches Institut der Universität Freiburg, 7800 Freiburg, FRG
Prof. Dr. M.-R. Kula, Institut für Enzymtechnologie der Universität Düsseldorf, KFA Jülich, Postfach 2050, 5170 Jülich, FRG
Prof. Dr. K.D. Kulbe, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. B. Kunz, Fraunhofer-Institut, Schragenhofstraße 35, 8000 München, FRG
Dr. G. Kupferschmitt, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Dr. U. Kulozik, Institut für Lebensmittelverfahrenstechnik und Molkereitechnik, Wüttingerstraße 45, 8050 Freising, FRG
Dr. M. Larsson, Department of Biotechnology, University of Lund, P.O. Box 124, 22100 Lund, Sweden
Dr. W. Leuchtenberger, Degussa AG, Postfach 1345, 6450 Hanau, FRG
Dr. E. Logemann, Institut für Rechtsmedizin der Universität Freiburg, 7800 Freiburg, FRG
Dr. P. Malinowsky, Millipore GmbH, Hauptstraße 71-79, 6236 Eschborn, FRG
Dr. A.S. Matthei, IMA GmbH, Feodor-Luenen-Straße 5, 3000 Hannover 61, FRG
Dr. B. Mattiasson, Department of Biotechnology, University of Lund, P.O. Box 124, 22100 Lund, Sweden
Dr. W. Merk, Dr. Karl Thomae GmbH, Birkendorferstraße 65, 7950 Biberach/Riß, FRG
Dr. M. Metz, Gewürzmüller, Klagenfurterstraße 1-3, 7000 Stuttgart 30, FRG
Dr. J. Metzger, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. H.E. Meyer, Institut für Physiologische Chemie, Ruhr-Universität, 4630 Bochum, FRG
Dr. S. Mizuno, Mitsubishi Chem. Ind. Research, 227 Yokohama- Shi, Japan
Prof. Dr. A. Moser, Technische Universität Graz, Institut für Biotechnologie, 8054 Graz, Austria
Dr. C.P. Muller, Medizinische Universitätsklinik Tübingen, 7400 Tübingen, FRG
Dr. A. Murakami, Snow Brand Milk Products, 519, Shimoishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi, 329-05, Japan

