Patents and Literature

Biocatalysis in Nonaqueous Media

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ABSTRACT

Biocatalysis in nonaqueous media is being used in increasing regularity both in academic and industrial research. A variety of biocatalysts have been used in organic media including enzymes, multienzyme systems, and whole cells. In addition, the nonaqueous media has encompassed both monophasic and biphasic solvent systems, enzymes and whole cells in reversed micelles, enzymes and cells in nearly anhydrous (no added water) solvents, and enzymes catalytically active in supercritical fluids and the gas phase. Recent US and overseas patents and scientific literature on biocatalysis in nonaqueous media are surveyed. Patent abstracts are summarized individually, and literature references are divided into major subheadings.

INTRODUCTION

The objective of the Patents and Literature Section is to summarize and cite recent developments in industrial and academic research as portrayed within the scope of current patents and literature and to highlight emerging biotechnological research areas. Three subject areas will be surveyed in 1988: biocatalysis in nonaqueous media; enzymatic and microbial production of optically active compounds; and monoclonal antibodies for clinical applications. The subject of the first Patent and Literature Section of 1988 is Biocatalysis in Nonaqueous Media.

PATENTS

This section covers the patents (including US and foreign) concerning biocatalysis in nonaqueous media from the period of January 1985 to December 1987. The major search headings were Enzymes or Cells or Bioconversions, with the crossterms: organic solvents, organic media, supercritical fluids, gas phase, and reverse micelles. Chemical Abstracts data base was used. The major patents recovered under the conditions of the search are described below. Copies of US patents can be obtained for \$1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Crook, S., Macrae, A. R., and Moore, H. EDIBLE FATS EP 170431, Feb. 5, 1986 Assignee: Unilever, Ltd.

A hardstock fat for use in manufacturing of margarine or other emulsified food spreads is prepared by fat or vegetable oil interesterification by a microbial lipase. In particular, a hardstock fat was manufactured by treating soybean oil plus 0.45 parts of lauric acid dissolved in 2.9 parts hexane with immobilized *Mucor miehei* lipase at 40°C.

Diamond, S. E., and Regina, F. R.
BINDING ASSAY WITH AMPLIFIED READ-OUT GAS-PHASE
DETECTION
EP 173055, Mar. 5, 1986
Assignee: Allied Corp.

An immunoassay procedure is described that uses gas-phase sensing of detectable moieties. The procedure consists of performing a specific binding reaction for the target binding pair in the presence of an enzyme, the concentration or activity of which being a function of the amount of target binding pair member, and in the presence of a substrate that can be converted into a detectable species in a volatile form by gas chromatography.

Drouzd, J. W., and Bailey, M. L.
BIOTRANSFORMATIONS
US 4,560,657, Feb. 1, 1984
Assignee: Shell Internationale Research Maatschappij B.V.

Alkylene oxides are produced from alkenes by an ethylene utilizing microorganism *Mycobacterium* NCIB 11626. The organism was cultured at 30°C and pH 6.6 in a mineral salts medium containing (NH₄)₂SO₄ to which 25 mL ethylene/min were added as C source. After the cells were harvested,

100 mg by dry weight were added to a 2-phase system of 0.5 mL phosphate buffer and 10 mL isooctane containing 5% 1-octene. A total of 0.2 mL ethylene were added to the sealed system which was shaken for 160 h. The result was 1,2-epoxyoctane with a specific production of 127 mol/g cell-h.

Empie, M. W. RESOLUTION OF RACEMATES OF AMINO ACIDS EP 171862, Feb. 19, 1986 Assignee: Stauffer Chem. Co.

Racemates of optically active amino acids can be resolved in a 2-phase solvent system in which the racemate is dissovled in a water-immiscible solvent and contacted with water. The equilibrium produced in the aqueous and organic phases enables an enzyme dissolved in the aqueous phase to selectively hydrolyze one isomer. The resulting optically pure amino acid can be recovered from the aqueous phase.

