Symposium Committee

Elias Greenbaum, General Chairman
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Charles E. Wyman, Cochairman
Solar Energy Research Institute
Golden, Colorado

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Helena L. Chum
Solar Energy Research Institute
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Arthur E. Humphrey
Lehigh University
Bethlehem, Pennsylvania

Donald L. Johnson
Grain Processing Corporation
Muscatine, Iowa

Leonard Keay
U.S. Department of Energy
Washington, D.C.

Frank R. Landsberger
Alan Patricof Associates
New York, New York

Richard F. Moorer
U.S. Department of Energy
Washington, D.C.

Michael L. Shuler
Cornell University
Ithaca, New York

E. James Whitehead
Badger Engineers, Inc.
Cambridge, Massachusetts

Jonathan Woodward
Oak Ridge National Laboratory
Oak Ridge, Tennessee

SOCIAL PROGRAM AND TOURS

- **Monday, May 7**

  — Evening reception for attendees and their guests (light snacks—no charge).

- **Tuesday, May 8**

  — Morning guest program for guests of attendees (continental breakfast and appropriate excursions). The guest program fee includes participation on Wednesday and Thursday mornings as well.

  — Evening banquet at the Riverside Motor Lodge (preceded by a social hour). The Symposium fee includes the social hour and banquet, but guests of attendees must purchase tickets.

- **Wednesday, May 9**

  — Wine tasting and luncheon buffet for those who are registered (guests may purchase tickets).

  — Afternoon tour to Oconaluftee Indian Village, a scenic trip over the mountains to a living American Indian Village in North Carolina.

  — Afternoon tour of Oak Ridge National Laboratory, with an emphasis on bioprocessing research facilities.

  — Hiking trips to be arranged at no additional cost.

- **Thursday, May 10**

  — Afternoon tour of the Cades Cove area of the Great Smoky Mountains National Park. Ticket price includes transportation and a box lunch. Cades Cove is an open-air museum that preserves some of the material culture of settlers who lived there as long ago as 1819. An eleven-mile loop road follows many of the old wagon roads; along the way you see homesteads, other buildings typical of a pioneer community, and abundant wildlife. (Tickets must be purchased.)

PROGRAM

Monday Evening, May 7, 1990

6:00-10:00 PM Registration (*Main Lobby*)

7:00-10:00 PM Reception (*Hideaway Lounge*)

Tuesday Morning, May 8, 1990

8:00 AM-5:00 PM Registration (coffee) (*Main Lobby*)

9:00 Guest program (*Mural Room,*

8:30 Welcome and Introduction to the Symposium (*Whaley Hall*)—E. Greenbaum, Oak Ridge National Laboratory, Oak Ridge Tennessee

Session 1—Thermal, Chemical, and Biological Processing

Chairman: **A. O. Converse**
Dartmouth College
Hanover, New Hampshire

Cochairman: **Robert J. Evans**
Solar Energy Research Institute
Golden, Colorado

8:40 Introduction (*Whaley Hall*)

8:45 Paper 1. "Development of a Differential Volume Reactor System for Soil Biodegradation Studies," **O. F. Webb,*** T. J. Phelps, P. R. Bienkowski, P. M. DiGrazia, G. D. Reed, J. M. H. King, D. M. White, and G. S. Sayler, University of Tennessee, Knoxville, Tennessee

9:10 Paper 2. "Biomass-to-Gasoline: Catalyst and Feedstock Effects," **R. J. Evans,** F. Agblevor, and T. A. Milne, Solar Energy Research Institute, Golden, Colorado

9:35 Paper 3. "The Effect of Carbonization Heating Rate on Charcoal and Activated Carbon Yields," C. E. Martin, **K. R. Purdy,** C. P. Kerr, S. Dubayeh, and T. A. Garr, Tennessee Technological University, Cookeville, Tennessee

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*Boldface denotes speaker.

10:00 Intermission

10:20 Paper 4. "Avoiding Digester Imbalance Through Real-Time Feedback Control of Dilution Rate," P. Pullammanappallil, J. Harmon, **D. P. Chynoweth**, G. Lyberatcs, and S. A. Svoronos, University of Florida, Gainesville, Florida

10:45 Paper 5. "Methane Production from Beet Pulp," **K. M. Ghanem**, A. H. El-Refai, and M. A. El-Gazaerly, Alexandria University, Alexandria, Egypt

11:10 Paper 6. "Effect of Temperature on the Glucose Yield Kinetics and HF-Adsorption/Desorption Profiles of the Solvolysis of a Single Lignocellulose Wafer in Anhydrous Hydrogen Fluoride (HF) Vapor," **G. L. Rorrer**, M. C. Hawley, and D. Lamport, Oregon State University, Corvallis, Oregon

11:35 Paper 7. "The Ammonia Freeze Explosion (AFEX) Process: A Practical Lignocellulose Pretreatment," **M. T. Holtzapple**, J-H. Jun, and B. E. Dale, Texas A&M University, College Station, Texas

Noon Session Adjournment

Tuesday Afternoon, May 8, 1990

Session 2—Applied Biological Research I

Chairwoman: **N. W. Y. Ho**
Purdue University
West Lafayette, Indiana

Cochairman: **K. Grohmann**
Solar Energy Research Institute
Golden, Colorado

1:30 Introduction (*Whaley Hall*)

1:40 Paper 8. "Ethanol Fermentation of Lignocellulose Hydrolysates," **B. Hahn-Hägerdal**, T. Lindén, T. Senac, and K. Skoog, Lund University, Lund, Sweden

2:05 Paper 9. "Synergism of Endo- and Exoglucanase and β-Glucosidase that Compose Cellulase on Hydrolysis of Cellulose," **M. Fujii**, T. Homma, K. Ooshima, and M. Taniguchi, Niigata University, Niigata, Japan

2:30 Paper 10. "Improvement of Cellulase Production in *Trichoderma*, **D. K. Sandhu** and S. Bawa, Guru Nanak Dev. University, Amritsar, India

2:55 Paper 11. "Cloning of the *Clostridium acetobutylicum* Acetoacetate Decarboxylase Gene and Its Expression in *Escherichia coli*," **D. J. Petersen** and G. N. Bennett, Rice University, Houston, Texas

3:20 Intermission

3:45 Paper 12. "Glutamate Production from CO_2 and NO_3^- by a New Biosolar Reactor Using Immobilized Marine Cyanobacteria," **T. Matsunaga**, N. Nakamura, and H. Takeyama, Tokyo University of Agriculture and Technology, Tokyo, Japan

4:10 Paper 13. "Oil Production by Wild, Micropropagated Plants, Calli, and Suspended Cells of *Euphorbia characias* L.," **M. Fernandes-Ferreira**, Universidade do Minho, Braga Codex, Portugal; M. S. S. Pais, Fac. Ciências, Lisboa Codex, Portugal; and J. M. Novais, LNETI, Lisboa Codex, Portugal

4:35 Paper 14. "Microbial Extraction of Beet Pulp Pectin," **K. M. Ghanem**, A. H. El-Refai, and M. A. El-Gazaerly, Alexandria University, Alexandria, Egypt

5:00 Session Adjournment

Tuesday Evening, May 8, 1990

6:30 Social Hour (*Hideaway Lounge*)

7:30 Banquet (*Pearl Room*)

8:30 After-dinner address: "Trekking with a Wildlife Photographer," **F. Alsop**, East Tennessee State University, Johnson City, Tennessee

Wednesday Morning, May 9, 1990

8-Noon Registration (coffee) (*Main Lobby*)

9:00 Guest Program (*Mural Room*)

Session 3—Applied Biological Research II

Chairman: **S. Lien**
Office of Technology Development/
Office of Environmental Restoration
and Waste Management
Germantown, Maryland

Cochairman: **G. W. Strandberg**
Oak Ridge National Laboratory
Oak Ridge, Tennessee

8:40 Introduction (*Whaley Hall*)

8:45 Paper 15. "Growth of Aerobic Bacteria on Alkali-Solubilized Lignite," **J. K. Polman**, C. R. Breckenridge, P. R. Dugan, and D. R. Quigley, Idaho National Engineering Laboratory, Idaho Falls, Idaho

9:10 Paper 16. "Production of Lignin-Peroxidase by *Streptomyces viridosporus*," **T. P. Adhi**, R. A. Korus, and D. L. Crawford, University of Idaho, Moscow, Idaho

9:35 Paper 17. "Utilization of Cheese Whey for Production of Alpha-Amylase Enzyme," P. Bajpai, N. Verma, J. K. Neer, and **P. K. Bajpai**, Thapar Corporate R&D Centre, Patiala, India

10:00 Intermission

10:20 Paper 18. "Characterization of Four Purified Extracellular Ligninases from the Lignin-Solubilizing Actinomycete, *Streptomyces viridosporus* T7A," T. S. Magnuson, M. A. Roberts, **D. L. Crawford**, and G. Hertel, University of Idaho, Moscow, Idaho

10:45 Paper 19. "Bioconversion of a L-carnitin Precursor in a Mono- or Two-Liquid Phase System," G. Bare, J.-B. Hubert, Ph. Jacques, R. Rikir, and **Ph. Thonart**, C.W.B.I.-Unité de Bio-Industrie, Gembloux, Belgium

11:10 Paper 20. "Performance of Trickle-Bed Bioreactors for Converting Synthesis Gas to Methane," D. E. Kimmel, K. T. Klasson, E. **C.** Clausen, and J. L. Gaddy, University of Arkansas, Fayetteville, Arkansas

11:35 Paper 21. "Metabolic Modeling of Fumaric Acid Production by *Rhizopus arrhizus*," **I. C. Gangl**, Illinois Institute of Technology, Chicago, Illinois; W. A. Weigand, University of Maryland, College Park, Maryland; and F. A. Keller, Cambridge BioScience Corporation, Worcester, Massachusetts

Noon Session Adjournment

Noon Wine tasting and luncheon buffet

Wednesday Afternoon, May 9, 1990

1:30-5 PM Tour to Oconaluftee Indian Village

1:00-6 PM Tour of Oak Ridge National Laboratory

Wednesday Evening, May 9, 1990

7:00-9 PM Special Topic Discussion Groups

"Venture Capitalists' View of Biotechnology for Fuels and Chemicals," Leader, **F. R. Landsberger**, Alan Patricof Associates, New York, New York

"Emerging Biotechnologies," Leader, **R. M. Busche**, Bio En-Gene-Er Associates, Inc., Wilmington, Delaware

Thursday Morning, May 10, 1990

8-Noon Registration (coffee) (*Main Lobby*)

9:00 Guest program (*Mural Room*)

Session 4—Bioengineering Research

Chairman: **H. R. Bungay**
Rensselaer Polytechnic Institute
Troy, New York

Cochairman: **J. S. Watson**
Oak Ridge National Laboratory
Oak Ridge, Tennessee

8:40 Introduction (*Whaley Hall*)

8:45 Paper 22. "A Critical Review and Evaluation of Bioproduction of Organic Chemicals," **S. A. Leeper** and G. F. Andrews, Idaho National Engineering Laboratory, Idaho Falls, Idaho

9:10 Paper 23, "Biological Production of Acetaldehyde from Ethanol Using Non-Growing Pichia Pastoria Whole Cells," H.-K. Chiang, W. W. Fish, and **G. Foutch**, Oklahoma State University, Stillwater, Oklahoma

9:35 Paper 24, "Enzymatic Hydrolysis of Starch in a Fixed-Bed Pulsed Flow Reactor," A. Sanromán, R. Chamy, M. J. Nuñez, and **J. M. Lema**, University of Santiago de Compostela, Spain

10:00 Intermission

10:20 Paper 25, "The Design of a Membrane-Based Integrated Ethanol Production Process," **W. J. Groot**, R. G. J. M. van der Lans, and K. Ch. A. M. Luyben, Delft University of Technology, Delft, The Netherlands

10:45 Paper 26, "Thermophilic Ethanol Production: Investigation of Ethanol Yield and Tolerance in Continuous Culture," **L. R. Lynd**, H-J. Ahn, G. Andersen, P. Hill, and D. S. Kersey, Dartmouth College, Hanover, New Hampshire

11:10 Paper 27, "Evaluation of a Multicompartment Bioreactor for Ethanol Production Using *in situ* Extraction of Ethanol," **M. L. Shuler**, D. E. Steinmeyer, A. P. Togna, S. Gordon, P. Cheng, and S. J. Letai, Cornell University, Ithaca, New York

11:35 Paper 28, "Non-Dispersive Extraction for Recovering Lactic Acid from Fermentation Broth," C. J. Wang and **R. K. Bajpai**, University of Missouri, Columbia, Missouri

Noon Session Adjournment

Thursday Afternoon, May 10, 1990

1-5 PM Cades Cove tour

2-4 PM Special Topic Discussion Groups

"Biotechnology and Specialty Chemicals," Leader, **M. L. Shuler**, Cornell University, Ithaca, New York

"In Situ Bioremediation: Why Is It So Difficult?" Leader, **M. E. Reeves**, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Thursday Evening, May 10, 1990

6:30 PM Social Hour (*Hideaway Lounge*)

7:00 PM **Poster Session** (*Pearl Room*). Chairman: **B. H. Davison**, Oak Ridge National Laboratory, Oak Ridge, Tennessee; Cochairman: **A. A. Antonopoulos**, Argonne National Laboratory, Argonne, Illinois

10:00 PM Refreshments (*Hideaway Lounge*)

Friday Morning, May 11, 1990

8-Noon Registration (coffee) (*Main Lobby*)

Session 5—Biotechnology, Bioengineering, and the Solution of Environmental Problems

Chairman: **J. R. Geiger**
Olin Research Center
Cheshire, Connecticut

Cochairman: **D. A. Graves**
International Technology Corporation
Knoxville, Tennessee

8:40 Introduction (*Whaley Hall*)

8:45 Paper 29. "Risk Assessment of Microorganisms Used in Pollution Control," **H. S. Strauss**, H. Strauss Associates, Inc., Natick, Massachusetts, and Massachusetts Institute of Technology, Cambridge, Massachusetts

9:10 Paper 30. "Anaerobic Dechlorination of Chlorinated Biphenyls by Mixed Bacterial Cultures from PCB-Contaminated Sediments," **M. E. Reeves**, C.-C. Chang, and T. L. Donaldson, Oak Ridge National Laboratory, Oak Ridge, Tennessee

9:35 Paper 31. "In-Situ Soil Aeration for Enhanced Biodegradation of Hydrocarbons," **R. E. Hinchee**, D. C. Downey, and R. Miller, Battelle Columbus Division, Columbus, Ohio

10:00 Intermission

10:20 Paper 32. "Biological Oxygen Consumption as a Measure of Biodegradation of Mixed Organic Wastes," **D. A. Graves**, International Technology Corporation, Knoxville, Tennessee

10:45 Paper 33. "Iron Oxidation by *Thiobacillus ferrooxidans*," S. K. Kang and **R. D. Sproull**, Oregon State University, Corvallis, Oregon

11:10 Paper 34. "Novel Biotreatment Process for Glycol Waters," **L. M. V. Raja**, G. Elamvaluthy, R. Palaniappan, and R. M. Krishnan, Southern Petrochemical Industries Corporation, Ltd., Tamilnadu, India

11:35 Paper 35. "Gas Hydrate Formation in Reversed Micelles—Applications to Bioseparations and Biocatalysis," **V. T. John**, H. Nguyen, M. Rao, and J. B. Phillips, Tulane University, New Orleans, Louisiana

Noon Session Adjournment

POSTER SESSION

Chairman: **B. H. Davison**
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Cochairman: **A. A. Antonopoulos**
Argonne National Laboratory
Argonne, Illinois

Thermal, Chemical, and Biological Processing

Poster 36. "Fermentation Studies on Sugars Recovered from Waste Cellulosics Via Acid Hydrolysis," **M. J. Beck**, R. D. Johnson, and C. S. Baker, Tennessee Valley Authority, Muscle Shoals Alabama

Poster 37. "Active Protein and Substrate Flow Effects in a Tubular Immobilized Invertase Reactor," **F. N. Onyezili**, University of Agriculture, Makurdi, Nigeria

Poster 38. "Evaluation of Dilute Sulfuric Acid Hydrolysis and Autohydrolysis for Sugar Production from Hardwood Hemicellulose," **R. C. Strickland**, D. S. Tuten, and M. D. Hardy, Tennessee Valley Authority, Muscle Shoals, Alabama

Poster 39. "Conversion of Newsprint and MSW-Cellulosics to Sugar by Dilute Sulfuric Acid Hydrolysis," **R. C. Strickland**, D. S. Tuten, and M. D. Hardy, Tennessee Valley Authority, Muscle Shoals, Alabama

Poster 40. "Effect of Medium Composition and Growth Intervals on Growth, Total Lipids, and the Fatty Acid Composition of *Fusarium solani*," M. K. Tahoun, Z. El-Merheb, and **A. E. Salem**, Alexandria University, Alexandria, Egypt

Poster 41. "Delignification of Non-Woody Biomass," **G. J. Tyson**, Xylan, Madison, Wisconsin

Poster 42. "The Study of Treating Pharmaceutical Wastewater in the Upflow Anaerobic Sludge Blanket (UASB) Reactor," **G. Datian**, F. Zihua, and H. Xiaogang, Cheng-Du University of Science and Technology, Cheng-Du, China