Dr. D. Müller-Schulte, Institut für Biologie IV (Mikrobiologie), Worringer Weg, 5100 Aachen, FRG
Dr. A. Niehoff, Institut für Technische Chemie, Rehbockstraße 38, 3000 Hannover 1, FRG
Dr. H. Ohkishi, Mitsubishi Chem. Ind. Research, 227 Yokohama- Shi, Japan
Prof. Dr. T. Oolman, Department of Chemical Engineering, University of Utah, 3064 MEB, Salt Lake City, UT 84412, USA
Dr. N. Pavlovic, Faculty of Technology, Dept. of Reaction Engineering, 41000 Zagreb, Yugoslavia
Dr. D. Petrides, MIT, Dept. Chem. Engineering, Room 66-468, Cambridge, Ma 02139, USA
Dr. P. Péringer, EPFL, 1051 Lausanne, Switzerland
Dr. H. Plainer, Röhm GmbH, Postfach 4242, 6100 Darmstadt, FRG
Dr. U. Plöcker, Degussa AG, Postfach 1345, 6450 Hanau, FRG
Dr. A. Purgstaller, Technische Universität Graz, Institut für Biotechnologie, 8054 Graz, Austria
Dr. E. Reinhard, Pharmazeutisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG
Prof. Dr. M. Reuß, Technische Universität Berlin, FG Biotechnologie, 1000 Berlin, FRG
Dr. U. Rinas, Institut für Technische Chemie, Rehbockstraße 38, 3000 Hannover 1, FRG
Dr. W. Rodatz, Bio-Rad Laboratories, 8000 München, FRG
Dr. H. Rüterjans, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Dr. R. Sasaki, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto, Japan
Dr. I. Saschewag, Lehrstuhl für Allg. Chemie und Biochemie der Universität München, 8050 Freising-Weihenstephan, FRG
Dr. W. Schaubschläger, Forschungsinstitut Borstel, Parkallee 35, 2061 Borstel, FRG
Dr. Th. Scheper, Institut für Technische Chemie, Rehbockstraße 38, 3000 Hannover 1, FRG
Dr. M. Schlaak, Forschungsinstitut Borstel, Parkallee 35, 2061 Borstel, FRG
Prof. Dr. M. Schlingmann, Hoechst AG, Hauptlabor 3, Postfach 800320, 600 Frankfurt 80, FRG
Dipl. Chem. E. Schmidt, Institut für Biotechnologie, KFA Jülich, Postfach 1913, 5170 Jülich, FRG
Prof. Dr. H.-L. Schmidt, Lehrstuhl für Allg. Chemie und Biochemie der Universität München, 8050 Freising-Weihenstephan, FRG
Dr. J. Schmidt, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Prof. Dr. G. Schmidt-Kastner, Bayer AG, Friedrich-Ebert-Straße 217, 5600 Wuppertal 1, FRG
Dr. M. Schmitz, RWTH Aachen, Worringer Weg 1, 5100 Aachen, FRG
Dr. G. Schneider, Biochemie GmbH, 6250 Kundl/Tirol, Austria
Dr. U. Schneider, Institut für Mikrobiologie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Prof. Dr. K. Schügerl, Institut für Technische Chemie, Universität Hannover, Callinstraße 3, 3000 Hannover, FRG
Dr. H. Schütte, Gesellschaft für Biotechnologische Forschung, 3300 Braunschweig, FRG
Dr. D. Schwantag, Pharmazeutisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. J.-P Schwitzguébel, EPFL, 1051 Lausanne, Switzerland
Dr. A.H. Scragg, Wolfson Unit of Plant Cell Biotechnology, University of Sheffield, Sheffield, UK
Dr. U. Seitz, Pharmazeutisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG

Dr. M. Seki, Mitsubishi Chem. Ind. Research, 227 Yokohama- Shi, Japan
Dr. B. Singrün, Institut für Biophysikalische Chemie, Universitätsklinikum, Haus 75 a, Theodor-Stern-Kai 7, 6000 Frankfurt 70, FRG
Dr. H. Somitsch, Technische Universität Graz, Institut für Biotechnologie, 8054 Graz, Austria
Dr. H. Spieler, Pharmazeutisches Institut, Auf der Morgenstelle, 7400 Tübingen, FRG
Dr. B.G. Sprößler, Röhm GmbH, Postfach 4242, 6100 Darmstadt, FRG
Dr. E. Stoppok, GH Essen, Fachbereich 13/Verfahrenstechnik, 4300 Essen, FRG
Dr. H. Strathmann, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. G. Street, Teesside Polytechnic, Cleveland TS1 3BA, Middlesbrough, UK
Dr. J.E. Strong jr., MIT, Dept. Chem. Engineering, Room 66-468, Cambridge, Ma 02139, USA
Dr. A.R. Thomson, Research Division Kodak, Headstone Drive, HA72MY Harrow Mid., UK
Dr. S. Tramm-Werner, RWTH Aachen, Worringer Weg 1, 5100 Aachen, FRG
Dr. I. Tripatzis, IMA GmbH, Feodor-Luenen-Straße 5, 3000 Hannover 61, FRG
Dr. J.M. Tuffnell, Teesside Polytechnic, Cleveland TS1 3BA, Middlesbrough, UK
Dr. M. Ueda, Snow Brand Milk Products, 13 Honshio-Cho, Shinyuku, Tokio, Japan
Dr. Z. Uzon, IMA GmbH, Feodor-Luenen-Straße 5, 3000 Hannover 61, FRG
Dr. D. Vasic-Racki, Faculty of Technology, Dept. of Reaction Engineering, 41000 Zagreb, Yugoslavia
Dipl. Phys. E. Walitza, Fraunhofer-Institut für Grenzflächen und Bioverfahrenstechnik, Nobelstraße 12, 7000 Stuttgart 80, FRG
Dr. F. Walz, Dr. Karl Thomae GmbH, Birkendorferstraße 65, 7950 Biberach/Riß, FRG
Prof. Dr. C. Wandrey, Institut für Biotechnologie II, Kernforschungsanlage Jülich, Postfach 1913, 5170 Jülich, FRG
Prof. Dr. R.G. Werner, Dr. Karl Thomae GmbH, Birkendorferstraße 65, 7950 Biberach/Riß, FRG
Dr. W. Westphal, Forschungsinstitut Borstel, Parkallee 35, 2061 Borstel, FRG
Dr. K.-H. Wiesmüller, Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG
Prof. Dr. J.H. Wissler, Institut für Physiologische Chemie, Ruhr-Universität, 4630 Bochum, FRG
Dr. N. Yoshikawa, Mitsubishi Chem. Ind. Research, 227 Yokohama- Shi, Japan
Prof. Dr. H. Zähner, Institut für Mikrobiologie, Universität Tübingen, Auf der Morgenstelle, 7400 Tübingen, FRG