Klibanov, A. M., and Kirchner, G.
ENZYMIC PRODUCTION OF OPTICAL ISOMERS OF
2-HALOPROPIONIC ACIDS
US 4,601,987, Jul. 22, 1986
Assignee: Massachusetts Institute of Technology

A method is described for resolving racemic 2-halopropionic acids by lipase catalyzed asymmetric esterifications in an organic medium. The prefered lipase is from *Candida cylindracea* and is used for selective esterification to yield R isomers of 2-phenoxy propionic acid and its esters. These compounds have herbicide activities. Thus 4.34 g 2-chloropropionic acid, and 2 g lipase plus 11 mL 1-butanol were added to 400 mL hexane at 30 °C and vigorously agitated. The resulting Bu(+)-R-2-chloropropionate was produced in 80.8% yield after 6 h. The S isomer can be isolated from the resulting solution optimally after 68% of the R ester is formed.

FATTY ACID ESTER PRODUCTION CATALYZED BY LIPASE JP 60 78587, May 4, 1985
Assignee: Lion Corporation

Fatty acid esters are produced by the alcoholisis of an oil or fatty acid monoalkylester catalyzed by lipases from *Alciligenes, Arthrobacter,* or *Pseudomonas* immobilized to silica gel. Methyl oleate and methyl palmitate were produced in yields approaching 100%.

Munk, B. H. PRE-WASH COMPOSITIONS CONTAINING ENZYMES EP 177183, Apr. 9, 1986 Assignee: Chlorox Co.

Prewash enzyme compounds, effective in removing oil and grease stains are described that contain 60–70% organic solvents mixed with nonionic surfactants and aqueous enzyme solutions dispersed as reverse micelles. Encapsulating the enzymes in reverse micelles protects them from solvent degradation and prolongs their shelf-life.

Pokora, A. R., and Cyrus, W. L. PHENOLIC DEVELOPER RESINS US 4,647,952, Mar. 3, 1987 Assignee: The Mead Corporation

The peroxidase-catalyzed synthesis of formaldehyde-free phenolic resins for use as developer aids is described. The reactions are carried out in a number of solvents including both water-miscible and immiscible organic solvents. The phenolic units of the resins are directly bonded to one another through positions ortho or para to the hydroxyl group and are selected from the group consisting of an alkyl group, a halogen atom, an aryl group, a phenylalkyl group, an allyl group, a carboxyl group, and an amino group. The resulting resins are reacted with a metal salt to give the final products, which are useful as developer resins in forming colored images by reaction with substantially colorless electron donating compounds.

LITERATURE

This section surveys the literature in the area of biocatalysis in non-aqueous media published from January 1985 to December 1987. The headings and crossterms are the same as listed in the patent search, above. Once again, Chemical Abstracts data base was used. In addition to the literature recovered under the search constraints, citations referenced in several recent reviews on the subject are listed. The literature section is divided into the following headings: reviews; books; and research articles. The latter is further subdivided into nearly anhydrous solvent systems, reverse micelles, aqueous-organic biphasic systems, water-miscible organic-aqueous cosolvent systems, supercritical fluids, gas phase, and enzymatic stability in nonaqueous media.

Reviews

- 1. Cesti, P., Francalanci, F., and Foa, M. (1986), Enzyme Catalysis in Organic Solvents. *Chim. Ind.* (Milan), **68**, 130–133.
- 2. Duarte, J. C. (1987), Bioconversions in Organic Solvents. *Nato ASI Ser.*, *Ser A*, **128** (Perspect. Biotechnol.), 23–41.
- 3. Doddema, H. J. (1987), Enzymes Also Function in Organic Solvents. *PT-Procestech* **42**, 44–47.