Poster 43. "Single-Cell Protein Production from Acid-Hydrolyzed Lignocellulosics," **T. A. McCaskey** and A. H. Stephenson, Auburn University, Auburn University, Alabama; and R. C. Strickland, Tennessee Valley Authority, Muscle Shoals, Alabama

Poster 44. "Dilute Acid Pretreatment of Corn Residues and Short Rotation Crops," **R. Torget**, P. Werdene, M. E. Himmel, and K. Grohmann, Solar Energy Research Institute, Golden, Colorado

Poster 45. "A Technical and Economic Analysis of Ethanol Production from Wheat Straw or Aspen Wood Chips Using Steam Explosion or Dilute Acid Pretreatments," **R. Torget**, A. Power, D. J. Schell, P. J. Walter, K. Grohmann, and N. Hinman, Solar Energy Research Institute, Golden, Colorado

Poster 46. "Effect of Pretreatment on SSF of Hardwood into Acetone/Butanol," **M. Shah** and Y. Y. Lee, Auburn University, Auburn University, Alabama; and R Torget, Solar Energy Research Institute, Golden, Colorado

Poster 47. "Continuous Production of Biogas from Cheese Whey Using a pH Controlled Two-Stage Mesophelic Reactor," **A. E. Ghaly** and J. Pyke, Technical University of Nova Scotia, Halifax, Nova Scotia, Canada

Poster 48. "Thermal Degradation of Cereal Straws in Air and Nitrogen," **A. E. Ghaly** and A. Ergudenler, Technical University of Nova Scotia, Halifax, Nova Scotia, Canada

Applied Biological Research

Poster 49. "Influence of Macronutrients, Auxins, and Biosynthetic Precursors on Rosmarinic Acid Synthesis in Cell Suspension Cultures of *Salvia officinalis*," K. Shetty, D. L. Crawford, and **R. Korus**, University of Idaho, Moscow, Idaho

Poster 50. "Isolation and Characteristics of Plasmids from *Clostridium thermosaccharolyticum*," **S. Kalyuzhnyy**, N. Belogurova, T. Mosolova, and S. Varfolomeyer, M. V. Lomonosov Moscow University, Moscow, USSR

Poster 51. "Kinetic Investigation and Mathematical Modelling of Methanogenesis," S. Kalyuzhnyy, **S. Varfolomeyer**, V. Gachok, and V. Sklyar, M. V. Lomonosov Moscow University, Moscow, USSR

Poster 52. "Biodegradation of Mixtures of Hazardous Organic Compounds," **G. W. Strandberg**, D. J. Larson, and T. L. Donaldson, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 53. "Induction of Mutation in *Trichoderma viride* and on Laboratory-Scale Conversion of Natural Cellulose into Fuel," **M. K. Tahoun**, A. Khalil, S. Helmi, and A. H. Khairy, University of Alexandria, Alexandria, Egypt

Poster 54. "Induction of Mutation in *Aspergillus niger* for Conversion of Cellulose into Fuel Precursor," S. Helmi, A. Khalil, **M. K. Tahoun**, and A. H. Khairy, University of Alexandria, Alexandria, Egypt

Poster 55. "Characterization of a Photosynthetic Shear-Resistant Glycine Max Cell Line," J. Castillon, V. N. Coleman, F. Kong, V. Piriyapan, W. J. Treat, C. R. Engler, and **E. J. Soltes**, Texas A&M University, College Station, Texas

Poster 56. "Immobilization of Enzyme to a Platinum Electrode and Its Use as an Enzyme Electrode," **S. Gondo**, M. Kawakami, and H. Koya, Fukuoka Institute of Technology, Fukuoka, Japan

Poster 57. "Ethanol Production by Recombinant *Escherichia coli* Carrying Genes from *Zymomonas mobilis*," **H. G. Lawford** and J. D. Rousseau, University of Tororito, Toronto, Ontario, Canada

Poster 58. "Degradation of Organic Sulfur Compounds by a Coal-Solubilizing Fungus," **B. D. Faison** and C. A. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee; T. M. Clark, University of Detroit, Detroit, Michigan; and D. M. Sharkey, California Polytechnic State University, San Luis Obispo, California

Poster 59. "Physiological Aspects of the Regulation of Extracellular Enzymes of *Phanerochaete chrysosporium*," **C. G. Dosoretz**, A. H. C. Chen, and H. E. Grethlein, Michigan State University and Michigan Biotechnology Institute, Lansing, Michigan

Poster 60. "Biochemical Oxidation of D-Sorbitol to L-Sorbose by Immobilized *Gluconobacter oxydans* Cells," **S Stefanova**, A. Triphonov, I. Tepavicharova, Chr. Konstantinov, National Bank of Industrial Microorganisms/Cell Culture, Sofia, Bulgaria

Poster 61. "Microbial Desulfurization of Fossil Fuels," **T. Omori**, L. Monna, and T. Kodama, The University of Tokyo, Tokyo, Japan; and A. Hiratsuka, K. Hon-nami, and N. Nishikawa, Tokyo Electric Power Company, Tokyo, Japan; and T. Koana, Institute of Research and Innovation, Tokyo, Japan

Poster 62. "Butanol Production from Carbon Monoxide by *Butyribacterium methylotrophicum*, **A. J. Grethlein**, R. M. Worden, M. K. Jain, and R. Datta, Michigan State University, East Lansing, Michigan

Poster 63. "Kinetics of Growth and Catechol Production by *Bacillus stearothermophilus* BR 219," **R. M. Worden**, R. Subramanian, M. J. Bly, S. Winter, and C. L. Aronson, Michigan State University, East Lansing, Michigan

Poster 64. "Performance of Immobilized Enzyme on Saccharification and Fermentation of Agricultural Wastes and Wood Residues," **E. Wilkins** and Y. Ramesh, University of New Mexico, Albuquerque, New Mexico

Poster 65. "Cell Associated β-Galactosidase Activity in Mycelial Pellets of *Aspergillus* and *Penicillium* sp." K. Réczey, Inst. Agr. Chem. Techn., Techn. Univ. Budapest, Budapest, Hungary; H. Stålbrand, F. Tjerneld, and **B. Hahn-Hägerdal**, Lund University, Lund, Sweden

Poster 66. "Studies on Cellulase Production Using Spent Sulfite Liquor and Paper Mill Waste," Z. Xin, Q. Yinbo, **G. Peiji**, and W. Zunong, Shandong University, Jinan, Shandong, China

Poster 67. "Hydrolysis of Cellobiose by Immobilized β-Glucosidase Entrapped in Maintenance-Free Gel Spheres," **J. Woodward**, Oak Ridge National Laboratory, Oak Ridge, Tennessee; and K. M. Clarke, Marquette University High School, Milwaukee, Wisconsin

Poster 68. "Fiber-Optic Based Apparatus for the Nondestructive, in vivo Measurement of Photosynthesis: Application to Environmental Monitoring," **J. E. Coffield**, University of Tennessee, Knoxville, Tennessee; D. A. Graves, International Technology Corporation, Knoxville, Tennessee; and E. Greenbaum, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 69. "Anaerobic Degradation of Furfural (2-Furaldehyde) to Methane and Carbon Dioxide," **C. J. Rivard** and K. Grohmann, Solar Energy Research Institute, Golden, Colorado

Poster 70. "Isolation and Characterization of Two Chromatographic Forms of β-D-Glucosidase from *Aspergillus niger*," M. E. Himmel, D. J. Mitchell, J. O. Baker, W. S. Adney, K. Tatsumoto, and **K. Grohmann**, Solar Energy Research Institute, Golden, Colorado; and J. W. Fox, University of Virginia, Charlottesville, Virginia

Poster 71. "Toxic Effects of Selected Industrial Solvents in Batch and Continuous Anaerobic Reactors," **L. J. Schwartz**, University of Wisconsin, Green Bay, Wisconsin

Poster 72. "Genetic Transformation of Xylose-Fermenting Yeast *P. stipitis*," **Nancy W. Y. Ho**, D. Petros, and X. X. Deng, Purdue University, West Lafayette, Indiana

Poster 73. "Selection of Thermotolerant Yeasts for Simultaneous Saccharification and Fermentation of Cellulose," I. Ballesteros, M. Ballesteros, A. Cabañas, J. Carrasco, C Martin, M. J. Negro, F. Saez, and **R. Saez**, Instituto de Energías Renovables, C.I.E.M.A.T., Madrid, Spain

Poster 74. "Dual Inoculation with VA *Mycorrhiza* and *Rhizobium* is Beneficial to *Leucaena* Growth," **R. P. Gupta** and V. Punj, Punjab Agricultural University, Ludhiana, India

Poster 75. "The Effects of Nutrients and Temperature on Biomass, Growth, Lipid Production, and Fatty Acid Composition of *Cyclotella cryptica*," **S. Sriharan**, D. Bagga, and M. Nawaz, Selma University, Selma, Alabama

Poster 76. "Entrapped Thermoalkalophilic *Bacillus* and Endoglucanase Production," **K. B. Krishna** and A. Varma, Jawaharlal Nehru University, New Delhi, India

Poster 77. "Purification of *Trichoderma reesei* Cellobiohydrolase I by Preparative Native Gel Electrophoresis," **N. E. Lee** and J. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 78. "Strain Development of the Alcohol Production from Hemicellulose Hydrolyzate," **T. Seki**, N. Nakashima, W-Z. Xy, C-X. Pan, I. Urabe, T. Yoshida, and H. Okada, Osaka University, Osaka, Japan

Poster 79. "Use of Cyanobacterial Diazotrophic Technology in Rice Agriculture," **D. N. Tiwari**, A. Kumar, and A. K. Mishra, Banaras Hindu University, Varanasi, India

Poster 80. "Plant Extract Estimulation of Biofilm Production by the 'Tea Fungus,' *Acetobacter xylinum*," **J. D. Fontana** and A. M. de Souza, UFPR, Curitiba, Brazil

Poster 81. "Seed Gum of *Stiphnodendron barbatiman*," F. Reciher, S. C. S. Leitner, M. R. Sierakowski, **J. D. Fontana**, and J. B. C. Correa, UFPR, Curitiba, Brazil

Bioengineering Research

Poster 82. "Extractive Fermentation of Acetic Acid: Economic Trade-Off Between Yield of *Clostridium* and Concentration of *Acetobacter*," **R. M. Busche**, Bio En-Gene-Er Associates, Inc., Wilmington, Delaware

Poster 83. "Production of Ethanol and Coproducts from MSW-Derived Cellulosics Using Dilute Sulfuric Acid Hydrolysis," J. W. Barrier, **M. M. Bulls**, R. O. Lambert, and J. D. Broder, Tennessee Valley Authority, Muscle Shoals, Alabama

Poster 84. "A New Kinetic Approach to the Fermentation of Multisubstrate and Complex Media," A. Converti and **M. Del Borghi**, Genoa University, Genova, Italy

Poster 85. "Modelling of D-Sorbitol to L-Sorbose Biotransformation by Immobilized Cells of Gluconobacter Suboxydans in a Bubble Column," V. Beschov and **M. Kosseva**, Bulgarian Academy of Sciences, Sofia, Bulgaria

Poster 86. "Microbial Removal of Sulfur Dioxide (SO_2) and Nitric Oxide (NO) from a Gas," **K. L. Sublette** and K. H. Lee, The University of Tulsa, Tulsa, Oklahoma

Poster 87. "An Economic Analysis of the Microbial Reduction of Sulfur Dioxide (SO_2) as a Means of By-Product Recovery from Regenerable Processes for Flue Gas Desulfurization," **K. L. Sublette**, The University of Tulsa, Tulsa, Oklahoma; and K. Gwozdz, Lummus-Crest, Inc., Bloomfield, New Jersey

Poster 88. "Determination of the Partition Coefficient for Protein Separation in an Air-Fluidized Bioreactor," P. B. Kokitkar and **R. D. Tanner**, Vanderbilt University, Nashville, Tennessee

Poster 89. "The Effect of pH and Gas Composition on the Bubble Fractionation of Proteins," A. H. G. DeSouza, W. T. Effler, Jr., and **R. D. Tanner**, Vanderbilt University, Nashville, Tennessee

Poster 90. "Bioreactor Design Considerations in the Production of High-Quality Microbial Exopolysaccharide," **H. G. Lawford** and J. Rousseau, University of Toronto, Toronto, Ontario, Canada

Poster 91. "Modeling of an Immobilized Cell, Three-Phase Fluidized-Bed Bioreactor," **J. N. Petersen**, Washington State University, Pullman, Washington; and B. H. Davison, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 92. "MSW Volume Reduction, Value Recovery, and Value Addition," **M. Eley**, University of Alabama, Huntsville, Alabama; J. Watson, M. J. Beck, and R. D. Johnson, Tennessee Valley Authority, Muscle Shoals, Alabama; and T. McCaskey, Auburn University, Alabama

Poster 93. "A Structured Model for Vegetative Growth and Sporulation in *Bacillus thuringiensis*," **M. Starzak** and R. Bajpai, University of Missouri, Columbia, Missouri

Poster 94. "Prediction of Bed Height in a Self-Aggregating Yeast Ethanol Tower Fermenter," **C. S. Chen**, E. C. Chang, C. S. Gong, and L. F. Chen, Purdue University, W. Lafayette, Indiana

Poster 95. "Theoretical and Experimental Investigation of an Upflow Solids-Retaining Bioreactor for Cellulose Conversion," **D. A. Hogsett**, G. Spieles, and L. R. Lynd, Dartmouth College, Hanover, New Hampshire

Poster 96. "The Use of an Aqueous Two-Phase System for ABE Fermentation," **Y. J. Kim** and W. A. Weigand, University of Maryland, College Park, Maryland; and J. Hong, University of California, Irvine, California

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Poster 102. "COS Degradation by Selected CO-Utilizing Bacteria," K. D. Smith, K. T. Klasson, M. D. Ackerson, **E. C. Clausen**, and J. L. Gaddy, University of Arkansas, Fayetteville, Arkansas

Poster 103. "Parameters Affecting the Kinetics of Ethanol Production from CO, CO_2, and H_2 by *Clostridium ljungdahlii*," K. M. O. Lundbäck, B. B. Elmore, S. B. Baker, T. K. Klasson, **E. C. Clausen**, and J. L. Gaddy, University of Arkansas, Fayetteville, Arkansas

Poster 104. "A Comparison of Inhibitory Effects on *S. cerevisiae* and *Z. mobilis* in Batch and Continuous Culture," J. J. Wu, **E. C. Clausen**, and J. L. Gaddy, University of Arkansas, Fayetteville, Arkansas

Poster 105. "Studies on Whole Cell Immobilization of Yeast *Saccharomyces cerevisiae* NSI 113 on a Packed-Bed Calcium Alginate Support on Cane Molasses," **K. Pande** and P. K. Agrawal, National Sugar Institute Kanpur, Kanpur, India

Poster 106. "Comparative Evaluation of Catalase Immobilization Methods on Different Supports," **V. G. Artenie** and D. C. Cojocaru, A. I. Cuza University, Jassy, Romania

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Poster 110. "Study on BOD Microbial Sensor for Wastewater Treatment Control," **L. Yourong** and C. Ju, East China University of Chemical Technology, Shanghai, P. R. China

Poster 111. "Comparative Microbial Degradation of Organic Cyanide," M. S. Nawaz, **J. H. Davis**, and K. D. Chapatwala, Selma University, Selma, Alabama

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Poster 113. "Production of Oil-Degrading Bacteria and Their Use in Microbial Remediation of Contaminated Soils," **H.-P. Rohns**, S. Schalenbach, and L. E. Webb, Nuclear Research Centre, Jülich, West Germany

Poster 114. "Methanol Suppression of *Trichloroethylene* Degradation by *M. trichosporium*," **A. V. Palumbo** and W. Eng, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 115. "Isolation of Amoebic-Bacterial Consortia Capable of Degrading Trichloroethylene," **R. L. Tyndall**, K. Ironside, C. D. Little, S. Katz, and J. Kennedy, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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Abstracts for Oral Sessions

DEVELOPMENT OF A DIFFERENTIAL VOLUME REACTOR SYSTEM FOR SOIL BIODEGRADATION STUDIES

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A bench-scale experimental system was developed for the systems analysis of polyaromatic hydrocarbon (PAH) degradation by mixed microbial cultures in PAH-contaminated Manufactures Gas Plant (MGP) soils. This reactor system was developed to provide a fundamental protocol for evaluating the performance of specific, mixed microbial cultures on specific soil systems by elucidating the salient system variables and their interactions. The reactor design and peripherals are described. A plug flow differential-volume reactor was used in order to remove performance effects which are related to reactor type, as opposed to system structure. This well-defined reactor system could be represented mathematically and provide insight on one potentially important system variable, macroscopic mass transfer. Two methods for the quantitative determination of PAH liquid-phase concentrations were developed. The mathematical models and experimental data are presented for the biodegradation of naphthalene on artificial and MGP soils.