Preface

In 1986, the Minister for Research and Technology of the Federal Republic of Germany and the Minister of State of Baden-Württemberg agreed to establish and finacially support a research center in Stuttgart concentrating on biochemical engineering. The motivation for this joint venture is to complement biotechnological activities in industry as well as at universities and other research institutes of Baden-Württemberg by research activities concentrating on biochemical engineering and applied biosciences. Cooperation among different biochemical disciplines and a transfer of know-how to industry from fundamental university research will be facilitated via applied research undertaken by the Fraunhofer-Gesellschaft.

An international congress, attended by over 500 delegates representing industry and universities from more than 15 countries, was held in Stuttgart in September 1986, to mark the start of this new activity. The overall themes of the congress were the opportunities and the challenges of the future, to integrate advances in biology, chemistry, and process engineering to promote the industrial practice of biotechnology. Some new products and new processes will not be commercially viable unless engineers and scientists can cooperate to combine synergistically the complete spectrum of genetic, chemical, and process methods for optimizing the product and the bioprocess.

In this volume, which contains most of the papers presented at the congress, new developments and future problems in biochemical engineering and in the related biological sciences are highlighted. These papers address the following main themes in biochemical engineering:

- Bioprocess technology with special attention to scale-up
- The application of bioprocesses for the production of foodstuffs, fine chemicals, and pharmaceuticals
- Optimization of existing types of bioreactors and development of a new generation of bioreactors
- Downstream processing, including product separation, concentration and purification

Hopefully this volume will aid the international biochemical engineering community to identify new opportunities for cooperation and for future contributions to the emerging biotechnology industry.

The State Government of Baden-Württemberg, especially the Ministry of Science and Arts are acknowledged for their generous financial support. Mrs. R. Walitza and Mr. E. Walitza prepaired the typoscript, many thanks.

H. Chmiel, W.P. Hammes, J.E. Bailey — Stuttgart, December 1987

Contents

Bailey, J.E.
Growth and Product Formation Kinetics in Recombinant Microbes 1

Hammes, W.P.
Biotechnology, Biochemical Engineering and Food Technology 11

Zähner, H.
Secondary Microbial Metabolism and Biotechnology 36

Wandrey, C.
The Growing Importance of Biochemical Engineering 43

Blenke, H.
Process Engineering Contribution to Bioreactor Design and Operation 69

Schügerl, K.
Interrelation Between Fluid Dynamics, Medium Properties and Biology of Cells in Bioreactors 92

Behie, L.A.; Kalogerakis, N. and Gaucher, G.M.
The Application of Continuous Three Phase Fluidized Bed Bioreactors to the Production of Pharmaceuticals 95

Schmidt-Kastner, G.
Bioreactors - Production of Bioproducts and Biotransformation by Enzymes and Immobilized Enzymes 111

Schmidt, H.-L. and Saschewag, I.
Possibilities of Process Control with Biosensors 132