- 4. Klibanov, A. M. (1986), Enzymes that Work in Organic Solvents. *Chemtech* **16**, 354–359.
- 5. Fukui, S. and Tanaka, A. (1985), Enzymic Reactions in Organic Solvents. *Endeavor*, **9**, 10–17.
- 6. Halling, P. J. (1987), Biocatalysis in Multi-Phase Reaction Mixtures Containing Organic Solvents. *Biotechnol. Adv.* **5,** 47–84.
- 7. Martinek, K., Berezin, I. V., Khmel'nitskii, Yu. L., and Levashov, A. V. (1987), Micellar Enzymology: Potentialities in Applied Areas (Biotechnology). Collect. Czech. Chem. Commun. 52, 2589–2602.
- Martinek, K., Berezin, I. V., Khmel'nitskii, Yu. L., Klyachko, N. L., and Levashov, A. V. (1987), Enzymes Entrapped into Reversed Micelles of Surfactants in Organic Solvents: Key Trends in Applied Enzymology (Biotechnology). Biocatalysis 1, 9-15.
- 9. Seno, M. and Noritomi, H. (1986), Enzyme Reactions in Reversed Micelle Systems. *Kagaku to Seibutsu* 24, 569–575.
- Luisi, P. L. and Magid, L. J. (1986), Solubilization of Enzymes and Nucleic Acids in Hydrocarbon Micellar Solutions. CRC Crit. Rev. Biochem. 20, 409– 474.
- 11. Luisi, P. L. and Laane, C. (1986), Solubilization of Enzymes in Apolar Solvents via Reverse Micelles. *Trends Biotechnol.* **4**, 153–161.
- 12. Berezin, I. V., Khmel'nitskii, Yu. L., Klyachko, N. L., Levashov, A. V., and Martinek, K. (1984), 3rd Eur. Congr. Biotechnol, vol. 3, 449-454.
- 13. Mattiasson, B. and Larsson, M. (1985), Extractive Bioconversions with Emphasis on Solvent Production. *Biotechnol. Genetic Eng. Rev.* 3, 137–174.

Books

- 1. Laane, C., Tramper, J., and Lilly, M. D., eds. (1987), *Biocatalysis in Organic Media*, Elsevier, Amsterdam, 426 pp.
- 2. Schneider, M. P., ed. (1986), Enzymes as Catalysts in Organic Solvents, Nato ASI Series, Series C: *Mathematical and Physical Sciences vol.* 178, D. Reidel, Dordrecht, 421 pp.

Research Articles

Nearly Anhydrous Systems

- 1. Boeriu, C. G., Dordick, J. S., and Klibanov, A. M. (1986), Enzymatic Reactions in Liquid and Solid Paraffins: Application for Enzyme-Based Temperature Abuse Sensors. *Bio/technology* **4**, 997–999.
- 2. Cesti P., Zaks, A., and Klibanov, A. M. (1985), Preparative Regioselective Acylation of Glycols by Enzymatic Transesterification in Organic Solvents. *Appl. Biochem. Biotechnol.* 11, 401-407.
- 3. Effenberger, F., Ziegler, T., and Foerster, S. (1987), Enzyme-Catalyzed Cyanohydrin Preparation in Organic Solvents. *Angew. Chem.* **99**, 491–492.
- 4. Goderis, H. L., Ampe, G., Feyten, M. P., Fouwe, B. L., Guffens, W. M., van Cauwenbergh, S. M., and Tobback, P. P. (1987), Lipase-Catalyzed Ester Exchange Reactions in Organic Media with Controlled Humidity. *Biotechnol. Bioeng.* 30, 258-266.
- 5. Grunwald, J., Wirz, B., Scollar, M. P., and Klibanov, A. M. (1986), Asym-

metric Oxidoreductions Catalyzed by Alcohol Dehydrogenase in Organic Solvents. J. Amer. Chem. Soc. 108, 6732-6734.