Paper 2

BIOMASS-TO-GASOLINE: CATALYST AND FEEDSTOCK EFFECTS

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The rapid screening of biomass feedstocks by pyrolysis mass spectroscopy[1] coupled with multivariate data analysis techniques[2] has been used to characterize samples of herbaceous and woody biomass. The results of this characterization were correlated with the performance of these feedstocks in the pyrolysis and zeolite conversion to gasoline-range hydrocarbons. We have studied the conversion of five herbaceous species, six hardwoods, and two softwoods, as well as samples of cellulose and lignin. The yield of gasoline-range hydrocarbons depended on the concentration of lignin and the relative distribution of the two main types of carbohydrate pyrolysis products. These two main carbohydrate pyrolysis products are the anhydrosugars (e.g., levoglucosan) and the C1 and C4 oxygenates (e.g., hydroxyacetaldehyde). Higher yields of hydrocarbons from the catalytic step were obtained from samples that gave higher yields of anhydrosugars in the pyrolysis step. Both the hydrocarbon product yields and the primary carbohydrate pyrolysis products were dependent on the potassium and calcium content of the samples, which catalytically control the pyrolysis product composition.

1. Evans, R. J., and T. A. Milne, *Energy and Fuels* 1, 312-314 (1987).
2. Evans, R. J., and T. A. Milne, 1988, "Research in Thermochemical Biomass Conversion," London: Elsevier Applied Science, pp. 264-279.

THE EFFECT OF CARBONIZATION HEATING RATE ON CHARCOAL AND ACTIVATED CARBON YIELDS

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Simulation of the conversion of hardwood chips to activated carbon in a char-recirculation, fixed bed reactor requires a prediction of the effect of the carbonization heating rate on the charcoal and activated carbon yields. The pyrolysis of white oak chips was studied with an electrically heated batch reactor. Heating rates were varied from 1 to 10°C per minute for maximum carbonization temperatures of 490 and 650°C. Solid, liquid, and gaseous pyrolysis product yields were measured. Gas composition was determined with gas chromatography. Liquids were "separated" into light oil, heavy oil, and water. The fixed-carbon-, volatile-matter-, and ash contents of the charcoal were determined. Each of the charcoal products was subsequently gasified with water vapor to produce activated carbon with various levels of activity. Significant effects of heating rate on pyrolysis product yields were found. However, except for very low rates, the yield of active carbon is essentially independent of heating rate.

Paper 4

AVOIDING DIGESTER IMBALANCE THROUGH REAL-TIME FEEDBACK CONTROL OF DILUTION RATE

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Imbalance in anaerobic digesters is generally attributed to overloading or feed inhibitors. A method is presented that relies on the on-line measurement of methane production rate to automatically adjust the dilution rate to avoid digester imbalance. An on-line identified model is used to detect whether or not the digester is being overfed due to unknown causes. The control algorithm uses this information to select among two possible modes of operation. If the digester is not being overfed, the dilution rate is adjusted so that, the reactor yield (volumetric ratio of methane produced to substrate fed) is constant (Mode 1). If methane production rate drops due to inhibition or changes in environmental conditions, the control system responds by reducing the substrate feeding rate, thus stabilizing the digester. Experimental results support this claim. If an overfeeding condition is detected, the digester is automatically switched to Mode 2, in which the dilution rate is adjusted so as to maintain the methane production rate at its value preceding the onset of overfeeding. The reactor yield is continually monitored and, when it drops to near the normal value, operation is switched back to Mode 1.

Paper 5

METHANE PRODUCTION FROM BEET PULP

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The application of untreated or alkali treated beet pulp (BP), a waste product of the sugar industry in Egypt, to methane production was examined. The daily methane yields varied in a stochastic manner. The yield values fluctuated throughout the experiment after an initial increase which occurred in the first 10 to 15 d. The average composition of the gas produced was from 42 to 72% methane and from 58 to 28% CO_2 for the alkali-treated BP. On the other hand, it was inferior for the untreated BP, since the average gas composition varied from 30 to 56% methane and from 70 to 44% CO_2.

The volatile fatty acid profiles were also variable and were not as stochastic as the methane yields.

Paper 6

EFFECT OF TEMPERATURE ON THE GLUCOSE YIELD KINETICS AND HF-ADSORPTION/DESORPTION PROFILES OF THE SOLVOLYSIS OF A SINGLE LIGNOCELLULOSE WAFER IN ANHYDROUS HYDROGEN FLUORIDE (HF) VAPOR

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This investigation studied the effect of temperature on solvolysis of a single lignocellulose wafer by a 100% HF vapor stream under carefully controlled laboratory conditions. Anhydrous hydrogen fluoride vapor at ambient conditions readily absorbed onto lignocellulose and solvolytically cleaved the cellulose fraction to solid glucosyl fluoride, which, upon posthydrolysis, produced a glucose yield of 94% of theoretical. A reactor equipped with a resealable sampling probe generated single HF-reacted lignocellulose wafers (0.4 mm x 1.0 cm/side) as a function of reaction time (0 to 12 min), HF vapor flow rate, and HF vapor temperature of 30 to 80°C. Water-soluble products from the HF-reacted lignocellulose wafers were analyzed both before posthydrolysis (reversion oligomers) and after posthydrolysis (free sugars) by HPLC. The physical adsorption and desorption of HF vapor onto a single lignocellulose chip was also measured over the same process conditions by a novel microbalance reactor.

Adsorption of HF vapor onto the lignocellulosic matrix was necessary for cellulose solvolysis. The HF loading (g HF adsorbed/g lignocellulose) decreased with increasing temperature (1.5 g HF/g lignocellulose at 30°C, versus 0.4 g HF/g lignocellulose at 60°C), whereas the intrinsic reaction rate constant for cellulose solvolysis increased with increasing temperature. This coupling caused the glucose yield vs time curve (after posthydrolysis) to decrease with increasing temperature (3 min to complete conversion at 30°C versus 6 min to complete conversion at 60°C). Adsorbed HF was also the limiting reagent for cellulose solvolysis; the minimum HF loading was 0.4 g HF/g lignocellulose. The extent of glucosyl fluoride repolymerization, as evidenced by HPLC oligosaccharide profiling, increased dramatically with increasing temperature, probably due to the combined effects of lowered HF loading and higher temperature.

Paper 7

THE AMMONIA FREEZE EXPLOSION (AFEX) PROCESS: A PRACTICAL LIGNOCELLULOSE PRETREATMENT

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The Ammonia Freeze Explosion (AFEX) process treats lignocellulosic biomass with high-pressure liquid ammonia for about ten minutes. Then, the pressure is quickly released allowing the liquid ammonia to explosively vaporize and disrupt the biomass structure. The combined chemical effect (cellulose decrystallization) and physical effect (increase in accessible surface area) make the cellulose extremely reactive to enzymatic hydrolysis. Sixfold increases in enzymatic digestibility have been demonstrated. This process has been in laboratory development for eight years but has only recently become commercially viable, due to a novel ammonia recovery scheme which minimizes capital and energy expenditures. Recently developed process improvements have dramatically increased the biomass reactivity compared to past achievements. The economic implications of these new developments will be discussed.

Paper 8

ETHANOL FERMENTATION OF LIGNOCELLULOSE HYDROLYSATES

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The fermentation of lignocellulose hydrolysates to ethanol involves two major problems: (1) the fermentation of the pentose sugar xylose and (2) the presence of microbial inhibitors. Xylose can be directly fermented with yeasts such as *Pachysolen tannophilus, Candida shehatae* and *Pichia stipitis* or with an isomerization of xylose to xylulose with the enzyme glucose (xylose) isomerase (XI) and subsequent fermentation with baker's yeast, *Saccharomyces cerevisiae.* The direct fermentation requires a low and carefully controlled oxygenation, as well as removal of inhibitors. Also, the xylose fermenting yeasts have a limited ethanol tolerance. The combined isomerization and fermentation with Xi and *S. cerevisiae* gives yields and productivities comparable to those obtained in hexose fermentations without oxygenation and removal of inhibitors. However, the enzyme is not very stable in a lignocellulose hydrolysate, and *S. cerevisiae* has a poorly developed pentose phosphate shunt. Different strategies, involving (1) strain adaptation, (2) protein, and (3) genetic engineering to overcome these different obstacles, are discussed.

SYNERGISM OF ENDO- AND EXOGLUCANASE AND β-GLUCOSIDASE THAT COMPOSE CELLULASE ON HYDROLYSIS OF CELLULOSE

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A kinetic model representing the synergistic action of the three components that compose cellulase on hydrolysis of solid cellulose particles is proposed.

The model consists of three simultaneous differential equations: (1) one representing the endoenzyme's own action, (2) another one representing the exoenzyme's own action, and (3) the rest representing the β-glucosidase's own action. A simultaneous solution of these three equations expresses the synergism.

Endoenzyme splits the substrate molecules at random on the surface of the solid cellulose particles to supply new nonreducing endgroups to exoenzyme, but most of the split molecules still remain on the surface of the solid particles. Exoenzyme produces soluble sugar successively from a molecule on the surface; in other words, it peels the molecule from the surface, revealing new glucoside bonds on the next layer of the particle, which are to be supplied to the endoenzyme. β-Glucosidase produces glucose from the soluble sugar released by the exoenzyme in order to reduce the inhibition of cellobiose to both glucanases.

The cooperation, performed by each one's own action of the different types of the three enzymes, is the synergism.

The fit between theory and experimental data is shown in the presentation.

Paper 10

IMPROVEMENT OF CELLULASE PRODUCTION IN *TRICHODERMA*

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Genetically marked strains of *Trichoderma reesei* QM 9414 were crossed using protoplast fusion. In the crosses attempted, the diploids showed enhanced yields of exoglucanase while the yield of endoglucanase and β-glucosidase was intermediate as compared to their respective parents. In some of the segregants, the yields were further enhanced, indicating the use of protoplast fusion techniques in developing breeding strategies for strain improvement. The study of cellulase mutants, as such and the segregants, revealed that the three cellulase components appear to be regulated independent of each other. Genetic analysis of the segregants showed that the three components are present on separate linkage groups.

CLONING OF THE *CLOSTRIDIUM ACETOBUTYLICUM* ACETOACETATE DECARBOXYLASE GENE AND ITS EXPRESSION IN *ESCHERICHIA COLI*

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The economics of petroleum distillation, combined with environmental concerns, have sparked renewed interest in the production of acetone and butanol from renewable biomass sources. *Clostridium acetobutylicum* has long been studied for its ability to convert simple organic acids to solvents, yet the commercial viability of this fermentation has been hindered by the inability to control the solventogenic switch. To enhance the industrial potential of this microorganism's fermentation capabilities, we have cloned the gene encoding acetoacetate decarboxylase, the enzyme responsible for the final conversion of acetoacetate to acetone, and expressed it in *E. coli*. A λEMBL3 phage library of *C. acetobutylicum* DNA was screened by plaque hybridization, using [^{32}P]-radiolabelled oligonucleotide probes, designed and based on the N-terminal amino acid sequence of the purified protein from *C. acetobutylicum* ATCC 824. Restriction mapping and subsequent subcloning have localized the gene to an ~1.8 kb *EcoRI/BglII* fragment. Enzyme assays confirm that the enzyme is well expressed in *E. coli*, in both orientations, from a promoter of Clostridia origin. Maxicell analysis and Western blots of SDS-PAGE whole cell extracts, using primary antibodies raised to the purified protein, have corrected the molecular weight of the protein to ~28 Kd *vs* 30 Kd in earlier reports.

GLUTAMATE PRODUCTION FROM CO_2 AND NO_3^- BY A NEW BIOSOLAR REACTOR USING IMMOBILIZED MARINE CYANOBACTERIA

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Recently, use of marine photosynthetic microorganisms has received much attention. Seawater in its natural state abounds with nutrients, including magnesium and potassium, which are essential for the growth of photosynthetic microorganisms. For several years, marine photosynthetic microorganisms have been isolated from the coastal areas of Japan. Among more than 200 strains of marine cyanobacteria, *Synechococcus* sp. selectively produce glutamate under light irradiation in artificial seawater containing nitrate and carbon dioxide. It was found that 82.6% of the total amino acid production was glutamate. *Synechococcus* sp. was immobilized in calcium alginate gel. Glutamate production by immobilized cells was double that of native cells. Immobilized marine *Synechococcus* sp. produced 0.26 mg/cm gel of glutamate for 7 d.

Then, immobilized marine *Synechococcus* sp. was used in a biosolar reactor consisting of a fiber optic light-diffusing unit. The upper end of the unit was formed into a light-receiving bundle, and the lower end was shaped into a bundle with a built-in light reflector.

OILS PRODUCTION BY WILD, MICROPROPAGATED PLANTS, CALLI, AND SUSPENDED CELLS OF *EUPHORBIA CHARACIAS* L.

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Oil yields obtained from wild *Euphorbia characias*, in neutral fractions extracted by n-hexane, vary between 4.5% and 7.5% while in acid fractions they vary between 0.44% and 0.60%, depending on the season. Callus afforded yields of 1.7% of neutral oils and 1.06% of acid oils, while suspended cells gave yields of 1.9% and 1.3%, respectively. The highest neutral oil yield (8.3%) was obtained from micropropagated plants transferred to the field and grown there during three months. However, those plants gave the lowest acid oils yield (0.21%). Analysis, performed by GLC and GC-MS, revealed that acid oils were composed mainly of free fatty acids. Neutral oil fractions, either from the wild or micropropagated in nature-growing plants, are composed mainly of hydrocarbons, triterpenols, and lipids. Neutral fractions from calli and suspended cells are composed mainly of lipids and triterpenols of a different type than those from the wild and micropropagated plants. Micropropagation techniques appear to have induced some profitable physiological modification in the micropropagated plants. They show an apical dominance much lower than those growing wild. The high oil yield afforded by micropropagated plants may be associated with the type of growth. As these plants produce a great number of short low-lignified stems, they may synthesize and accumulate oil compounds in higher amount than the wild ones. In the wild plants, the biosynthesis of lignin and structural carbohydrates may compete with the biosynthesis of other compounds, namely those of neutral oils, and reduce their accumulation.

MICROBIAL EXTRACTION OF BEET PULP PECTIN

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Beet pulp (BP), a waste product of the sugar industry in Egypt, is about 24% pectin. Different yeasts were tested for their ability to produce protopectin-solubilizing enzymes, of which *Kluyveromyces marxianus* was the most active culture from the standpoint of pectin released (about 14% on the dry BP basis, which represents about 60% of the whole pectin in BP). The microbial pectin extraction was influenced by the physical and chemical pretreatment of BP, the BP levels (solid/liquid and liquid/solid), the size of seed culture, incubation period, pH value, some salts, and some growth and enzymatic activators (NAD, NADP, ATP, ascorbic acid) as well as with the presence of some natural additives. At those conditions, the pectin released was as much as 99% of the whole pectin in BP.

The microbiologically extracted pectin was chemically characterized and appeared to be of superior qualities to those of chemically extracted pectin.

GROWTH OF AEROBIC BACTERIA ON ALKALI-SOLUBILIZED LIGNITE

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Several strains of aerobic bacteria were isolated from environmental enrichment cultures on media containing alkali-solubilized lignite. Some of these strains, including strain KPA16, show enhanced growth when coal is present in the medium. Growth experiments with KPA16 on media containing different molecular fractions of lignite indicated that the growth of this organism is enhanced by the inclusion, in the basal medium, of any of these fractions: (1) alkali-solubilized whole lignite, (2) alkali-solubilized THF-insoluble lignite, and (3) the THF-soluble portion of lignite. Growth was analyzed by turbidimetric and protein determinations. In addition to improving the growth of KPA16, inclusion of alkali-solubilized whole coal in the basal medium extends the stationary phase of KPA16 cultures. Our results suggest that KPA16, and other aerobic strains that were isolated, may be capable of lignite degradation.

Paper 16

PRODUCTION OF LIGNIN-PEROXIDASE BY *STREPTOMYCES VIRIDOSPORUS*

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Streptomyces viridosporus T7A produces extracellular cellulolytic and ligninolytic enzyme systems. One major component of the ligninolytic enzyme system is lignin-peroxidase, which, in the presence of hydrogen peroxide, oxidizes lignin and lignin substructure model compounds. However, further investigation on the detailed characterization of this enzyme and assessment of its potential in biotechnological applications has been hindered by poor enzyme yield. In this paper, we report the development of a suitable defined culture medium for production of lignin-peroxidase *by Streptomyces viridosporus* T7A. Although this enzyme can be produced in yeast extract-mineral salt medium containing lignocellulose or xylan powder, it was found that the use of mono-, di- and trisaccharides will repress lignin-peroxidase synthesis. However, peroxidase activity was enhanced with the addition of a combination of glutamic acid, asparagine, and proline, using yeast extract as a source of nitrogen and additional carbon. The optimum conditions for cell growth and enzyme production were determined, as were the preferred conditions for enzyme synthesis. The kinetics of cell growth, total carbon consumption, and lignin-peroxidase production in batch reactors will be discussed as well as the characterization of lignin-peroxidase activity.