Gilles, E.D.
Advanced Control Techniques in Bioengineering 139

Reuß, M.
Process Computer Coupled Substrate Feeding for Fermentation Processes 149

Cooney, C.L.; Strong, J.E. jr.; Petrides, D. and Evans, L.
Computer-Aided Design of Biochemical Recovery Processes 169

Schlingmann, M. and Keller, R.
Technical Aspects of the Workup and Purification of Nucleic Acids 181

Plöcker, U. and Leuchtenberger, W.
Process Development for the Production of α–Amino Acids and α-Hydroxycarboxylic Acids 186

Hill, F.F.
Dry Living Microorganisms - Products for the Food Industry 199

Hunger W.
Starter Cultures for the Food Industry - New Developments in Dairy Industry 216

Bauer, W. and Kunz, B.
Solid-State Fermentation in Food Industry 228

Chmiel, H.
Downstream Processing in Biotechnology 242

Thomson, A.R.
Adsorption in Downstream Processing 252

Janson, J.-C.
Process Chromatography for Protein Purification 261

Brunner, K.H.
Extraction in Downstream Processing 272

Strathmann, H.
Membranes in Downstream Processing- State-of-the-Art and
Future Developments 288

Billig, D.
Animal Cell Cultures for the Production of Bioproducts 301

Heath, C. and Belfort, G.
Modeling of Substrate Mass Transfer and Uptake in Hollow Fiber and Microcapsule
Bioreactors 307

Werner, R.G.; Merk, W. and Walz F.
Fermentation with Immobilized Cell Cultures 327

Fowler, M.W.; Bond, P. and Scragg, A.H.
Developments in Plant Cell Culture Technology 333

Adler, I.
Equipments and Bioreactors 342

Metz M. und Hammes, W.P.
Optimum Conditions for Growth and Nitrate Reductase Activity of
Micrococcus varians 355

Wissler, J.H.
Bioreactors with Laminar and Gradient Hydrodynamics:
Assessement of *in-vivo*-Hydraulics of Tissue Structures in Biochemical Engineering
of Cell Cultures *in vitro* 361

Plainer, H. and Sprößler, B.G.
Immobilized Enzymes in the Sugar Industry 368

Kolvenbach, M.; Call, H.P.; Müller-Schulte, D. and Emeis, C.C.
Affinity Chromatographic Separation of Proteins with Novel Gels and Simple Ligands 372

Holst, O.; Kaul, R.; Larsson, M. and Mattiasson, B.
Some Model Studies on the Integration of Bioprocesses and Downstrean Processing 376

Schwitzguébel, J.-P. and Péringer, P.
An Assessment of Separated-Phase Anaerobic Digestion to Improve Methane Production 381

Wissler, J.H.; Logemann, E.; Meyer, H.E.; Krützfeld, B. and Höckel, M.
An Endogenous Bioactive Metallo-Ribonucleo-Polypeptide: A Copper-Containing Monocytic Blood Vessel Morphogen as Novel Type of "Wound-Hormone" 385

Hummel, W.; Schmidt, E.; Schütte, H. and Kula, M.-R.
Isolation of Microorganisms Containing High Levels of Phenylalanine Dehydrogenase 392

Gosling, I.S.
The Design of Adsorptions in Downstream Processing: The Importance of Data from Small-Scale Experiments 396

Howaldt, M.; Kulbe, K.D. and Chmiel, H.
Optimization of Performance of an Enzyme Membrane Reactor: Computer Simulation and Experimental Results 402

Kulozik, U. and Kessler H.G.
Factors Influencing Solute Permeation Through Reverse-Osmosis Membranes in Food and Biochemical Engineering 407

Gudernatsch, W.; Kimmerle, K.; Strathmann, H. and Chmiel, H.
Continuous Removal of Ethanol from Fermentation Broths by Pervaporation 409

Oolman, T.; Walitza, E.; Hanssmann, E. and Chmiel, H.
Viscoelasticity and Mass Transfer in Bioreactors 416