- 6. Gutman, A. L., Zuobi, K., and Boltansky, A. (1987), Enzymic lactonization of gamma-hydroxy esters in organic solvents. Synthesis of optically pure gamma-methylbutyrolactones and gamma-phenylbutyrolactone. *Tetrahedron Lett.* 28, 3861–3864.
- 7. Hoq, M. M., Tagami, H., Yamane, T., and Shimizu, S. (1985), Characteristics of Continuous Glyceride Synthesis by Lipase in a Microporous Hydrophobic Membrane Bioreactor. *Agr. Biol. Chem.* **49**, 335–342.
- 8. İnada, Y., Takahashi, K., Yoshimoto, T., Ajima, A., Matsushima, A., and Saito, Y. (1986), Application of Polyethylene Glycol-Modified Enzymes in Biotechnological Processes: Organic Solvent-Soluble Enzymes. *Trends Biotechnol.* 4, 190–194.
- Kazandjian, R. A., and Klibanov, A. M. (1985), Regioselective Oxidation of Phenols Catalyzed by Polyphenol Oxidase in Chloroform. J. Amer. Chem. Soc. 107, 5448-5450.
- 10. Kazandjian, R. Z., Dordick, J. S., and Klibanov, A. M. (1986), Enzymatic Analyses in Organic Media. *Biotechnol. Bioeng.* 28, 417-421.
- 11. Kirchner, G., Scollar M. P., and Klibanov, A. M. (1985), Resolution of Racemic Mixtures via Lipase Catalysis in Organic Solvents. *J. Amer. Chem. Soc.* **107**, 7072–7076.
- 12. Kise, H. and Shirato, H. (1985), Synthesis of Aromatic Amino Acid Ethyl Esters by Chymotrypsin in Solutions of High Ethanol Concentration. *Tetrahedron Lett.* **26**, 6081–6084.
- 13. Koshiro, S., Sonomoto, K., Tanaka, A. and Fukui, S. (1985), Stereoselective Esterification of d,l-Menthol by Polyurethane-Entrapped Lipase in Organic Solvent. *J. Biotechnol.* 2, 47–57.
- 14. Kresze, G. and Sabuni, M. (1987), Experiments on the Optical Resolution of Conduramine Analogs by Enzymic Transesterification in Organic Solvents. *Z. Naturforsch.*, C: Biosci. 42, 446–448.
- 15. Laane, C., Boeren, S., Vos, K., and Veeger, C. (1987), Rules for Optimization of Biocatalysis in Organic Solvents. *Biotechnol. Bioeng.* **30**, 81–87.
- Langrand, G., Secchi, M., Buono, G., Baratti, J., and Triantaphylides, C. (1985), Lipase-Catalyzed Ester Formation in Organic Solvents: An Easy Preparative Resolution of Alpha-Substituted Cyclohexanols. *Tetrahedron Lett.* 26, 1857–1860.
- 17. Langrand, G., Baratti, J., Buono, G., and Triantaphylides, C. (1986), Lipase Catalyzed Reactions and Strategy for Alcohol Resolution. *Tetrahedron Lett.* 27, 29–32.
- 18. Legoy, M. D., Kim, H. S., and Thomas, D. (1985), Use of Alcohol Dehydrogenase for Flavor Aldehyde Production. *Proc. Biochem.* 20, 145-148.
- 19. Marlot, C., Langrand, G., Triantaphylides, C., and Baratti, J. (1985), Ester Synthesis in Organic Solvent Catalyzed by Lipases Immobilized on Hydrophobic Supports. *Biotechnol. Lett.* 7, 647–650.
- Margolin, A. L. and Klibanov, A. M. (1986), Peptide Synthesis Catalyzed by Lipases in Anhydrous Organic Solvents. J. Amer. Chem. Soc. 109, 3802– 3804.
- 21. Margolin, A. L., Tai, D. F., and Klibanov, A. M. (1987), Incorporation of D-amino acids into peptides via enzymic condensation in organic solvents. *J. Amer. Chem. Soc.* **109**, 7885–7887.