Paper 17

UTILIZATION OF CHEESE WHEY FOR PRODUCTION OF ALPHA-AMYLASE ENZYME

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Annually, approximately 1.2 million tons (1.1 Ggm) of lactose and 200,000 tons (0.18 Ggm) of milk proteins are transferred into whey worldwide, of which less than 60% are utilized for human food and animal feed. Thus, a large quantity (40%) of nutritionally valuable food resource is wasted. Considering the magnitude of the pollution problem and the nutritive value of whey solids, there is a need to develop new uses for whey and its derivatives in order to fully realize the benefits of this wasted resource.

We have found that it could be efficiently fermented into alpha-amylase enzyme by *Bacillus* sp. 25 AM1. The results of batch studies of flasks, about 1030 U/mL of enzyme was obtained in 72 h from cheese whey supplemented with 2% defatted soya flour and nutrient salts. Supplementation of cheese whey with 1% corn starch increased enzyme production to 1232 U/mL. However, further increase of corn starch from 1 to 2% did not result in appreciable change in enzyme production. In a fermenter, about 1590, 1780, and 1990 U/mL of enzyme was obtained in 30 h from media containing cheese whey, cheese whey + 1% corn starch and cheese whey + 2% corn starch, respectively.

CHARACTERIZATION OF FOUR PURIFIED EXTRACELLULAR LIGNINASES FROM THE LIGNIN-SOLUBILIZING ACTINOMYCETE, *STREPTOMYCES VIRIDOSPORUS* T7A

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Each of the four isoforms of the extracellular lignin peroxidase of *Streptomyces viridosporus* T7A (ALip-P1, P2, P3, and P4) were purified to homogeneity by ultrafiltration and ammonium sulfate precipitation, followed by electroelution using polyacrylamide gel electrophoresis. The purified peroxidases were compared for their substrate specificities towards lignin and lignin substructure model compounds and for their immunogenic relatedness by use of Western blot assays with a polyclonal antibody preparation produced in rabbits against purified isoform ALip-P3. This antibody preparation was also tested for its reactivity towards purified lignin peroxidases from the white-rot fungus *Phanerochaete chrysosporium* and several different *Streptomyces* species. Results showed that peroxidases P1 through P4 are probably derived from the same protein. They have similar substrate specificities but differ in molecular weights and probably in carbohydrate content. ALip-P3 is the dominant form of the enzyme. All four isoforms reacted strongly with the antibody. Anti-ALip-P3 antibody was reactive with peroxidases from some, but not all, of the other organisms tested. The results are discussed in relation to their use in characterizing *Streptomyces* lignin peroxidases, which may be useful in industrially useful bioconversions of lignocellulose.

BIOCONVERSION OF A L-CARNITIN PRECURSOR IN A MONO- OR TWO-LIQUID PHASE SYSTEM

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We have investigated the ability of the yeast, *Saccharomyces cerevisiae* to bioconvert octyl-4-chloroacetoacetate (OCA) stereoselectively into the corresponding chiral alcohol, a precursor of L-carnitin and an important physiological agent. In a monophase system and in free-cell reactors, more than 90% OCA (18 mmol/L) bioconversion has been reached after 6 h (enantiometric excess for the R-form - eeR:97%). Immobilized cells in alginate beads were less efficient to bioconvert OCA than free cells. In a two-phase system, the level of reduction of OCA (18 mmol/L) reached 85% in 48 h. With a medium containing a higher OCA concentration (270 mmol/L), 41% was bioconverted after the same period. On the other hand, immobilized cells didn't show any significant bioconversion of OCA transformation in two-phase reactors.

The regeneration of the cofactors involved in the OCA reduction has been investigated; they were regenerated at the beginning of the bioconversion.

PERFORMANCE OF TRICKLE BED BIOREACTORS FOR CONVERTING SYNTHESIS GAS TO METHANE

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Carbon monoxide, H_2, and CO_2 in synthesis gas can be converted to CH_4 by employing a tri-culture of *Rhodospirillum rubrum, Methanosarcina barkeri,* and *Methanobacterium formicicum.* Trickle bed reactors have been found to be effective for this conversion because of plug flow behavior and high mass transfer coefficients. This paper compares results obtained for the conversion of synthesis gas to methane in 2-in. (5 cm) and 6.5-in. (16.5 cm) diam. trickle bed reactors. Mass transfer for scaleup parameters are defined and light requirements for *R. rubrum* are considered in bioreactor design.

METABOLIC MODELING OF FUMARIC ACID PRODUCTION BY *RHIZOPUS ARRHIZUS*

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Fumaric acid has a variety of uses in the chemical, paper, food, and pharmaceutical industries. The fungus, *Rhizopus arrhizus*, utilizes glucose, carbonate (from the neutralizing agent), and oxygen (from aeration for respiration) to produce fumaric acid, carbon dioxide, cell mass, and five other byproducts. We have developed a metabolic model of the pathway kinetics and the reaction network producing these products. The metabolic model has two functions: (1) to predict rates (mol/g cell/h) or extents (mol/g cell) of individual reactions in the pathway in terms of measurable quantities, and (2) to provide relationships among the measurable quantities in addition to those given by a macroscopic balance. The rates of the individual reactions are found by taking the carbon and reductance degree balances on all the species in the proposed pathway. Additional relationships among the measurable quantities are found by invoking a pseudo-steady-state assumption on the non-accumulating species in the pathway. Several applications resulting from these two functions of the model will be discussed. From an analysis of the reaction network, the theoretical yield of fumaric acid from glucose and the maximum selectivity are determined. From the pathway kinetics, limiting reaction and possible ways to enhance fumaric acid productivity are identified.

A CRITICAL REVIEW AND EVALUATION OF BIOPRODUCTION OF ORGANIC CHEMICALS

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In this paper, over 60 organic chemicals (not including polysaccharides, nucleic acids, amino acids, proteins, etc.) that can be produced via bioconversion were identified. Current market values and production rates were compiled for several chemicals. In addition, previous analyses of the future of a biomass-based chemicals industry were surveyed. These data were used to identify promising potential production using bioconversion.

Organic chemicals can be made by a variety of bioconversion routes, including direct microbial and enzymatic conversions and combined biological/synthetic (chemical) conversions. Bioconversion routes can compete with synthetic routes through (1) direct substitution (i.e., production of chemicals presently produced and used by the U.S. chemical industry), and (2) indirect substitution (i.e., production of new, alternate chemicals that perform the same, or similar, functions as chemicals that are displaced). Each route has advantages and disadvantages. Examples are discussed.

Several bulk organic chemicals can be produced via bioconversion and provide potential examples of direct substitution. They include acetone, acrylic acid, adipic acid, 1,3-butadiene (via 2,3-butanediol or ethanol), n-butanol, ethanol, ethylene (via ethanol), glycerol, i-propanol, maleic anhydride (via malic acid), methanol (via methane), methyl ethyl ketone, and propylene (via i-propanol). Large quantities of citric acid, ethanol, and acetic acid are also produced at present by fermentation. Chemicals that could enter the marketplace by indirect substitution include lactic acid, 2,3-butanediol, butyric acid, fumaric acid, gluconic acid, and malic acid.

BIOLOGICAL PRODUCTION OF ACETALDEHYDE FROM ETHANOL USING NON-GROWING *PICHIA PASTORIS* WHOLE CELLS

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Acetaldehyde is produced commercially from the catalytic oxidation of ethyl alcohol or ethylene. Recently, interest has focused on the production of acetaldehyde by biological methods as a natural additive for various foods. Alcohol oxidase, from the peroxisome of *Pichia pastoris* yeast, can be used to produce "food grade" acetaldehyde without expensive isolation of the enzyme. A batch bioreactor with continuous aeration and liquid ethanol feed has been tested. The highly volatile acetaldehyde is removed with the air stream and condensed. This paper discusses the reaction parameters required to design a commercial bioreactor and presents enzyme stability data over a wide range of potential operating conditions. A preliminary process design and economic evaluation will be presented.

Paper 24

ENZYMATIC HYDROLYSIS OF STARCH IN A FIXED-BED PULSED-FLOW REACTOR

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One of the most important problems in the design and operation of fixed-bed biological reactors is the limitation of the process rate by mass transfer control. In order to overcome this problem, we have tried to develop a new technology based on the use of pulsed reactors. A new type of pulsing device, giving a see-saw disturbance, was assayed. To quantify the possible improvement obtained, we have chosen, as an example, the hydrolysis of concentrated starch solutions by glucoamylase (from *Aspergillus niger*) immobilized on chitin slabs, because of the high diffusional limitations of this system. The reactor has an internal diameter of 50 mm and a 200-mm bed height. Temperature was controlled at 25°C, and the working flow rates were 0.035 to 0.27 L/h. The results revealed that pulsation helps to attenuate the diffusional difficulties, since the maximum reaction velocity increased 5%. Additional improvements, up to 21% in some cases, were achieved when a recycling pulsed flow was introduced in the reactor.

THE DESIGN OF A MEMBRANE-BASED INTEGRATED ETHANOL PRODUCTION PROCESS

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The performance of an ethanol fermentation can be improved by integration with membrane separation steps. Microfiltration can be used for cell retention to increase the productivity, and pervaporation or perstraction can be used for in situ alcohol recovery. In this study, experimental results and a process evaluation are presented for an alcohol fermentation coupled to crossflow microfiltration and pervaporation. The fermentation was carried out with bakers' yeast in a stirred fermentor, coupled to external membrane modules. Pervaporation was carried out with commercially available high-flux hollow fiber modules. The mass transfer characteristics of these modules as a function of the operating conditions were investigated. Three different reactor/membrane configurations were investigated for ethanol production from a glucose feed. In each system, the productivity and substrate consumption in the fermentation could be increased. The fermentation kinetics was modeled, which enabled optimization of the integrated process. Furthermore, this model was incorporated into an economics evaluation model. With this model, membrane areas can be calculated for the different reactor configurations and operating conditions. The most important boundary conditions for an economical operation of the process were high membrane fluxes and a high selectivity of ethanol/water separation with pervaporation.

THERMOPHILIC ETHANOL PRODUCTION: INVESTIGATION OF ETHANOL YIELD AND TOLERANCE IN CONTINUOUS CULTURE

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Thermophilic bacteria are among the leading candidates for direct microbial conversion of biomass to ethanol. In addition to the advantageous properties of cellulose utilization, xylose utilization, and high fermentation rates, thermophiles are thought to have the disadvantages of low ethanol yield and low ethanol tolerance. In spite of over a decade of research, basic understanding and even description of yield and tolerance is incomplete. For example, high-yielding strains have often proved unstable, and apparently identical input conditions often result in varying yields. Furthermore, limitation by endogenously produced ethanol has yet to be documented.

This study reports ethanol yields as a function of input substrate concentration and dilution rate in continuous xylose-fed cultures of *Clostridium thermosaccharolyticum*. In addition, increasing substrate transients with this organism was found to increase ethanol yields, dramatically resulting in ratios of ethanol:organic acids >10:1. Such transients provide a useful model system for the study of end-product control and also a cultivation mode with considerable applied potential. Ethanol tolerance for *C. thermosaccharolyticum* was investigated using exogenous addition of ethanol to continuous cultures, endogenous ethanol at high feed xylose concentrations, and continuous ethanol removal. Results are considered from both practical and fundamental perspectives.

EVALUATION OF A MULTICOMPARTMENT BIOREACTOR FOR ETHANOL PRODUCTION USING IN SITU EXTRACTION OF ETHANOL

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A multicompartmented reactor can be used for ethanol production from a starch hydrolysate using yeast (*Saccharomyces cerevisiae*). By separating a solvent layer (tri-normal-butylphosphate or TBP) from a cell layer, ethanol can be extracted in situ while preventing phase toxicity. Pressure cycling of the gas phase can be used to reduce significantly mass transfer limitations that often limit entrapped cell systems. A detailed model for *S. cerevisiae* has been developed that can be incorporated into a model of the reactor system. The predictions of this model compare well to data obtained from laboratory-scale reactors. This reactor model has been used as the basis of a preliminary economic evaluation. The multicompartment reactor is generally more economically favorable at lower volumes while the traditional system is more favorable at high volumes. The cross-over point is sensitive to how compartmentation is obtained (e.g., membranes), the pore size of the membrane entrapping the cells, and the distribution coefficient and physical characteristics of the extractant.

NON-DISPERSIVE EXTRACTION FOR RECOVERING LACTIC ACID FROM FERMENTATION BROTH

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A number of fermentation products are toxic to cellular activity. In these fermentations, in situ removal of the product should improve the productivity of the microbial process. With this as a driving force, a non-dispersive extraction process is being developed for recovering lactic acid from broth. Of the many factors being investigated, choice of an appropriate solvent and that of the membrane are critical. This paper will discuss our results in these two areas. Specifically, the criteria for selection of the solvent, distribution coefficients of lactic acid, and the role of hydrogen ion concentration will be discussed. The rate of extraction of lactic acid across the membrane, longevity, frequency of cleaning, and reusability of polypropylene hydrophobic membrane will also be discussed.

Using a mathematical model, results of simulation of a reactive-extractive process for recovery of lactic acid and a comparison of its performance with those of a competing electrodialytic process will be presented.

RISK ASSESSMENT OF MICROORGANISMS USED IN POLLUTION CONTROL

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Microorganisms have been proposed as a solution to a variety of environmental problems including the cleanup of toxic waste sites (bioremediation), coal and oil desulfurization, and wastewater pretreatment. One important question to ask is whether these potential solutions themselves cause risks; and if so, what is the magnitude of those risks?

Several frameworks for evaluating the potential human health and ecological risks of planned releases of genetically engineered microorganisms (GEMS) have been proposed. Each of these frameworks can be applied to microorganisms used in bioremediation and other aspects of pollution control. The earlier proposals, which are currently the basis of risk evaluation at the US EPA, are analogous to chemical risk assessment frameworks. Recently, two frameworks specifically for genetically engineered organisms were proposed, one by the National Academy of Sciences and the other by the Ecological Society of America.

The chemical risk-assessment-like frameworks start with the assumption that risk is a product of exposure and hazard, and that they incorporate components, such as hazard identification and exposure assessment. For microbes used for pollution control, the hazard identification should include consideration of the hazard posed by the microorganism itself and of the toxicity of the products and by-products of microbial metabolism. One fundamental difference in the exposure assessment for microbes compared to chemicals, is that fate and transport models must account for the ability of microorganisms to reproduce. In addition, microbes can be transported in potentially environmentally significant quantities on surfaces and by vectors, such as insects.

Paper 30

ANAEROBIC DECHLORINATION OF CHLORINATED BIPHENYLS BY MIXED BACTERIAL CULTURES FROM PCB-CONTAMINATED SEDIMENTS

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Mixed cultures of anaerobic bacteria were isolated by enrichment with biphenyl, from PCB-contaminated sediments collected on the U.S. Department of Energy's Oak Ridge Reservation. These cultures were tested for their ability to remove chlorine from chlorinated biphenyls under anaerobic conditions. Single congeners of chlorinated biphenyls containing five or more chlorines were used to evaluate the dechlorination potential of the bacteria. With certain congeners, one bacterial culture from these enrichments demonstrated the ability to remove chlorines from the biphenyl ring structure, resulting in the production of a range of lower-chlorinated species. Dechlorination activity could be detected after as little as one week and increased with further incubation. Dechlorination activity was noted even in the presence of an added carbon source (e.g., glucose). Data on effects of added carbon sources will be discussed in relation to their potential implications for laboratory maintenance of such cultures for use in possible bioremediation technologies for PCB-contaminated sites. This mixed culture has been characterized microbiologically and found to contain a small variety of common soil genera.

IN SITU SOIL AERATION FOR ENHANCED BIODEGRADATION OF HYDROCARBONS

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Conventional technology for enhancing in situ biodegradation of petroleum contamination typically depends upon aerobic biodegradation in groundwater. For the more readily degradable fuels, oxygen (or an alternative electron acceptor) is typically the limiting factor. Oxygen is introduced in injection water either by air sparging, pure oxygen sparging, or hydrogen peroxide. Even with 500 mg/L of hydrogen peroxide approximately 30,000 gallons (114,000 L) of water must be pumped to deliver sufficient oxygen to fully degrade 1 gallon (3.8 L) of fuel. With the use of air pumping in the vadose zone to supply oxygen, pumping requirements are decreased by more than 3 orders of magnitude.

Data will be presented from two sites at which air pumping has been utilized to stimulate in situ biodegradation. One site is located at Hill Air Force Base, Utah, and the other at Tyndall Air Force Base, Florida. It will be shown that even in the arid environment of Utah's high desert, significant biodegradation can be induced by simply pumping air into the vadose zone. Bench-scale studies indicated that biodegradation rates may be substantially increased by the addition of nutrients and increasing soil moisture content. At the Tyndall site, with very shallow groundwater, the site was dewatered to increase the vadose zone depth and expose the majority of the contamination to air flow.

These demonstrations illustrate the feasibility of soil aeration to stimulate in situ biodegradation of petroleum fuels in two very different environments and show the technology to be a very cost-effective alternative to more conventional groundwater-based, enhanced, biodegradation technologies.