Dauner-Schütze, C.; Borchert, A. and Buchholz, K.
High Capacity Adsorbent for Enzyme Isolation 421

Murakami, A.; Akai, K.; Ueda, M.; Kawanisi, G.; Katayama, S.; Chiba, H. and Sasaki, R.
Production and Characterization of Human Erythropoietin 424

Schneider G.; Purgstaller, A.; Somitsch, H. and Moser A.
Approach to Mixing in Bioreactors - Experimental Verification, Methodology and Model Bioreactor 428

Yoshikawa, N.; Seki, M.; Mizuno, S.; Imada, Y. and Ohkishi, H.
Microbial Production of Muconic Acid 431

Malinowsky, P.
Viable Cell Harvesting by Tangential Flow Filtration 434

Seitz, U. and Reinhard, E.
Cryostorage of Plant Cell Cultures and Preservation of Biochemical Capacities 437

Becker, W.-M.; Schaubschläger, W.; Westphal, W. and Schlaak, M.
Purification of Grass Pollen Allergens by Means of Immobilized Monoclonal Antibodies 440

Breuling, M.; Spieler, H.; Schwantag, D.; Alfermann, A.W. and Reinhard, E.
Large Scale Cultivation of Plant Cells for Production of Natural Products 443

Brückner, H. ; Gehrlein, B. and Kieß M.
The Polypeptide Mycotoxin Paracelsin from *Trichoderma reesei:* Scaled-Up Isolation and Possible Consequences 450

Fera, B.; Singrün, B.; Kupferschmitt, G.; Schmidt, J.; Buck, F. and Rüterjans, H.
Preparation of ^{15}N Labeled Nucleosides and Large Scale Synthesis of Labeled Oligonucleotides with a New Type DNA-Synthesizer 454

Ahlmann, N.; Niehoff, A.; Rinas, U.; Scheper, Th. and Schügerl, K.
Continuous On-Line Monitoring of Intracellular Enzyme Activity 459

Gibert, E.
Production of Single Cell Protein (SCP) with Reduced Nucleic Acid Content 464

Hartmeier, W. and Schmitz, M.
Peptide Alkaloids using Immobilized Mycelia of *Claviceps purpurea* 469

Tramm-Werner, S. and Hartmeier, W.
Heterogeneous Continuous Fermentation Using a Flocculent Strain of *Zymomonas Mobilis* 474

Hartmeier, W.; Heinrichs, A. and Döppner, T.
Coimmobilization of Gluconate Forming Bacteria and Fungal Catalase 479

Vasic-Racki, D.; Husadzic, B.; Drazic, M.; Pavlovic, N. and Cizmek, S.
The Estimation of Kinetic Constants for Glucose Isomerase in the *Streptomyces bambergiensis* Whole Cells 483

Schmidt, E.; Hummel, W.; Wandrey, C. and Kula, M.-R.
Continuous Production of L-Phenylalanine from Phenylpyruvate Using Isolated L-Phenylalanine Dehydrogenase from *Rhodococcus* sp. 487

Jung, G.; Kabatek, U.; Kern, A.; Metzger, J.; Wiesmüller, K.-H.; Bühring, H.-J.; Muller, C.P. and Bessler, W.
Efficient Production of Antibodies by Antigen Presentation via a New Carrier/Adjuvans System 491

Jung, G.; Allgaier, H.; Kellner, R.; Schneider U.; Hörner, Th.; Zähner, H. and Werner, R.G.
Isolation, Purification and Structure Elucidation of Epidermin, a Ribosomally Synthesized Polypeptide Antibiotic 494

Cumming, R.H.; Street, G. and Tuffnell, J.M.
Optimisation of Autolysis for the Release of Intracellular Proteins 498

Bartz, U.; Stoppok, E. and Buchholz, K.
Pilot Scale Investigations on Anaerobic Conversion of Solid Substrates 506

Czuppon, A.B.; Tripatzis, I.; Matthei, A.S.; Uzon, Z.; Rodatz, W.
One Step Purification of Monoclonal Antibodies and Antibody-Methotrexate Conjugates by HPLC 509

Subject index 514

Growth and Product Formation Kinetics in Recombinant Microbes

Bailey, J.E.