- 22. Ooshima, H., Mori, H., and Harano, Y. (1985), Synthesis of Aspartame Precursor by Solid Thermolysin in Organic Solvents. *Biotechnol. Lett.* 7, 789–792.
- Saunders, R., Cheetham, P. S. J., and Hardman, R. (1986), Microbial Transformation of Crude Fenugreek Steroids. Enzyme Microbial Technol. 8, 549–555.
- 24. Takahashi, K., Nishimura, H., Yoshimoto, T., Okada, M., Ajima, A., Matsushima, A., Tamaura, Y., Saito, Y., and Inada, Y. (1984), Polyethylene Glycol-Modified Enzymes Trap Water on Their Surface and Exert Enzymic Activity in Organic Solvents. *Biotechnol. Lett.* 6, 765–770.
- 25. Tanaka, A. and Fukui, S. (1985), Bioconversion of Lipophilic Compounds by Immobilized Biocatalysts in the Presence of Organic Solvents. *Biotechnol. Ser.* 5 (Enzymes Immobilized Cells Biotechnol.), 149–176.
- 26. Therisod, M. and Klibanov, A. M. (1986), Facile Enzymatic Preparation of Monoacylated Sugars in Pyridine. J. Amer. Chem. Soc. 108, 5638-5640.
- Therisod, M. and Klibanov, A. M. (1987), Regioselective Acylation of Secondary Hydroxyl Groups in Sugars Catalyzed by Lipases in Organic Solvents.
 J. Amer. Chem. Soc. 109, 3977–3981.
- 28. Ueda, M., Mukataka, S., Sato, S., and Takahashi, J. (1986), Conditions for the Microbial Oxidation of Various Higher Alcohols in Isooctane. *Agric. Biol. Chem.* **50**, 1533–1537.
- 29. Wingard, L. B., Brackin, J. S., and Silver, R. (1986), Formation of Propylene Oxide by *Nocardia corallina* Immobilized in Liquid Paraffin. *Biotechnol. Bioeng.* 28, 343–348.
- 30. Wisdom, R. A., Dunnill, P., and Lilly, M. D. (1985), Enzymic Interesterification of Fats: The Effect of Non-Lipase Material on Immobilized Enzyme Activity. *Enzyme Microbial Technol.* 7, 567–572.
- 31. Zaks, A. and Klibanov, A. M. (1986), Substrate Specificity of Enzymes in Organic Solvents vs. Water is Reversed. J. Amer. Chem. Soc. 108, 2767–2768.
- 32. Zaks, A. and Klibanov, A. M. (1985), Enzyme Catalyzed Processes in Organic Solvents. *Proc. Natl. Acad. Sci. USA* 82, 3192–3196.

Reverse Micelles

- 1. Eremin, A. N. and Metelitsa, D. I. (1985), Regulation of the Catalytic Activity of Peroxidase in Surfactant Mixed Reversed Micelles. *Biokhimiya* (Moscow) **50**, 102–109.
- 2. Giovenco, S., Verheggen, F., and Laane, C. (1987), Purification of Intracellular Enzymes from Whole Bacterial Cells using Reverse Micelles. *Enzyme Microbial Technol.* 9, 470–473.
- 3. Han, D. and Rhee, J. S. (1986), Characteristics of Lipase-Catalyzed Hydrolysis of Olive Oil in AOT-Isooctane Reversed Micelles. *Biotechnol. Bioeng.* **28**, 1250–1255.
- 4. Haering, G., Luisi, P. L., and Meussdoerffer, E. (1985), Solubilization of Bacterial Cells in Organic Solvents via Reverse Micelles. *Biochem. Biophys. Res. Commun.* 127, 911–915.
- Kabanov, A. V., Klyachko, N. L., Pshezhetskii, A. V., Nametkin, S. N., Martinek, K., and Levashov, A. V. (1987), Kinetic Mechanisms of Enzymatic Catalysis in Systems of Surfactant Reversed Micelles in Organic Solvents. *Mol. Bio.* (Moscow) 21, 275–286.
- 6. Luisi, P. L. (1985), Enzymes Hosted in Reverse Micelles in Hydrocarbon Solutions. *Angew. Chem. Int. Ed.* **24,** 439–450.

7. Mevkh, A. T., Sud'ina, G. F., Lagutina, I. O., and Levashov, A. V. (1985), Catalytic Properties of the Membrane Enzyme, Prostaglandin H Synthetase, in a System of Aerosol-OT Reverse Micelles in Octane. *Biokhimiya* (Moscow) **50**, 1719–1723.