Paper 32

BIOLOGICAL OXYGEN CONSUMPTION AS A MEASURE OF BIODEGRADATION OF MIXED ORGANIC WASTES

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Biological oxygen demand has been used to continuously monitor bacterial respiration during growth on mixed organic wastes from contaminated water and soil. Continuously collected oxygen consumption data provide information on the overall metabolic activity of the resident bacterial population and permit direct observation of the cessation of microbial respiration, and thus, the termination of aerobic biodegradation. The correlation of biological oxygen utilization with biodegradation has been confirmed using independent analytical methods. Continuous, long-term biological oxygen demand analysis has been applied to bench-scale studies to assess the biodegradability of mixed organic wastes from contaminated sites and industrial waste effluents. Case studies have demonstrated the utility of respirometry as a means of reliably determining the aerobic biodegradation potential of hazardous organic wastes using native bacterial populations.

IRON OXIDATION BY *THIOBACILLUS FERROOXIDANS*

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The bacterium most frequently used in microbial leaching operations and studies is *Thiobacillius ferrooxidans*. Applications of this bacterium include the treatment of coal, low-grade ores, and solids wastes (e.g., from hot-brine geothermal power plants) for the solubilization of pyrite and other metal sulfides. The goal of this study is the development of a standard procedure that can be used for screening or comparing various strains of *Thiobacillius ferrooxidans*.

Measuring the oxidation of ferrous iron is a quick and simple method for predicting the relative effectiveness of a given strain of *Thiobacillius ferrooxidans* in microbial leaching operations. When conducting experiments for the relative determination of bacterial activity, 9K medium with a low initial ferrous iron concentration and a pH of approximately 2.0, is recommended. Also, it is important to begin with the same ferric iron concentration in bacterial cultures, while minimizing the amount of precipitated iron. The effect of inoculum size on the maximum oxidation rate of ferrous iron is negligible; however, lag time can be reduced by using larger inocula.

NOVEL BIOTREATMENT PROCESS FOR GLYCOL WATERS

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Propylene oxide (PO), propylene glycol (PG), and polyols are produced from propylene via propylene chlorohydrin. Effluent from the plants producing these contain high BOD/COD loads in addition to high chloride concentrations. The high salinity poses a severe problem to adapt to conventional methods like activated sludge processes. Also, the conventional processes are marked by large sludge production, a higher sensitivity to pH, large residence time, high capital investment, and a higher treatment cost.

Presently, a simple, economically viable, and versatile microbiological process has been developed to get more than 90% biodegradation in terms of BOD/COD, utilizing specially developed cultures of *Pseudomonas* and *Aerobacter*. The process can tolerate high salinity (up to 10 wt% $CaCl_2$) and can withstand wide variations in pH (6.5 to 11.0) and temperature (15 to 45°C). There is no need to aerate the bioreactor contents, unlike activated sludge processes. Also, the capital and operating costs of the process are low compared to that of the activated sludge processes.

The process involves enzymatic conversion of glycols to carboxylic and hydroxy-carboxylic acids and further degradation to CO_2 and H_2O. In this study, various process parameters obtained at the lab (50-L bioreactor) and pilot stages (20 m^3 bioreactor) are discussed. A commercial plant to treat 120 m^3/h of PO/PG - polyol effluent is under construction and is expected to be commissioned in the first quarter of 1990.

GAS HYDRATE FORMATION IN REVERSED MICELLES - APPLICATIONS TO BIOSEPARATIONS AND BIOCATALYSIS

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Reversed micelles are water-in-oil microemulsions which are capable of solubilizing a variety of compounds in the microaqueous phase. Protein-containing reversed micelles are extensively studied for their biomembrane mimetic properties and for their potential in biocatalysis and protein extraction processes.

We describe a novel technique to control protein solubility and to optimize enzyme activity in reversed micelles. The technique is based on the ability of natural gas hydrates to form in the microaqueous phase. Clathrate hydrates are crystalline inclusions of water and gas, and their formation in bulk water has been traditionally studied with relevance to natural gas recovery. We have found that hydrates can form in the new environment of the microaqueous pools of reversed micelles and that their extent of formation can be precisely controlled through the thermodynamic variables of temperature and pressure. Additionally, formation of hydrates affects the size and aggregation number of the micelles and thus influences the solubility and conformation of encapsulated proteins. We demonstrate how the concept can be used in two applications: (a) protein extraction from fermentation broths and (b) optimization of lipase activity during ester synthesis in reversed micellar systems.

Abstracts for Poster Session

Poster 36

FERMENTATION STUDIES ON SUGARS RECOVERED FROM WASTE CELLULOSICS VIA ACID HYDROLYSIS

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Fermentation studies were conducted on sugars recovered from various waste cellulosics, including newsprint and cellulosics from municipal solid wastes. Sugars were recovered by a dilute sulfuric acid hydrolysis of the cellulosics designed to optimize glucose recovery at the expense of more acid-labile sugars. However, the acid processing produced toxins which interfered with fermentation. Toxins were identified and studied to determine inhibitory levels as well as a means to alleviate their inhibition of glucose fermenting yeasts. Several strains of yeasts were evaluated for their ability to produce acceptable ethanol yields from the acid hydrolyzates. Parameters of fermentations, including growth rates, sugar utilization rates, ethanol production rates, fate of inhibitors, and yield data were determined.

Results from the fermentation studies of the various waste cellulosics will be presented.

ACTIVE PROTEIN AND SUBSTRATE FLOW EFFECTS IN A TUBULAR IMMOBILIZED INVERTASE REACTOR

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Laboratory-scale investigations of invertase (EC 3.2.1.26) immobilized inside modified nylon tubes showed that between 4% and 20% (w/w) of the enzyme protein exposed to binding sites on the tube was immobilized. An enhanced activity consistent with enzyme purification during immobilization was also evident, suggesting that, in commercial applications, nylon tube invertase would be a more economical converter of sucrose than the free enzyme. The quantity and specific activity of the immobilized protein were not stoichiometrical with the concentration of invertase used in the coupling solution and, in the system studied, an invertase concentration of 2 mg mL^{-1} was optimal.

K_m and V_{max} values confirmed higher rates of immobilized invertase catalysis at higher rates of substrate flow through the reactor. These higher catalysis rates, attributable to a reduction of the diffusion barrier between enzyme and substrate, would not translate into improved economy in the commercial flow-through processes at which the reactor is aimed. Higher rates of substrate flow imply a shortened residence time in the reactor and would lower the fractional conversion of the substrate per pass, thus reducing the efficiency of the reactor in flow-through situations.

Poster 38

EVALUATION OF DILUTE SULFURIC ACID HYDROLYSIS AND AUTOHYDROLYSIS FOR SUGAR PRODUCTION FROM HARDWOOD HEMICELLULOSE

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The Tennessee Valley Authority (TVA) is evaluating dilute sulfuric acid hydrolysis for sugar production from newsprint and the cellulosic fraction of municipal solid waste (MSW). The objective of this work is to provide information essential for the assessment of the technical and economic feasibility of a process to produce ethanol from these waste cellulosics. A one-step hydrolysis process to maximize glucose production from cellulose coupled with zero or negative feedstock cost could be a viable alternative to the landfilling and mass burning of a significant portion of MSW. Tests have been performed at temperatures between 150°C and 210°C, retention times of 1 to 180 min, and acid concentrations between 1% and 4% (w/w). This paper will discuss the effect of different hydrolysis variables on glucose production and will examine the use of the hydrolysis residue as an acceptable boiler fuel.

Poster 39

CONVERSION OF NEWSPRINT- AND MSW-CELLULOSICS TO SUGAR BY DILUTE SULFURIC ACID HYDROLYSIS

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The Tennessee Valley Authority (TVA) is developing a two-stage dilute sulfuric acid hydrolysis process to produce sugar from hardwood hemicellulose (first stage) and cellulose (second stage). The objective of this work has been to maximize the possible process changes and reduced costs associated with using hydrolysis. To compare sugar production using autohydrolysis or dilute acid, tests have been performed at temperatures between 130°C and 210°C, several liquid-to-solid ratios, retention times from 1 to 180 min and a sulfuric acid concentration of 0.75% for the tests with acid. This paper presents data on sugar production, sugar degradation, residue composition, and nonsugar hydrolyzate components resulting from the two types of hemicellulose hydrolysis.

Poster 40

EFFECT OF MEDIUM COMPOSITION AND GROWTH INTERVALS ON GROWTH, TOTAL LIPIDS, AND THE FATTY ACID COMPOSITION OF *FUSARIUM SOLANI*

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Growth rate and total lipids of *Fusarium solani* cultivated on lactose as the sole source of carbon in a media of carbon to nitrogen source ratio of 33:1 or 2:1 were investigated. In nitrogen-limited media (C:N of 33:1), the mold gave the highest growth rate of 9.01 g/L after 12 d and total lipids of 13 gm/L after 3 d. However, cells grown in carbon-limited media (C:N of 2:1) gave a maximum growth rate (1.06 g/L) after 12 d and total lipids (112 mg/L) after 6 d.

The fatty acid composition of polar lipids and triglycerides from cells grown for different intervals were studied. The results obtained indicated that polar lipids contained lower values of unsaturated and C_{18} fatty acids than triglycerides, whether the mold was grown in nitrogen- or carbon-limited media.

Poster 41

DELIGNIFICATION OF NON-WOODY BIOMASS

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Xylan, Inc., is a biotechnology R&D company that has developed a proprietary process which can be used effectively for the conversion of lignocellulosic materials into value-added products. Our initial work focused on the production of dietary fiber from milling byproducts such as oat hulls, wheat bran, brewers grains, and other agricultural waste substrates.

It was this work that led us to a number of other commercial applications for this technology. These include the conversion of biomass into (1) fermentation feedstocks, (2) fiber material for co-polymerization into composites, and (3) into ruminant livestock feed.

A non-woody biomass is delignified through extrusion technology, utilizing hydrogen peroxide and an alkali agent to break down complex biomass materials. The process is useful in forming a highly absorbent fiber material for use as a dietary fiber or an absorbent fiber. Alternatively, the process is useful for preparing dietary feeds for ruminant animals, as well as to produce a broad range of alcohols or polymers from the non-woody lignocellulosic substrate.

Poster 42

THE STUDY OF TREATING PHARMACEUTICAL WASTEWATER IN THE UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTOR

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In this paper, we expound on the study of a method for treating pharmaceutical wastewater in a UASB reactor using the mesophilic digestion (35 to 38°C). We have obtained a series of data from start up to operation and analyzed the technological parameters which affect operation of the reactor. This experiment was done with a UASB reactor which had a volume of 5 L, and we had very good results. The COD removal rate and COD loading rate achieved 80 to 90% and 8 to 10 kg COD/m^3•d, respectively, when the hydraulic retention time equaled 6 to 8 h. Also, this paper discusses the structure and type of gas-liquid-solid separator in a UASB reactor and various factors that affect separator efficiency. Lastly, we used fuzzy optimization on the design structure of the reactor, which provided some insight for the engineering design of a UASB reactor.

Poster 43

SINGLE-CELL PROTEIN PRODUCTION FROM ACID-HYDROLYZED LIGNOCELLULOSICS

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Production of single-cell protein can be used as a means of recovering nutrients contained in acid hydrolyzates of hardwood and crop residues. Three yeasts, *Pichia (Hansenula) anomala* NRRL, Y-656, *Candida tropicalis* NRRL Y-11860, and *Candida utilis* NRRL Y-900, and four variants of the mold *Paecilomyces varioti* were screened for their ability to utilize the nutrients in these hydrolyzates for cell growth and for their ability to decrease the potential pollution load of these hydrolyzates. Culture conditions were varied to determine optimum incubation parameters, including pH, nitrogen, phosphorus levels, and aeration rate. Compositional analyses were performed on the cell biomass to determine the suitability of the product as an animal feed. Chemical oxygen demand and other wastewater analyses were performed on the hydrolyzate media, before and after fermentation, to determine the amount of reduction in the nutrient load of the hydrolyzate. Overall, *C. tropicalis* and one variant of *P. varioti* (NRRL 1116) produced the most cell biomass from acid hydrolyzates. The pH necessary for optimum growth was about 5.5, while the amount of supplemental nitrogen and phosphorus needed was different, depending on the type of hydrolyzate. All the cultures grew poorly in unsupplemented hydrolyzates, indicating that one or more nutrients may need to be added to achieve appreciable cell growth.

Poster 44

DILUTE ACID PRETREATMENT OF CORN RESIDUES AND SHORT ROTATION CROPS

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Expansion of alcohol fuel production will require incorporation of lignocellulosic feedstocks into existing industrial processes, which are based on the bioconversion of starch, in corn and other grains, to ethanol. Abundant resources of lignocellulosic materials exist in the form of agricultural, forestry, and municipal wastes. These resources can be expanded in the future by the production of short rotation woody and herbaceous crops. We have extended previous investigations of dilute acid pretreatment of wheat straw, and a few short rotation crops, to corn stover, corn cobs, and additional short rotation hardwoods - black locust, silver maple, and sycamore. These hardwood samples were provided by the Biomass Production Program at Oak Ridge National Laboratory. The milled, debarked hardwoods and corn residues were subjected to prehydrolysis with dilute sulfuric acid at 140 and 160°C for reaction times ranging from 5 to 60 min. Dilute sulfuric acid hydrolyzed all hemicelluloses in corn stover and silver maple within 30 to 60 min at 140°C and 5 to 10 min at 160°C. The hemicelluloses in corn cobs were unusually susceptible to dilute acid prehydrolysis, since all xylan was hydrolyzed in 10 min at 140°C. Cellulose, in both pretreated corn residues and in pretreated silver maple, was highly digestible by cellulase enzyme from *Trichoderma reesei*. The results of dilute acid pretreatment of the remaining two hardwoods will also be presented.

Poster 45

A TECHNICAL AND ECONOMIC ANALYSIS OF ETHANOL PRODUCTION FROM WHEAT STRAW OR ASPEN WOOD CHIPS USING STEAM EXPLOSION OR DILUTE ACID PRETREATMENTS

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The process economics of ethanol production from lignocellulosics has been shown to be very sensitive to the cost of the feedstock, as well as to the yields of the sugars produced chemically or enzymatically through pretreatment and saccharification, respectively. Agricultural residues, such as corn stover and wheat straw, and logging residues in the form of limbs, tree tops, and defective or broken logs, represent an underutilized and readily available resource for ethanol production. In order to obtain a comparison of the costs of production of ethanol, from either an herbaceous crop (wheat straw) or woody biomass (aspen), a preliminary technical and economic analysis of an enzymatic hydrolysis-based ethanol plant has been completed. This analysis addresses two promising pretreatment alternatives: (1) dilute sulfuric acid prehydrolysis and (2) sulfur-dioxide-impregnated steam explosion. Detailed process flow sheets, heat and material balances, and equipment cost information were generated. An economic analysis then compared the performance of the two chosen feedstocks for each of the pretreatment options. These results will be presented along with a sensitivity analysis, which was done to identify critical areas for further process improvements.

Poster 46

EFFECT OF PRETREATMENT ON SSF OF HARDWOOD INTO ACETONE/BUTANOL

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The effectiveness of various pretreatment methods on hardwood substrate was investigated in connection with its subsequent conversion by simultaneous saccharification and fermentation (SSF) using *Clostridium acetobutylicum*. The main objective of pretreatment was to minimize carbohydrate losses and to obtain maximum sugar yield upon enzymatic hydrolysis of pretreated wood. Two methods: (1) SO_2 treatment and (2) monoethanolamine treatment have shown promising results.

Under the scheme of the SSF, the pretreated hardwood is converted to acetone and butanol via single-stage processing by cellulase enzyme and *C. acetobutylicum* cells. The primary benefit of SSF is that the inhibition on cellulase by glucose and cellobiose is eliminated as sugars are consumed by microorganisms as soon as they are formed. The kinetics of the SSF process were studied, and the effects of various process parameters, including enzyme loading, were investigated. Hydrolysis was found to be a rate-limiting step in the conversion of cellulosics to solvents. Thus, fermentation was completed under a glucose-limited state. The ability of *C. acetobutylicum* to metabolize various 6- and 5-carbon sugars resulted in complete utilization of the available sugars from hardwood.

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CONTINUOUS PRODUCTION OF BIOGAS FROM CHEESE WHEY USING A pH-CONTROLLED, TWO-STAGE MESOPHILIC REACTOR

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A two-stage, no-mix anaerobic digester was used for the recovery of energy from acid cheese whey. The system was specially designed to act as a liquid-solid separator. The performance of the system was evaluated at four retention times (20, 15, 10, and 5 d). Mass and energy balances were also performed on the system. The effects of various regimes for controlling the pH of the methanogenic stage of the anaerobic digestion process were investigated in relation to biogas productivity, methane yield, and pollution potential reduction.

Controlling the pH of the methanogenic stage has significantly increased the biogas production rate and methane yield. The system was capable of reducing the COD, solids, and nitrogen concentrations by 60%, 55% and 39%, respectively. The sludge obtained from the digester was rich in protein content and can be used as a supplement in animal rations.

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THERMAL DEGRADATION OF CEREAL STRAWS IN AIR AND NITROGEN

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Cereal straws are one of the most commonly available lignocellulosic materials which can be converted to different types of fuels and chemical feedstocks through a variety of thermochemical conversion processes. Cellulose, hemicellulose, and lignin are the major components of straw. Different components of straw exhibit different thermal behavior, with hemicellulose being the least stable component. The natural impurities and ash content could also produce profound effects. Although cellulose, one of the major constituents of straws, has been extensively investigated for its thermal behavior, straw itself has not been given the same consideration.