Department of Chemical Engineering, California Institute of Technology, Pasadena, California 91125, U.S.A.

The central theme of this presentation is the set of exciting opportunities which exist for cooperation between biochemical engineers and molecular biologists to optimize organisms and processes for production of valuable compounds using genetically engineered cells. The examples which follow, which will illustrate productive interactions between information and methods from the biological and engineering sciences, focus on the cell as a complex chemical reactor. Understanding of the response of the cell to different types of genetic and environmental manipulations is central to bioreactor optimization and to placing bioreactor scale-up on a more reliable and systematic basis.

Considering the rate of synthesis of a single protein product expressed from a cloned gene, the prototype example through most of this presentation, it is apparent that the synthesis rate is given by the integral of product of the density of cells in the culture, the rate at which plasmid-containing cells synthesize the product protein as a function of the number of plasmid copies in the cell, and the distribution of plasmid content within individual cells in the population. In the following examples we will examine how engineering methods can be used to analyze and describe in quantitative terms the plasmid replication and segregation properties in a recombinant population, the rates of product synthesis, and the growth characteristics of plasmid-harboring cells.

Genetically Structured Models: Plasmid Replication

As an example of one type of analysis at the level of molecular interactions, we shall first consider plasmid replication kinetics. Like the kinetics of synthesis of many biological macromolecules, it is the frequency of initiation of synthesis which dictates the synthesis rates. As a particular example, we shall examine the replication of plasmid λdv and a detailed mathematical model that has been formulated to describe this system (1-3). This system serves as a prototype for a class of kinetic models which seek to describe the detailed interactions between regulatory molecules in the cell and the key processes which determine synthesis rates of classes of molecules of central interest. λdv is a deletion derivative of phage λ which replicates autonomously in *Escherichia coli*. There is a wealth of information on the control of replication initiation in λdv which is based upon the work of Matsubara, Ptashne, and others.

Examining the origin of replication of plasmid λdv, we see that replication depends upon interaction of a number of proteins with different domains of the DNA. A critical regulating factor in replication initiation of this plasmid is the frequency of transcription which begins at the promoter P_R which is upstream of the binding site for initiator proteins at the *ori* site in the origin of replication. Transcription activity at P_R is regulated at binding of the *cro* gene product, here designated R, at three different binding sites, two of which overlap and hence, when bound with R, inactivate the promoter P_R.

Based upon this mechanistic picture of the control system which has been provided by extensive molecular biology research, we can formulate material balances on the key components and complexes, applying the tools of thermodynamic and kinetic calculations and material balances from chemical engineering in a straightforward way. For example, calculation of the transcription efficiency at the promoter P_R can be accomplished by assuming equilibrium binding of the repressor R at the three binding sites designated O_R1, O_R2, O_R3. In addition to delineating this mechanism, our colleagues in molecular biology have also measured the binding constant for repressor association with each of these different binding domains, providing the needed parameters to calculate this transcription efficiency. Similarly, information from the molecular biology literature provides values of the other parameters which enter into the set of differential equations required to describe the replication of plasmid λdv during life cycle of single *E. coli* cell. All but one of the model parameters is so available, and this final parameter may be adjusted in order to make the calculated number of plasmids per cell agree with the observed value for the wild-type plasmid.

Calculations with the model converge to a repetitive, cyclic state after a few cell cycles. In this cyclic state, the concentrations of all species at the end and the beginning of the cell cycle are equal. After adjusting the single model parameter to obtain the correct number of plasmids, it is important to note that the corresponding calculated value of repressor content of the cell is very close to the experimentally measured value, suggesting that the model has the correct structure for consistent description of this replication apparatus.