- 8. O'Connor, C. J., Stockley, I. C., and Walde, P. (1986), Studies in Bile Salt Solutions. XXII. The Effect of Reversed Micelles and of Aerosol-OT Aqueous Micelles on the Esterase Activity of Bile-Salt-Stimulated Human Milk Lipase. Determination of Enzyme-Inhibitor Complex Dissociation Constants. *Aust. J. Chem.* 39, 2037–2048.
- 9. Steinmann, B., Jaeckle, H., and Luisi, P. L. (1986), A Comparative Study of Lysozyme Conformation in Various Reverse Micellar Systems. *Biopolymers* **25**, 1133–1156.

Aqueous-Organic Biphasic Systems

- 1. Brink, L. E. S. and Tramper, J. (1985), Optimization of Organic Solvent in Multiphase Biocatalysis. *Biotechnol. Bioeng.* **27**, 1258–1269.
- 2. Brookes, I. K., Lilly, M. D., and Drozd, J. W. (1986), Stereospecific Hydrolysis of d,l-Menthyl Acetate by *Bacillus subtilis*: Mass Transfer reaction Interactions in a Liquid-Liquid system. *Enzyme Microbial Technol.* **8**, 53–57.
- 3. Cho, T. and Shuler, M. L. (1986), Multimembrane Bioreactor for Extractive Fermentation. *Biotechnol. Prog.* **2**, 53–60.
- 4. Furahashi, K., Shintani, M., and Takagi, M. (1986), Effects of Solvents on the Production of Epoxides by *Nocardia corallina* B-276. *Appl. Microbiol. Biotechnol.* **23**, 218-223.
- 5. Harbron, S., Smith, B. W., and Lilly, M. D. (1986), Two-Liquid Phase Biocatalysis: Epoxidation of 1,7-Octadiene by *Pseudomonas putida*. *Enzyme Microbial Technol.* **8,** 85–88.
- Honda, H., Taya, M., and Kobayashi, T. (1986), Ethanol Fermentation Associated with Solvent Extraction Using Immobilized Growing Cells of Saccharomyces cerevisiae and its Lactose-Fermentable Fusant. J. Chem. Eng. Japan 19, 268–273.
- 7. Hoq, M. M. Yamane, T., Shimizu, S., Funada, T., and Ishida, S. (1985), Continuous Hydrolysis of Olive Oil by Lipase in Microporous Hydrophobic Membrane Bioreactor. *J. Amer. Oil. Chem. Soc.* **62**, 1016–1021.
- 8. Hoq, H. H., Koike, M., Yamane, T., and Shimizu, S. (1985), Continuous Hydrolysis of Olive Oil in Microporous Hydrophobic Hollow Fiber Bioreactor. *Agric. Biol. Chem.* 49, 3171–3178.
- 9. Hoq, H. H., Yamane, T., and Shimizu, S. (1986), Role of Oleic Acid Solubilized in Buffer-Glycerol Solution on Adsorbed Lipase During Continuous Hydrolysis of Olive Oil in a Microporous Hydrophobic Membrane Bioreactor. *Enzyme Microbial Technol.* 8, 236–240.
- 10. Ishii, S., Taya, M., and Kobayashi, T. (1985), Production of Butanol by *Clostridium acetobutylicum* in Extractive Fermentation Systems. *J. Chem. Eng. Japan* 18, 125–130.
- 11. Khar, H. T., Tan, N. H., and Chua, C. L. (1986), Lipase-Catalyzed Hydrolysis of Palm-Oil. J. Amer. Oil. Chem. Soc. 63, 538-540.
- 12. Laane, C., Boeren, S., and Vos, K. (1985), On Optimizing Organic Solvents in Multi-Liquid-Phase Biocatalysis. *Trends Biotechnol.* 3, 252–2.
- 13. Maidan, R. and Willner, I. (1986), Photochemical and Chemical Enzyme Catalyzed Debromination of Meso-1,2-dibromostilbene in Multiphase Systems. *J. Amer. Chem. Soc.* **108**, 1080–1082.