In this study, the thermogravimetric behavior of four cereal straws (wheat, barley, oats, and rye) at three heating rates (10, 20 and 50°C/min) in air was examined. The thermal degradation rate, the initial degradation temperature, the active and passive pyrolysis zones, and the residual weight at 600°C were determined. Increasing the heating rate increased the thermal degradation rate and decreased both the initial degradation temperature and the residual weight at 600°C. The higher the cellulosic content of the straw, the higher the thermal degradation rate and the initial degradation temperature. Also, higher ash content in the straw resulted in higher residual weight at 600°C.

INFLUENCE OF MACRONUTRIENTS, AUXINS, AND BIOSYNTHETIC PRECURSORS ON ROSMARINIC ACID SYNTHESIS IN CELL SUSPENSION CULTURES OF *SALVIA OFFICINALIS*

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The influence of macronutrients, auxins, and biosynthetic precursors on growth and rosmarinic acid (RA) synthesis was investigated in cell suspension cultures of *Salvia officinalis*. RA was synthesized maximally in the stationary phase, indicating growth was uncoupled to RA synthesis. A study of the effects of sucrose as a carbon source indicated a concentration of 3% (w/v) was optimum for both cell growth and RA synthesis. However, the optimum concentration of macronutrients (nitrate, phosphate, and calcium) varied for growth and RA synthesis. Also, the optimum concentrations of auxins and RA precursors differed for growth and for RA synthesis.

ISOLATION AND CHARACTERISTICS OF PLASMIDS FROM *CLOSTRIDIUM THERMOSACCHAROLYTICUM*

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It is shown that *C. thermosaccharolyticum*, strain DSM 571, was resistant to 5 to 20 mg/L streptomycin and kanamycin. The superproduction of hydrogen was recorded in the presence of their 5 to 50 mg/L concentrations. A few hypotheses are advanced to interpret the observed phenomenon.

The plasmid DNA was isolated from *C. thermosaccharolyticum*, strain DSM 571. Restriction analysis afforded a conclusion about the presence of two plasmids, 4.9 kb and 2.0 kb. We termed them pNB1 and pNB2, respectively. The copy number of the pNB1 per cell during cultivation of the bacteria on the medium without antibiotics is 1-2. On the selective media containing kanamycin or streptomycin, the plasmid copy number per cell increases up to 5-10. The pNB1 has one restriction site EcoR I, one site Pst I, and three sites Hind III. There are no restriction sites for Bgl II and BamH I. The pNB2 has at least one site Hind III and one site Pst I. The cloning of pNB1 was carried out in *E. coli*. A detailed restriction map and description of both plasmids will be reported. The plasmids pNB1 and pNB2 can be used as vectors for genetic study and exploration of thermophilic gram-positive bacteria.

KINETIC INVESTIGATION AND MATHEMATICAL MODELLING OF METHANOGENESIS

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We have studied the kinetics of the biomethanogenesis of various polysaccharides, monosaccharides, proteins, amino acids, volatile fatty acids (VFA), and alcohols in a batch process. As methane producers, both pure cultures and microbial associations were used. The most important chemical reactions were selected, main intermediates identified, and the kinetic schemes of the studied processes constructed. The processes studied were mathematically modeled in a few steps. First, a mathematical model of monosaccharide conversion was designed, which involved growth and metabolism of three groups of microorganisms: acid producers, as well as acetate- and hydrogen-utilizing methane producers. Then, the model was supplemented by cell lysis processes, induction, and repression of the enzyme responsible for β-oxidation of VFA, as well as by some inhibition processes related to VFA and hydrogen concentrations. The model satisfactorily describes the experimental results, can predict some disruptions of methanogenic process, and helps to estimate the kinetic parameters. Further improvement of the model is related to introduction of growth and metabolism parameters of the bacteria responsible for β-oxidation, pH-dependency, as well as of parameters of cellulase complex enzymes in the case of methanogenesis of cellulose. Studies of this trend are in process.

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BIODEGRADATION OF MIXTURES OF HAZARDOUS ORGANIC COMPOUNDS

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Numerous hazardous organic compounds are known to be biodegradable individually. However, hazardous waste sites usually contain a mixture of several of these compounds. Because of their chemical diversity and complexity, these sites are presently viewed as poor candidates for bioremediation. Our purpose has been to determine if complex mixtures of hazardous organics can be microbially degraded using a sequential batch process aided by adjustment and control of process conditions. Small-scale (1-L) batch reactors employing mixed cultures were used to study the biodegradation of mixtures of five hazardous organic compounds: naphthalene, benzene, bis(ethylhexyl)phthalate, acetonitrile, and 1,2,4-trichlorobenzene. The biodegradation, including kinetics and interactive effects, of these compounds alone and in various combinations will be discussed.

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INDUCTION OF MUTATION IN *TRICHODERMA VIRIDE* AND ON LABORATORY-SCALE CONVERSION OF NATURAL CELLULOSE INTO FUEL

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Mutants of *Trichoderma viride* ATCC 32630 were obtained by uv-irradiation [2400 erg/mm^2 (240 J/m^2)] for 60 s of conidia suspension to 6% survivors. The mutants were selected on the basis of their capacity to convert cellulose into reducing sugars. Six of the selected mutants revealed higher avicelase activity 0.0365, 0.0375, 0.0385, 0.0475, 0.0565, and 0.0605 μmol reducing sugars/mL/min than the wild type 0.033 μmol reducing sugars/mL/min. While five mutants exhibited higher CMCase activity values 4.35, 4.58, 4.61, 4.91, and 5.74 μmol reducing sugars/mL/min as compared with the parent strain 3.76 μmol reducing sugars/mL/min. However, only one mutant gave 29.95 μmol P-nitrophenol/mL/min compared to the wild strain 21.0 μmol P-nitrophenol/mL/min. Hydrolysis of natural cellulose (rice straw) and cellulose powder on a laboratory scale by the cell-free extract of *A. niger*, *T. viride*, or the mixture of both, indicated the superiority of *T. viride* liquid culture filtrate and the mixture of the culture filtrate of the two fungi species, in the saccharification of the cellulosic materials tested.

INDUCTION OF MUTATION IN *ASPERGILLUS NIGER* FOR CONVERSION OF CELLULOSE INTO FUEL PRECURSOR

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Induction of mutation in *Aspergillus niger* ATCC 1004 was achieved by exposure of conidia suspension to uv-light [2400 erg/m^2 (240 J/m^2) for 120 s to obtain 5% survivors. The mutants were tested for the activity of different cellulases and were selected according to (1) rate and percentage of germination, (2) mycelial dry weight, (3) colony morphology, and (4) diameter of conidia. The efficiency of the mutants to convert cellulose into reducing sugars was detected by the assay of the activity of different cellulases. Three of the selected mutants indicated higher Avicel activity, 0.0183, 0.0205, and 0.0295 μmol reducing sugars/mL/min compared to 0.0153 for the wild type. The uv-irradiation also induced mutants that gave elevated CMCase activity, 3.06, 3.44, 5.30, 6.47, and 7.20 μmol reducing sugars/mL/min as compared with the wild type, 2.94 μmol reducing sugars/mL/min. Highly significant (F-test) increases in the activity of β-glucosidase were obtained for some of the selected mutants, 196.50, 255.00, 255.70, and 267.5 μmol P-nitrophenol/mL/min compared to the parent strain, 167.20 μmol P-nitrophenol/mL/min.

CHARACTERIZATION OF A PHOTOSYNTHETIC SHEAR-RESISTANT *GLYCINE MAX* CELL LINE

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Two cell lines of photosynthetic suspension cultures of *Glycine max* were established under conditions which allowed one of the cell lines to adapt to higher shear, making it more suitable for growth in a stirred tank reactor or generally in any reactor under higher transfer rates. Increases in biomass, cell aggregate size distributions, and chlorophyll levels of the two cell lines were measured and compared, as well as the polysaccharide composition of cell wall materials. High shear conditions produce smaller cell aggregates. The higher shear cell wall material shows a lower cellulose content with an equivalent increase in hemicellulose content, and increases in galactose and xylose at the expense of mannose and glucose was shown in the analysis of cell wall composition after hydrolysis. Various interpretations of the data will be offered in the light of progress in developing functional photosynthetic plant cell suspensions.

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IMMOBILIZATION OF ENZYME TO A PLATINUM ELECTRODE AND ITS USE AS AN ENZYME ELECTRODE

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Glucose oxidase was covalently immobilized to a platinum electrode surface which was treated by (1) anodic oxidation, (2) aminosilanization, and (3) glutaraldehyde. The condition employed for anodization and aminosilanization was shown to affect the sensor response. The linear response of the sensor using the platinum enzyme electrode was found to range from 10 to 1000 μmol/L of glucose concentration.

Diffusion equations, which describe the material balances of the participating species, were integrated with the boundary conditions appropriate to the condition under which the sensor was used; and the expression which correlated the sensor output signal to the system properties was obtained. This equation was used to explain the dependency of the sensor response on the stirring speed of the sample solution.

The same method of immobilization of enzyme was applied to other enzymes such as galactose oxidase.

ETHANOL PRODUCTION BY RECOMBINANT *ESCHERICHIA COLI* CARRYING GENES FROM *ZYMOMONAS MOBILIS*

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Efficient utilization of lignocellulosic feedstocks offers an opportunity to reduce the cost of producing fuel ethanol. Although the bacterium, *Zymomonas mobilis*, is more efficient than yeast in converting glucose to ethanol, it is unable to utilize the C_5-sugars derived from the hemicellulose fraction of lignocellulosic materials. Using rDNA technology, *E. coli* has been genetically transformed with the PET operon-carrying pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*adhB*) genes from *Z. mobilis* CP4 (F. Alterthum and L. 0. Ingam, *Appl. Environ. Microbiol.* 55, 1943, 1989). The fermentation performance characteristics of recombinant *E. coli* ATCC 11303 (pLOI297) were assessed in batch and continuous processes with sugar mixtures designed to mimic process streams from lignocellulosic hydrolysis systems. Operating parameters were optimized with respect to both product yield and productivity. The results were compared to other pentose-utilizing microorganisms recently reported in the literature.

DEGRADATION OF ORGANIC SULFUR COMPOUNDS BY A COAL-SOLUBILIZING FUNGUS

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Certain fungi have been demonstrated to convert low-ranked coals to a water-soluble product. This product contains low-molecular-weight oxidized aromatic compounds which may provide carbon skeletons for subsequent biological or chemical processing. The coals suitable for biological conversion are typically contaminated with sulfur, which may be covalently incorporated into the polyaromatic coal structure. The fate of organic sulfur during fungal coal solubilization has not been studied.

Paecilomyces sp. TLi, a natural fungal isolate known to solubilize coal, has been shown to degrade low-molecular-weight, heterocyclic coal substructure compounds. Sulfur-containing compounds, including thiophene, dibenzothiophene, and thianthrene, were broken down to soluble products. Aryl sulfides were also degraded. Experimental evidence suggests the cleavage of carbon-sulfur bonds, with the potential for the release of free sulfate.

The degradation of sulfur-containing coal model compounds by fungi in general, and by coal-solubilizing fungi in particular, has not previously been reported. The ability of this organism to break down coal structural elements suggests its potential utility in a biological process for the desulfurization of coal or coal-derived materials.

*Operated by Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

PHYSIOLOGICAL ASPECTS OF THE REGULATION OF EXTRACELLULAR ENZYMES OF *PHANEROCHAETE CHRYSOSPORIUM*

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The production of ligninase (LiP) and Mn-peroxidase (MnP) by *Phanerochaete chrysosporium* was studied in shaken cultures. LiP was only found under high 0_2 tension (100%) and was always undetectable using air, regardless of the aeration conditions employed. In contrast, MnP reached significant levels under a very broad range of 0_2 tensions. Both enzymes displayed a maximum in N-limited media (2.2 mM) and were undetectable at 22 mM. However, at 11 mM, nitrogen 5% and 20% activity remained for LiP and MnP, respectively. The conditions that led to a decrease or depletion of LiP activity led to high levels of a primary and secondary extracellular protease activities and increased the formation of extracellular-free and mycelial-bound polysaccharides; whereas, MnP seems to be only affected by the primary protease activity. The results showed that, in spite of the constitutive similarities displayed by both lignin-degrading peroxidases, the environmental conditions controlling their activities are quite different.

BIOCHEMICAL OXIDATION OF D-SORBITOL TO L-SORBOSE BY IMMOBILIZED *GLUCONOBACTER OXYDANS* CELLS

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Gluconobacter oxydans cells were entrapped in alginate gel, stabilized with Al^{3+} ions, and in gels based on polyacrylamide hydrazide - Na - alginate, crosslinked with dialdehydes and Ca^{2+} ions.

The conditions for immobilization of cells were investigated. The optimum concentration of used polymers and crosslinked reagents was determined. The biochemical activity of *G. oxydans* cells immobilized in gels of Ca-alginate, Ca-alginate stabilized with Al^{3+} ions, polyacrylamidhydrazide - Na - alginate, crosslinked with polyvinylalcohol (oxidized by periodate), glutaraldehyde, and Ca^{2+} ions, and polyacrylamidchydrazide - Na - alginate crosslinked with glyoxal were established. Optimum pH, cultivation time of initial cell suspension, and optimum concentration of biocatalyst in the fermentation medium were discovered; and it was observed that immobilized cells of *G. oxydans* oxidized D-sorbitol to L-sorbose at a rate approximating that of free cells.

G. oxydans cells immobilized in Ca-alginate were stable for 6 d. Cells immobilized in Ca-alginate, treated with Al^{3+} ions, were stable more than 30 d; and cells immobilized in polyacrylamidehydrazide - Na - alginate were stable more than 50 d without loss in their activity.

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MICROBIAL DESULFURIZATION OF FOSSIL FUELS

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The technology of flue gas desulfurization is now established and generally used for the desulfurization of coal. In order to develop a more economical system, it is very important to obtain bacteria that are capable of efficiently removing the sulfur from coal before combustion. Two strategies were employed for the microbial desulfurization of fossil fuels. One approach was to establish an enrichment culture utilizing dibenzothiophene (DBT) as the growth-limiting source of sulfur. The sulfur-limited enrichment culture resulted in the isolation of a mixed bacterial culture capable of utilizing sulfur from DBT while growing on succinate as a carbon source. The mixed bacterial culture vigorously utilized sulfur from not only DBT but also DBT sulfone, benzothiophene, thiophene, benzyl phenyl sulfide, and thiophene-2-carboxylate. The mixed culture formed a sulfur-free yellow aromatic compound of molecular weight 215 from DBT. The other approach was co-metabolic oxidation of sulfur-containing organics to give water-soluble compounds which could be removed by leaching. The use of traditional shake flask enrichment culture techniques resulted in the isolation of a *Pseudomonas* sp. DBF63, which was capable of co-metabolizing DBT while growing at the expense of dibenzofuran (DBF). Based on the formation of benzocinnoline from DBF and yellow aromatic compounds from DBT, new metabolic pathways of those compounds are proposed.

BUTANOL PRODUCTION FROM CARBON MONOXIDE BY *BUTYRIBACTERIUM METHYLOTROPHICUM*

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A biological route for butanol production from carbon monoxide (CO) gas has been discovered in the anaerobe *Butyribacterium methylotrophicum.* Butanol production was observed in batch, continuous, and continuous-cell-recycle fermentations with CO gas as the sole carbon and energy source. Concentrations of butanol as high as 0.5 g/L have been obtained, with concurrent production of butyrate, ethanol, and acetate in larger quantities. The results of these fermentations represent the first evidence for a direct biological pathway for butanol production from CO. A speculative pathway for CO metabolism in *B. methylotrophicum* has been proposed. The biological significance and potential applications are discussed.

KINETICS OF GROWTH AND CATECHOL PRODUCTION BY *BACILLUS STEAROTHERMOPHILUS* BR219

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Bacillus stearothermophilus BR219, a phenol-tolerant thermophile, catabolizes phenol via the meta pathway. Tetracycline blocks this pathway at an intermediate point, resulting in the accumulation of catechol, a specialty chemical used in the photographic, pharmaceutical, and agricultural industries. A biological process for catechol production from phenol is under development.

This paper presents kinetic data on cell growth and catechol production by *B. stearothermophilus* BR219. Batch growth curves were found to be relatively unaffected by pH in the range of 6.0 to 8.0. Stable, continuous culture was achieved over a several-week period using a 10 mM (940 mg/L) phenol medium and dilution rates as high as 2.0 h^{-1}. No evidence of microbial contamination was observed during this period despite the use of nonsterile sampling techniques on several occasions. When entrapped in thermostable, polysaccharide-gel support particles, *B. stearothermophilus* cells grew rapidly, as indicated by an INT staining technique.