Of course, simpler models could be used to calculate the time progression of plasmid content for this plasmid. However, the model formulated at the molecular level serves a different function. It can be used to relate fine scale genetic changes to the corresponding cellular properties. To illustrate for the plasmid λdv, we note that a number of replication mutants of this plasmid have been isolated and the locus of several mutations in the repressor binding sites have been determined at the level of nucleotide sequence. Knowing the precise location of each location, we can, with a model formulated in molecular level detail, identify a corresponding specific model parameter with each mutation so that the effect of each mutation, in terms of the model, is to alter one or a very small number of clearly identified model parameters. Based upon this correspondence, simulations show consistently good agreement between calculated plasmid and repressor levels and the experimental values for these quantities for all mutants (2). This example illustrates an important theme of genetically structured models. These models provide the potential for a quantitative mapping from nucleotide sequence of key regulatory and functional regions to the eventual outcome in terms of cellular properties, in this case plasmid content of the cells. Using such models, biochemical engineers can cooperate with molecular biologists to probe and to alter in a systematic fashion the control systems used in recombinant cells to obtain the desired cellular levels of plasmid content and cloned gene expression.

Rapid Analysis of Plasmid Propagation Using Flow Cytometry

The plasmid content of a recombinant culture is determined not only by the replication characteristics of the plasmid in the host cell but also by the segregation properties of the plasmid. Unlike chromosomes which are almost always faithfully partitioned between dividing and offspring cells, plasmids often segregate in a less precise fashion upon cell division. The result is a population of cells which is heterogeneous with respect to plasmid content and which may contain a significant fraction of cells without any plasmids at all. The simplest model for growth of an unstable recombinant population, developed and studied by many different research groups over the past several years, divides the culture into two populations, one plasmid-free cells and the other plasmid-containing cells (e.g. 4,5). The central parameters which determine the dynamics and the eventual time-invariant properties of this model are the ratio of specific growth rates of plasmid-free to plasmid-containing cells and the probability that plasmid-free cells are formed upon cell division. The fraction of cells which contain plasmid after 25 generations have been evaluated in terms of these two parameters by Imanaka and Aiba (4).

Their results show clearly that, in the absence of selection which means that plasmid-free cells grow more rapidly than plasmid-containing cells, the fraction of plasmid-containing cells in the culture after 25 generations can be much less than 0.1 for even very small asymmetric partitioning probabilities. The choice of 25 generations for this calculation is based upon an approximate estimate of the number of generations involved between a stock culture and a production fermentor.

In order to understand and to minimize problems of plasmid loss during production using recombinant cells, it is important to determine the effects which different plasmid constructions, different hosts, and different growth conditions have on plasmid segregational stability. The traditional method of determining the fraction of plasmid-containing cells in the culture involves plating a sample of cells simultaneously on selective and nonselective solid media, growing up several such plates, counting colonies, and evaluating the results. This process takes in excess of one day for recombinant *Saccharomyces cerevisiae* cells, and a more rapid and more statistically rich measurement approach is necessary.

An alternative strategy for measuring plasmid replication and segregation characteristics has been developed in this laboratory based upon specific labeling of plasmid-containing cells and analysis of this labeling using flow cytometry (6 - 8). To summarize, a marker is placed in the plasmid which consists of a *lac Z* fragment, expression of which results in *E. coli* β-galactosidase activity in yeast. Expression of this gene in *S. cerevisiae* is regulated by an inducible *GAL10/CYC1* promoter, and a host strain containing the *reg1* mutation is used in order to eliminate catabolite repression of the *GAL* promoter. In order to label plasmid-containing cells, a sample from the growing recombinant population is induced to express β–galactosidase by addition of galactose and, after about one generation of growth in induction medium, the cells are chilled and permeabilized. Then, a fluorogenic substrate and trapping reagent are added. The substrate is hydrolyzed by β-galactosidase, and the product of this enzyme-catalyzed reaction subsequently undergoes reaction with a trapping reagent to form a precipate in the cell which can be detected in the microscope and in the flow cytometer. Examining a population of *S. cerevisiae* D603 containing the plasmid pLGSD5 shows very heterogeneous labeling of the cells, indicating significant cell-to-cell heterogeneity with