- 14. van der Meer, A. B., Beenackers, A. A. C. M., and Stamhuis, E. J. (1986), Microbial Production of Epoxides from Alkenes in Continuous Multi-Phase Reactors. *Chem. Eng. Sci.* 41, 607–616.
- 15. Miyano, S., Kawahara, K., Inoue, Y., and Hashimoto, H. (1987), A Convenient Preparation of Optically Active 1,1'-Binaphthyl-2,2'-diol via Enzymatic Hydrolysis of the Racemic Diester. *Chem. Lett.* 355–356.
- 16. Mukataka, S., Kobayashi, T., and Takahashi, J. (1985), Kinetics of Enzymatic Hydrolysis of Lipids in Biphasic Organic-Aqueous Systems. *J. Ferm. Technol.* **63,** 461-466.
- 17. Nakanishi, K., Kamikubo, T., and Matsuno, R. (1985), Continuous Synthesis of *N*-0(benzyloxycarbonyl)-*L*-aspartyl-*L*-phenylalanine Methyl Ester with Immobilized Thermolysin in an Organic Solvent. *Bio/technology* **3**, 459–463.
- 18. Nakanishi, K., Kimura, Y., and Matsuno, R. (1986), Design of Proteinase-Catalyzed Synthesis of Oligopeptides in an Aqueous Organic Biphasic System. *Bio/technology* **4**, 452-454.
- 19. Schutt, H., Schmidt-Kastner, G., Arens, A., and Preiss, M. (1985), Preparation of Optically Active D-Arylglycines for Use as Side Chains for Semisynthetic Penicillins and Cephalosporins Using Immobilized Subtilisins in Two-Phase Systems. *Biotechnol. Bioeng.* 27, 420–433.
- 20. Selvi, C., Baboulene, M., Speziale, V., and Lattes, A. (1985), Synthesis by Enzymic Catalysis II. Amino Acid Polymerization. *J. Chem. Technol. Biotechnol.* **35B**, 282–290.
- 21. Singh, M. and Thomas, M. (1985), Biocatalytic Oxidation of Hydroquinone to *p*-Benzoquinone in a Water-Organic Solvent Two-Phase System. *Biotechnol. Lett.* 7, 663–664.
- 22. Taylor, F., Panzer, C. C., Craig, J. C., and O'Brien, D. J. (1986), Continuous Hydrolysis of Tallow with Immobilized Lipase in a Microporous Membrane. *Biotechnol. Bioeng.* **28**, 1318–1322.
- 23. Weetall, H. H. (1985), Enzymatic Synthesis of Gallic Acid Esters. *Appl. Biochem. Biotechnol.* 11, 25–28.

Water-Miscible Organic-Aqueous Cosolvent Systems

- 1. Corley, E. and Wolosiuk, R. A. (1985), The Effect of Organic Solvents on the Activation and the Activity of Spinach Chloroplast Fructose-1,6-bisphosphatase. *J. Biol. Chem.* **260**, 3978–3983.
- 2. Dordick, J. S., Marletta, M. A., and Klibanov, A. M. (1986), Peroxidases Depolymerize Lignin in Organic Media But Not in Water. *Proc. Natl. Acad. Sci. USA* 83, 6255-6257.
- 3. Dordick, J. S., Marletta, M. A., and Klibanov, A. M. (1987), Polymerization of Phenols Catalyzed by Peroxidase in Non-Aqueous Media. *Biotechnol. Bioeng.* **30**, 31–36.
- 4. Korpela, M. and Tahti, H. (1986), Effect of Organic Solvents on Human Erythrocyte Membrane Acetylcholinesterase Activity in Vitro. Arch. Toxicol., Supp. 9, (Toxic Interfaces Neurones, Smoke Genes), 320–323.
- Korpela, M. and Tahti, H. (1986), The Effect of Selected Organic Solvents on Intact Human Red Cell Membrane Acetylcholinesterase in Vitro. Toxicol. Appl. Pharmacol. 85, 257-262.
- 6. Nagamoto, H., Yasuda, T., and Inoue, H. (1986), Effect of Organic Solvents on the Activity of Glucoamylase. *Biotechnol. Bioeng.* 28, 1172–1177.
- 7. Osetskii, A. I. (1986), Kinetic Properties of Enzyme Reactions in a Medium of Viscous Organic Solvents. *Kriobiologiya* 4, 36–42.