PERFORMANCE OF IMMOBILIZED ENZYME ON SACCHARIFICATION AND FERMENTATION OF AGRICULTURAL WASTES AND WOOD RESIDUES

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The saccharification of agricultural wastes and wood residues are studies using a commercial enzyme preparation, *Aspergillus niger*. The enzyme is immobilized onto a support matrix - glass beads. The performance of the immobilized enzyme on the rate of saccharification of the cellulose is studied. A comparison is made of the rate of saccharification using free enzyme with the immobilized enzyme system. The organism, media, and environments are identical in both systems, so that the performance can be compared directly. The study also deals with the fermentable sugars and its subsequent conversion to ethyl alcohol. The yeast, *Saccharomyces cerevisiae*, is used in the ethyl alcohol fermentation. Simultaneous saccharification-fermentation studies are also conducted. The present work aims at developing a reasonably stable model reaction system to saccharify untreated cellulose and cellulose pretreated physically or chemically. This work provides information on the nature of treatments given to the substrate, the enzyme stability, and optimum operating conditions.

CELL ASSOCIATED β-GALACTOSIDASE ACTIVITY IN MYCELIAL PELLETS OF *ASPERGILLUS* AND *PENICILLIUM* SP.

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It has previously been shown that mycelial pellets of the fungus *Aspergillus phoenicis* QM 329 retain β-glucosidase activity[1] and that such pellets can function as a self-immobilized enzyme preparation in a fluidized-bed reactor.[2] In the present study, 13 strains of *Aspergillus* and 2 strains of *Penicillium* sp. were screened for (1) β-galactosidase activity and (2) pellet formation. Lactose, glucose, and lactose glucose were used as carbon sources. Mycelial associated β-galactosidase activity was found in 8 strains and all of them were able to form pellets under certain circumstances. In 5 strains, the β-galactosidase activity remained associated with the mycelium even after sonication. In repeated batch hydrolyses of lactose at 60°C, the stability of the mycelial associated β-galactosidase activity was evaluated in relation both to the leakage from the mycelium and the thermal inactivation of the enzyme.

1. Réczey, K., Persson, I., Tjerneld, F., Hahn-Hägerdal, B. (1989), *Biotechnol. Techniques* 3, 205-310.
2. Réczey, K., Stålbrand, H., Persson, I., Hahn-Hägerdal, B., Tjerneld, F. (1990), *Appl. Biochem. Biotechnol.* (in press).

STUDIES ON CELLULASE PRODUCTION USING SPENT SULFITE LIQUOR AND PAPER MILL WASTE

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Spent ammonium sulfite liquor (SASL) and cellulosic waste from a paper mill were used as substrates to produce cellulase. The SASL was pretreated to reduce its toxic components by aeration at 80°C for 1 to 2 h. After adaptation on the SASL agar plates, a strain of *Penicillium decumbens* JU-A10, was selected from the parent strain JU-1, a catabolite repression-resistant mutant with the ability of cellulase overproduction. *P. decumbens* JU-A10 can grow fast and produce a high quality of cellulase in the SASL-waste fibril medium. In a 50-L fermentor with a 30-L working volume, a two-stage-temperature culture technique was used to enhance the productivity of cellulase. 31°C was used for growth of the fungus, and 28°C was used for cellulase production. 4.6 IU/mL of filter paper activity (FPA), 100 IU/L•h of productivity and 264 IU/g celluiose of yield were reached by these techniques in less than 72 h. Further experiments showed that the FPA can be increased to 7.2 IU/mL in a fed-batch culture.

HYDROLYSIS OF CELLOBIOSE BY IMMOBILIZED β-GLUCOSIDASE ENTRAPPED IN MAINTENANCE-FREE GEL SPHERES

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A crude preparation of *Aspergillus niger* β-glucosidase (27.5 cellobiase units mg^{-1} protein at 40°C, pH 5.0) was immobilized on concanavalin A-Sepharose (CAS). The cellobiase activity of the immobilized enzyme was 1334 units mg^{-1} dried CAS or 108 units mL^{-1} of CAS gel. The β-glucosidase-CAS complex was entrapped within cross-linked bone gelatin/propylene glycol alginate gel spheres (2 mm in diameter) that were determined to have 45 cellobiase units mL^{-1} spheres. The effect of cellobiose concentration (10 to 300 mM) on the activity of two sizes of spheres (3.08 and 1.85 mm in diameter) was determined; and unexpectedly, it was found that the rate of cellobiose hydrolysis oscillated when the concentration was above 20 mM cellobiose, present in the reaction mixture. The same phenomenon was found to occur with the native and immobilized enzyme, although to a lesser extent. Exogenous ion addition was not necessary to maintain the structural integrity of the spheres.

*Operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

FIBER-OPTIC BASED APPARATUS FOR THE NONDESTRUCTIVE, IN VIVO MEASUREMENT OF PHOTOSYNTHESIS: APPLICATION TO ENVIRONMENTAL MONITORING[1]

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A prototype contact-leaf electrode has been developed which allows nondestructive measurement of photosynthetic rates of plants. Photosynthetic production of oxygen is monitored using a Clark-type electrode placed in direct contact with the plant leaf. Illumination of the leaf is achieved using optical fibers positioned around the platinum cathode. The diameter of this bundle is approximately 3 mm, allowing for control of the location on the leaf where the measurement is being made. Due to the fact that this procedure requires no pretreatment of the plant and can be performed with the leaf still attached to the plant, it should prove very useful for environmental monitoring of plants. Calibration procedures and computerized data acquisition, necessary for use as a fully functional field instrument, will also be discussed.

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[1]Research sponsored by the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy.

[2]Post-Doctoral Research Associate, Chemistry Department, University of Tennessee, on assignment at Oak Ridge National Laboratory.

[3]Present Address: International Technology Corporation, 312 Director's Drive, Knoxville, TN 37923.

[4]Operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

ANAEROBIC DEGRADATION OF FURFURAL (2-FURALDEHYDE) TO METHANE AND CARBON DIOXIDE

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Furfural, a byproduct formed during the thermal-chemical pretreatment of hemicellulosic biomass, was degraded in an anaerobic packed-bed reactor system to methane and carbon dioxide. The consortium of anaerobic microbes responsible for the degradation were enriched using small continuously stirred tank reactor (CSTR) systems and continuous addition of furfural and a nutrient solution as feedstock. Although the continuous infusion of furfural was initially inhibitory to the CSTR system, adaption of the consortium occurred even at high furfural addition rates. Addition rates of 7.35 mg furfural per 700 mL reactor per day, resulted in biogas productions of 375% of the biogas produced from a control CSTR, fed a nutrient solution only. The performance of the CSTR system fed high levels of furfural was stable, with a sludge pH of 7.1 and methane gas composition of 69%, as compared to the control CSTR with a pH of 7.2 and 77% methane. CSTR systems in which furfural was continuously added resulted in 79 to 80% of the theoretical biogas expected. Intermediates in the anaerobic biodegradation of furfural were determined by spike additions in serum bottle assay systems, using the enriched consortium from the CSTR systems. Furfural was converted to several intermediates including furfuryl alcohol, furoic acid, butyric acid, and acetic acid, before final conversion to the endproducts methane and carbon dioxide.

ISOLATION AND CHARACTERIZATION OF TWO CHROMATOGRAPHIC FORMS OF β-D-GLUCOSIDASE FROM *ASPERGILLUS NIGER*

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β-D-Glucosidase activity [EC 3.2.1.21] was isolated from dialyzed preparations of NOVO SP188, which was produced from clarified supernatants of *Aspergillus niger* fermentations. β-D-Glucosidase activity was found to elute from high-performance-size exclusion chromatography (HPSEC) columns in a volume which corresponded to ~170,000 MW. This fraction was then subjected to DEAE ion-exchange chromatography, where activity eluted in two distinct peaks, one at 270 and one at 310 mM NaCl. These two forms of the enzyme were found to behave identically on SDS-PAGE, HPSEC, and isoelectric focusing. Also, the N-terminal amino acid sequence, amino acid composition, fingerprint of tryptic-digest peptides, circular dichroism spectra, and reaction kinetics appear identical for these forms. This feature of the *A. niger* enzyme is distinctly different from β-glucosidase isozymes reported from other sources, where multiple forms tend to differ in molecular weight and/or isoelectric pH. The attempts to resolve this dilemma by endoglycosylase treatment of the native enzyme will also be reported.

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TOXIC EFFECTS OF SELECTED INDUSTRIAL SOLVENTS IN BATCH AND CONTINUOUS ANAEROBIC REACTORS

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The potential toxic effects of methylene chloride, ethyl acetate, iso-amyl acetate, xylene, and acetone were examined in batch anaerobic culture, using anaerobic solids which had been acclimated on a carbohydrate/acetate carbon source. Methylene chloride caused some inhibition of the anaerobic process at concentrations as low as 3 mg/L in batch reactors. In contrast, ethyl acetate and acetone showed no inhibitory effects, and in fact, were degraded rather rapidly in batch culture reactors at concentrations as high as 1,000 mg/L. Xylene appeared to have no toxic effects, if the concentration was kept below 50 mg/L in a batch reactor. In most instances, where concentrations of a particular substance resulted in inhibition of the anaerobic process, recovery could be demonstrated within several days of feeding a stressed reactor with fresh medium.

Preliminary results of feeding a sludge blanket reactor, containing a high concentration of very active biological solids, shows no inhibitory effects from methylene chloride at a concentration of 10 mg/L.

GENETIC TRANSFORMATION OF XYLOSE-FERMENTING YEAST *P. STIPITIS*

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A plasmid-mediated transformation system has been developed for the xylose-fermenting yeast, *Pichia stipitis*. We found that shuttle vectors containing a proper replication origin and an effective selection marker can be introduced into *P. stipitis* by a modified procedure of *Saccharomyces cerevisiae* protoplast transformation. Furthermore, conditions for transformation of intact *Pichia* cells by electroporation have also been established. We found that recombinant vectors used for transformation of *P. stipitis* can be stably maintained in the *Pichia* transformants; and identical plasmids can still be recovered from the *Pichia* transformants, even after they have been cultured in selective medium for more than fifty generations. *P. stipitis* is one of a few yeasts which have the capability to directly ferment xylose derived from renewable biomass to ethanol. Furthermore, *P. stipitis* can also metabolize both glucose and xylose extremely effectively under aerobic conditions. This is a property that is not possessed by any of the yeasts currently used as hosts for the expression of heterologous genes and for the production of foreign recombinant products. Thus, the development of a plasmid-mediated genetic transformation system for such a yeast will allow further exploitation of *P. stipitis* as a host for the conversion of sugars derived from biomass to fuels and chemicals.

SELECTION OF THERMOTOLERANT YEASTS FOR SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF CELLULOSE

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Simultaneous saccharification and fermentation is a promising method to transform lignocellulosic biomass into ethanol with high yields. Selection of the microorganisms capable of growing and fermenting at the elevated temperatures required for the enzymatic hydrolysis by cellulases is a key step for the development of this type of process.

In this work, a total of 31 yeast strains from several genera have been tested for their ability to grow and ferment carbohydrates at 40°C. The influence of different variables in the fermentation yield was also evaluated for the selected yeasts. The effect of the nutritional supplementation, initial glucose concentration, inoculum size, as well as tolerance to cellulases (from *Trichoderma reesei* cultures) and ethanol concentration are reported as results of these studies.

SSF studies using the selected yeasts under the optimum conditions determined in the previous step have been finally carried out employing cellulosic substrate Solka Floc HW-200.

DUAL INOCULATION WITH VA MYCORRHIZA AND RHIZOBIUM IS BENEFICIAL TO *LEUCAENA* GROWTH

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Research work was undertaken to study the effect of dual inoculation of VA mycorrhiza and Rhizobium on growth of *Leucaena leucocephala*, a well-known fuel tree. The application of Rhizobium showed a significant increase in all the symbiotic parameters which resulted in 5% and 42% increases in plant height and plant dry weight accumulation of *Leucaena*. Similarly, the application of mycorrhiza increased the uptake of nitrogen and phosphorous, which in turn enhanced plant height and plant dry weight by 32% and 55%, respectively. Dual inoculation of mycorrhiza and *Rhizobium* interacted positively to enhance nitrogen fixation and plant growth. Due to the difficulties in raising mycorrhizal inoculum, it is now generally accepted that mycorrhizal inoculation has more practical utility in transplanted crops. Since *Leucaena leucocephala* is a transplanted crop, dual inoculation with VAM and Rhizobium can be exploited by the grower as a regular cultivation practice.

THE EFFECTS OF NUTRIENTS AND TEMPERATURE ON BIOMASS, GROWTH, LIPID PRODUCTION, AND FATTY ACID COMPOSITION OF *CYCLOTELLA CRYPTICA*

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Batch cultures of *Cyclotella cryptica* were grown at two different nutrient (nitrogen/silica) levels and at two different temperatures (20 and 30°C). Biomass and cellular yields decreased with decreased levels of both the nutrients (nitrogen and silica) at 20 and 30°C; whereas lipids (total, neutral, and polar) increased at these two temperatures. Changes in fatty acid composition were noted; the diatoms produced increased amounts of fatty acids C 16:0, C 16:1, C 18:0, and C 22:0 when grown in low levels of nitrogen. Similar observations were made when the diatoms were grown in silica deficient medium. The influence of nutrient stress and temperature is discussed.

*Supported by subcontract No. 5-05104 from Solar Energy Research Institute, Golden, Colorado.

ENTRAPPED THERMOALKALOPHILIC *BACILLUS* AND ENDOGLUCANASE PRODUCTION

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The organism, a thermoalkalophilic *Bacillus*, was an isolate from a higher termite (*Odontotermes obesus*) infested soil of a semiarid region of northern India. The optimum growth of the organism was at 60°C and a pH of 9.0. It was a good cellulolytic and xylanolytic organism. Endoglucanase production in the organism was found to be inducible and catabolite-repressible. In order to have a continuous synthesis of the enzyme, the cells were entrapped in 15% polyacrylamide gel. Cell leakage in the reactor conditions was nonsignificant. Morphologically there was not significant change, as evidenced by the critical point of dehydrated samples of the immobilized cells. Oxygen consumption was about 40% over that of free cells. The immobilized cells showed about 2 min nonconsumption of oxygen. Enzyme production by the immobilized cells was poor for the first 2 d, but increased thereafter to about 140 units per day. The immobilized cells showed continuous enzyme production for 3 weeks; whereas in free cells, the rate of enzyme production was negligible after 5 d.

PURIFICATION OF *TRICHODERMA REESEI* CELLOBIOHYDROLASE I BY PREPARATIVE NATIVE GEL ELECTROPHORESIS

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A simple procedure was developed for the routine separation of the major cellulase component, cellobiohydrolase I, from a crude commercial preparation of *Trichoderma reesei* cellulase. The crude enzyme [4 mL, ~80 mg protein, 9.6 *p*-nitrophenylcellobiosidase (PNPC) units] was initially separated by gel filtration on a 2.54 x 94-cm BioGel P-100 column. The fractions containing PNPCase activity were pooled, lyophilized, and reconstituted in nanopure water (5 mL); and the procedure was repeated. The filtered enzyme (4 mL, ~68 mg protein, 8.95 PNPCase units) was loaded into a single well (5 x 125 mm) and electrophoresis performed vertically on a 7.5% native polyacrylamide gel (3.75 mm x 13 cm x 14 cm) at 500 V and 150 mA until the dye marker (50% sucrose in 0.1% bromophenol blue) migrated to the bottom of the gel. The band containing CBH I enzyme was extracted from the gel by maceration of the gel in water, followed by centrifugation. After filtering and lyophilization, the enzyme was redissolved in 50 mM sodium acetate buffer, pH 5.0. The enzyme (11.2 mL, 22.5 mg, 3.23 PNPCase units) was purified 1.2 fold by this procedure, and analytical gel electrophoresis (native and SDS) indicated CBH I to be >95% homogenous. Using this method, mg quantities of CBH I can be obtained in three working days.

*Operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

STRAIN DEVELOPMENT FOR ALCOHOL PRODUCTION FROM HEMICELLULOSE HYDROLYZATE

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Xylan and its component, xylose, are hemicellulosic natural resources for which utilization has not been well developed. We made several attempts at the improvement of a traditional alcohol-fermenting yeast, *Saccharomyces cerevisiae*, for alcohol production from xylan and/or xylose. Xylanase and xylosidase genes, derived from *Bacillus pumillus*, were cloned and introduced into *S. cerevisiae* with a gap promoter. The genes were expressed, and remarkable enzyme activities were detected. Furthermore, xylose reductase gene of *Pichia stipitis* was cloned and introduced into *S. cerevisiae*, which originally was unable to assimilate xylose. The mRNA synthesis and the enzyme activity were observed constitutively in *S. cerevisiae*, whereas those of *P. stipitis* were inductively expressed. Since *S. cerevisiae* carrying the gene cannot assimilate xylose, further investigations for development of a strain which is able to assimilate xylose and convert it to ethanol is necessary.