8. Pungnieri, M., Skalli, A., Coletti-Previero, M. A., and Previero, A. (1986), Peptide and Ester Synthesis in Organic Solvents Catalyzed by Seryl Proteases Linked to Alumina. *Proteins: Struc., Funct., Genet.* 1, 134–138.

- Sakurai, H. and Hisabori, T. (1987), Effects of Organic Solvents on the Enzyme-Bound ATP Synthesis by Isolated CF₁. Prog. Photosynth. Res., Proc. Int. Congr. Photosynth., 7th, Meeting Date 1986, vol. 3, 13–16. Ed., Biggins, J. Nijhof, Dordrecht, Netherlands.
- Sigel, H., Martin, B. R., Tribolet, R., Haering, U. K., and Malini-Balakrishnan, R. (1985), An Estimation of the Equivalent Solution Dielectric Constant in the Active Site Cavity of Metalloenzymes. Dependence of Carbohydrate-Metal-Ion Complex Stabilities on the Polarity of Mixed Aqueous/Organic Solvents. Eur. J. Biochem. 152, 187-193.
- 11. Tahti, H. and Korpela, M. (1986), *In Vitro* Experiments on the Effects of Organic Solvents on Red Cell Membrane Acetylcholinesterase. *Food Chem. Toxicol.* **24**, 805–806.
- 12. Visuri, K. and Klibanov, A. M. (1987), Enzymatic Production of High Fructose Corn Syrup (HFCS) Containing 55% Fructose in Aqueous Ethanol. *Biotechnol. Bioeng.* **30**, 917–921.

Supercritical Fluids

- 1. van Eijs, A. M. M., Oostrom, W. H. M. and Wijsman, J. A. (1987), Enzymatic Transesterification in Supercritical Carbon Dioxide. *Proc. 4th Eur. Congr. on Biotechnol.* vol. 2., p. 211.
- 2. Hammond, D. A., Karel, M., Klibanov, A. M., and Krukonis, V. J. (1985), Enzymatic Reactions in Supercritical Gases. *Appl. Biochem. Biotechnol.* 11, 393-400.
- 3. Randolph, T. W., Blanch, H. W., Prausnitz, J. M., and Wilke, C. R. (1985), Enzymic Catalysis in a Supercritical Fluid. *Biotechnol. Lett.* 5, 325–328.
- 4. Randolph, T. W., Clark, D. S., Blanch, H. W., and Prausnitz, J. M. (1987), Enzymatic Oxidation of Cholesterol Aggregates in Supercritical Carbon Dioxide. *Science* 238, 387–390.

Gas Phase

1. Pulvin, S., Legoy, M. D., Lortie, R., Pensa, M., and Thomas, D. (1986), Enzyme Technology and Gas Phase Catalysis: Alcohol Dehydrogenase Example. *Biotechnol. Lett.* **8**, 783–784.

Enzymatic Stability in Nonaqueous Media

- 1. Aldercreutz, P. and Mattiasson, B. (1987), Aspects of Biocatalyst Stability in Organic Solvents. *Biocatalysis* 1, 99–108.
- 2. Ayala, G., Tuena de Gomez-Puyou, M., Gomez-Puyou, A., and Darszon, A. (1986), Thermostability of Membrane Enzymes in Organic Solvents. *FEBS Lett.* **203**, 41–43.
- Rodionova, M. V., Belova, A. B., Mozhaev, V. V., Martinek, K., and Berezin, I. V. (1987), Mechanism of Denaturation of Enzymes by Organic Solvents. Dokl. Akad. Nauk. SSR 292, 913–917.