USE OF CYANOBACTERIAL DIAZOTROPHIC TECHNOLOGY IN RICE AGRICULTURE

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The positive effect of diazotrophic rice cyanobacteria on the growth and yield of rice in the tropics has led to the development of small-scale biotechnology, involving inoculum of paddy soils with appropriate cyanobacterial strains as biofertilizers. Since the rice cyanobacteria are extremely sensitive to commonly used rice herbicides such as machete, basalin, propalin, and to some extent 2,4-D, a successful biotechnology involving cyanobacteria would require them to have the additional properties of resistance to various toxic herbicides. In this respect, several cyanobacteria, native to rice fields, were selected by growth in the presence of field doses of herbicides, either individually or collectively; and they were then grown under laboratory conditions. These isolates represented mainly unicellular cyanobacteria (e.g., *Gloeocapsa, Aphanothece, Chroococcum*) and a few non-heterocystous cyanobacteria. No heterocystous forms could be selected for isolation. Results obtained on the nitrogen fixation potential, photosynthetic oxygen evolution, and diazotrophic growth of these isolates showed that *Gloeocapsa* is capable of nitrogen fixation in the presence of machete and basalin, while *Aphanothece* could fix nitrogen in the presence of diuron and propalin. Similarly, growing cultures of *Gloeocapsa* exhibited 100-fold resistance to both basalin and machete, while *Aphanothece* showed 20 to 25-fold resistance to diuron and propalin, compared to that of heterocystous forms. Further attempts are being made to create a defect in an ammonia assimilating enzyme system (GS activities) in the herbicide-resistant strains, in order to make them doubly advantageous for rice field application.

PLANT EXTRACT ESTIMULATION OF BIOFILM PRODUCTION BY THE 'TEA FUNGUS' *ACETOBACTER XYLINUM*

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The unique ability of *Acetobacter xylinum* performing cellulose biosynthesis through activated cellulose synthase by a diguanyl cyclic nucleotide was clarified by Benziman et al. Biosynthesis control also involves a specific phosphodiesterase able to cleave this unusual nucleotide. Home culture of *A. xylinum* is a ubiquitous practice and hence the popular denominations of "algal jam" or "tea fungus" for the "zooglea" thus obtained (to be designated, more properly, as a "schizoglea"). A protective role for this polysaccharide mantle was recently proposed by Cannon et al., who also designed a synthetic medium for the maintenance of the bacterium in agar solidified media. The dried pellicle from an isolate of *A. xylinum* - BioFill® - is being exponentially used as a temporary human and animal skin substitute in the medical treatment of burns, esthetic dermabrasions, and other dermic injuries (Fontana et al., *App. Biochem. Biotechnol.* 24/25, 1990, in press). Several hundreds of cases of successful applications were reported for this commercial biofilm.

Data on activators from a plant origin (teaceae, sapindaceae, rubiaceae, aquifoliaceae, and esterculiaceae) as well as estimulators from a defined chemical origin on *A. xylinum* (strain JDF) cellulosic membrane formation will be presented.

*Funding: CNPq, PADCT-SBI0-FINEP, and CONCITEC-PR.

SEED GUM OF *STIPHNODENDRON BARBATIMAN*

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Stryphnodendron barbatiman ("barbatimão") is a ubiquitous legume tree in the "Cerrados" region of central Brazil. Its timber and bark tannins are explored for the construction and leather industries. Although legume seed polysaccharides are widely studied, nothing is known about them in this species, and thus our current focus on the chemical structure and rheological properties of barbatiman seed glycans.

Powdered seed lipids, pigments, and small molecular weight carbohydrates were discarded by extractions with hot benzene:ethanol (2:1) and methanol:water (4:1). The residue was then extracted with cold water (4°C) producing a viscous solution. Its precipitation with an excess of ethanol allowed the recovery of crude polysaccharides [yield: 28%, dry weight basis; intrinsic viscosity (η) = 750 centipoises (750 mPa•s)]. Viscosity proved to be very dependent on extraction procedures. The purified fraction after Sevag deproteinization and complexation with Fehling's reagent was submitted for structural analyses. It showed mannose and galactose in a 1.5:1.0 ratio by glc of alditol peracetate derivatives of the acid hydrolysis products. The ^{13}C-nmr peaks were fully resolved and identified, confirming a galactomannan structure. The anomeric configuration for D-mannose and L-galactose was confirmed through chromium trioxide oxidation. A structural and rheological comparison with galactomannans from conventional sources is being undertaken.

*Funding: CNPq, FINEP, and CONCITEC-PR.

EXTRACTIVE FERMENTATION OF ACETIC ACID: ECONOMIC TRADE-OFF BETWEEN YIELD OF *CLOSTRIDIUM* AND CONCENTRATION OF *ACETOBACTER*

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This technoeconomic study compares the extractive fermentation of acetic acid by two bacteria:

Acetobacter metabolizes ethanol aerobically to produce acid at 100 g/L in a low-pH medium. This insures that the product is in the form of extractable free acetic acid rather than as an unextractable salt. Conversely, yields from glucose by way of the ethanol fermentation are poor but near the biological limits of the organisms involved.

C. thermoaceticum is a thermophilic anaerobe that operates at high fermentation rates on glucose at neutral pH to produce acetic acid in substantially quantative yields. However, it is severely inhibited by the product, which restricts concentration to a dilute 20 g/L.

Both systems were projected to operate as immobilized cells in a continuous, fluidized bioreactor using solvent extraction to recover the product. The economic advantages of maintaining the fermenter at high cell densities and high product concentrations were determined.

An improved *Acetobacter* system appears closer to competing with synthetic acid, although this system has only a limited margin for improvement.

The present *Clostridium* system cannot compete. However, if the organism could be adapted to tolerate higher product concentrations at acid pH, cost would be reduced to about 80% of the price for synthetic acid.

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PRODUCTION OF ETHANOL AND COPRODUCTS FROM MSW-DERIVED CELLULOSICS USING DILUTE SULFURIC ACID HYDROLYSIS

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The Tennessee Valley Authority is conducting research to convert the cellulosic portion of municipal solid waste (MSW) to marketable products. A one-stage dilute sulfuric acid hydrolysis process is being evaluated in bench-scale research to produce sugars for the production of ethanol and other chemicals.

Preliminary economics of the process have been evaluated based on bench-scale results. Capital and operating costs for a base case commercial facility designed to process 500 t/d of processed MSW (the cellulosic portion) have been prepared. Sensitivity analyses were conducted to evaluate the effect of variables such as (1) tipping fee, (2) plant size, and (3) electricity selling prices on return on investment for a commercial facility.

A NEW KINETIC APPROACH TO THE FERMENTATION OF MULTISUBSTRATE COMPLEX MEDIA

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The aim of this work is the kinetic study of ethanol production from the sugars contained in multisubstrate complex media, such as hardwood hemicellulose hydrolysate. The study of the kinetic parameters associated with the fermentation usually allows one to formulate models able to describe the process development by means of adequate equations. For this substrate, however, all the efforts to carry out such a study rigorously ended in failure, mainly due to the difficulty in obtaining hydrolysates having exactly the same composition, because of the inevitable inconstancy of the hydrolysis conditions. Another cause of the failure of traditional kinetic approaches in the study of complex media is the simultaneous presence of more than one substrate which are consumed at the same time. In this particular case, glucose and xylose are fermented separately with different reaction rates. A new kinetic approach, presented in this study, is founded on the supposition that both the whole ethanol production and the biomass growth can be subdivided into two separated components imputable to glucose or xylose, respectively. For this purpose, the experimental data previously obtained using hardwood hemicellulose hydrolysate have been compared with those obtained from synthetic media, which allowed us to evaluate variations of the kinetic parameters reliable to eventual inhibition or stimulation factors. The reasonable agreement between continuous data calculated through the model and experimentally determined data gives confirmation of the validity of such a new kinetic approach.

MODELING OF D-SORBITOL TO L-SORBOSE BIOTRANSFORMATION BY IMMOBILIZED CELLS OF GLUCONOBACTER SUBOXYDANS IN A BUBBLE COLUMN

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The purpose of the present paper is to study experimentally in a bubble column and to model the process of biotransformation of D-sorbitol to L-sorbose by the bacteria *Gluconobacter suboxydans* immobilized in calcium alginate.

The mathematical model is based on unsteady diffusion in the gel particles and comprises four dimensionless parameters: the Thiele modulus, the ratio of product's to substrate's diffusivity in gel particles, and the Biot numbers.

The results show that, within a wide range of initial concentrations of dry substances and initial degrees of conversion, the determined values of the Biot numbers and the beads' sizes remain practically the same. Under these conditions, the gel beads remained stable during more than 14 fermentations. It was demonstrated that the process carried out with the immobilized bacteria runs more rapidly than the case of free culture at sufficiently high substrate concentrations. This fact is explained as being due to the autocatalytic character of the studied process and the retarded diffusion through the gel membranes.

The rate constants, calculated from the Thiele modulus after the parameter evaluations, decreased with the initial substrate concentrations, probably due to inhibition. The values of K_1a for the cases of free and immobilized cells also were determined.

MICROBIAL REMOVAL OF SULFUR DIOXIDE (SO_2) AND NITRIC OXIDE (NO) FROM A GAS

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A project is under way at the University of Tulsa to develop a viable process concept whereby a microbial process can impact on the problem of flue gas desulfurization and NO_x removal. We have previously reported studies of SO_2 reduction by *Desulfovibrio desulfuricans* and NO_x reduction *by Thiobacillus denitrificans.* One potential process concept is the simultaneous removal of SO_2 and NO_x from cooled flue gas by contact with cultures of sulfate-reducing bacteria ($SO_2 \rightarrow H_2S$) and *T. denitrificans* ($H_2S \rightarrow SO_4^{-2}$) as cultures-in-series or in co-culture in a single contacting stage. Each of these contacting schemes has been investigated.

In the reactors-in-series scheme, the first stage was a mixed culture of *D. desulfuricans* and fermentative heterotrophs operated on a SO_2-limited basis. The second stage was a septic culture of *T. denitrificans* operated on an H_2S-limited basis. Sulfur dioxide (1% in N_2) was completely reduced in the first stage to H_2S which was completely oxidized to sulfate in the second stage. The introduction of NO to the feed gas produced inhibition of SO_2 reduction at concentrations of 1000 to 1500 ppm (1 to 1.5 mL/L). Prior to the onset of upset conditions, about 50% removal of NO was observed with apparent reduction to N_2. Similar results were obtained with co-cultures of *D. desulfuricans and T. denitrificans.*

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AN ECONOMIC ANALYSIS OF THE MICROBIAL REDUCTION OF SULFUR DIOXIDE (SO_2) AS A MEANS OF BY-PRODUCT RECOVERY FROM REGENERABLE PROCESSES FOR FLUE GAS DESULFURIZATION

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We have previously reported a study of SO_2 reduction to H_2S by mixed cultures of *Desulfovibrio desulfuricans* and fermentative heterotrophs in which glucose served as the ultimate source of carbon and energy. We have also proposed that a concentrated stream of SO_2 [as may be obtained from a regenerable process for flue gas desulfurization (copper oxide process, for example)] could be split with two-thirds of the SO_2 reduced to H_2S by contact with a microbial culture and then combined with the remaining SO_2 to use as feed to a Claus reactor to produce elemental sulfur.

Based on laboratory data, we have designed and sized a microbial SO_2 hydrogenation plant for the case of a 1000 MW power plant burning 3.5% sulfur coal. The capital investment and operating costs of a microbial hydrogenation process have been estimated and compared to conventional catalytic hydrogenation.

These analyses indicate that in terms of capital investment, microbial and conventional catalytic hydrogenation of SO_2 are comparable. However, the operating costs of the microbial process are much higher, due almost entirely to the high cost of starch hydrolyzate as a carbon and energy source for the process culture. Other, more economical, carbon and energy sources for the process culture are discussed.

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DETERMINATION OF THE PARTITION COEFFICIENT FOR PROTEIN SEPARATION IN AN AIR-FLUIDIZED BIOREACTOR

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Baker's yeast was grown on a semi-solid substrate (homogenized whole potatoes) in an air-fluidized bioreactor. During the batch bioprocess, certain proteins were trapped by sparging the effluent air stream into a water chamber. Surprisingly, the proteins carried over were specific ones and not the most abundant ones available in the process mixture.

The combined protein production and separation process is modeled as being an equilibrium process between the extracellular protein in the bioreactor and the protein carried over in the air. A partition coefficient is incorporated in the model, which governs the transfer of proteins from the process mixture to the micro water droplets in the air. The value of this partition coefficient is estimated by fitting the model to the experimental data describing the air-fluidized bioreactor.

The partition coefficient is assumed to be specific to each protein. In order to verify the above model, separate experiments were carried out in a separatory funnel using various enzymes (proteins) at different pH values. The enzyme's isoelectric point is also included in order to obtain the best separation (at the greatest partition coefficient value).

THE EFFECT OF pH AND GAS COMPOSITION ON THE BUBBLE FRACTIONATION OF PROTEINS

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Studies were conducted to establish the effect of the variation of environmental factors on the separation occurring in protein systems due to bubble fractionation in a tall bioreactor. The measure of separation was selected to be the separation ratio. This is defined to be the ratio of either the top- or the middle-position concentration in the vessel to the bottom concentration of the vessel. Invertase and a-amylase were the two "model" enzymes considered. It was observed that under certain conditions, i.e., a combination of the nature of the sparging gas and the medium pH, varying degrees of protein separation were achieved. The pH of the system dramatically influenced the separation. It is assumed that the system is at, or near, its isoelectric point at the pH where the separation has its greatest value. Thus, the pI of crude a-amylase is ~8 while the pI for crude invertase is ~5, since these are the pH values at which the respective enzyme systems exhibited their greatest separations. Further, it was observed that systems sparged with CO_2 exhibited higher separation than systems sparged with air. In fact, in the case of invertase, almost threefold greater separation was observed at the top port when the solution was sparged with CO_2.

BIOREACTOR DESIGN CONSIDERATIONS IN THE PRODUCTION OF HIGH-QUALITY MICROBIAL EXOPOLYSACCHARIDE

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Biotechnology has responded to the increasing demand for inexpensive, high-quality, viscosifiers and biodegradable polymers; and today, the large-scale production of microbial exopolysaccharides (EPS) represents a new fermentation industry with global markets representing many hundreds of million dollars annually. Through controlled fermentations, the potential exists to inexpensively produce a variety of different high-quality microbial polysaccharides of consistent composition and quality. In contrast to the extensive literature relating to the chemistry of these fermentation biopolymers (EPS), it would appear that much less attention has been paid to the influence of physical parameters on biological response and the quality of the recovered polymer. One of the first steps in developing a commercial fermentation product involves the transition from shake flask to stirred-tank reactor. The use of an STR offers greater potential for controlling the physicochemical environment, and the oxygen-limitation observed with shake flask cultures (H. G. Lawford & J. D. Rousseau, *Biotech. Letts.* 11, 125, 1989) can be addressed through standard engineering strategies relating to increasing oxygen mass transfer for the purpose of increasing specific productivity. In the case of β-1,3-glucan produced by selected strains of *Alcaligenes faecalis and Agrobacteriwn radiobacter,* the exopolymer represents a barrier to oxygen transfer to the culture, thereby necessitating an unusually high DOT for maximal rates of polymer biosynthesis. Shake flask cultures have been observed to produce a higher MW polymer with superior gelling and rheological characteristics. Therefore, our approach (a kind of reverse engineering) has attempted to simulate the low-shear mixing environment of the shake flask in an STR. Our studies have focused on bioreactor designs employing various types of agitation devices, in which the objective was to provide sufficiently high rates of oxygen transfer for maximal rates of polymer biosynthesis, without creating a detrimental shear environment that would compromise the quality of the recoverable EPS product.

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MODELING OF AN IMMOBILIZED CELL, THREE-PHASE FLUIDIZED-BED BIOREACTOR*

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Zymomonas mobilis was immobilized and used to convert glucose to ethanol and CO_2 in a fluidized-bed bioreactor. The modeling of such systems requires both an intrinsic understanding of reaction and diffusion within the biocatalyst, and an understanding of the hydrodynamic effects and interphase processes in the bed.

Within the biocatalyst bead, the substrate and products are transported by molecular diffusion. Thus, an effectiveness factor is used to account for the effects of concentration gradients within the bead. To account for the hydrodynamics within the bed, a dispersion model was used to describe the glucose and ethanol concentrations; and a plug-flow model was used to determine the amount of CO_2 produced as a function of axial bed position. In addition, because of the additional mixing induced by the gaseous CO_2 flowing through the bed, the dispersion coefficient was assumed to be a function of the gas flow rate (amount of CO_2 produced). Model predictions were compared with steady-state concentration profiles obtained from a typical reactor using a wide range of feed substrate concentrations and flow rates.

Using this model, optimal feed concentrations and flow rates for various bed configurations were then determined.

*Research sponsored by the Energy Conservation and Utilization Technologies Program, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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MSW VOLUME REDUCTION, VALUE RECOVERY, AND VALUE ADDITION

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Technologies are needed to reduce dependency on landfilling and mass burning as our primary means of disposal of municipal solid wastes (MSW). Source separation and recycling is desirable; however, significant quantities of wastes will remain for disposal.

This paper describes a number of processing steps which might be included in a technical scheme for volume reduction, value recovery, and value addition in processing paper, MSW, and sewage sludge. The substrates processed were from one municipality but appear to be representative of documented MSW analyses.

The separation and pulping of cellulosics step was accomplished with steam classification. Non-cellulosics were identified and assigned value based on regional markets for glass, plastics, and metals. Three technologies for the glucose recovery from recovered-cellulosics step were compared: enzymatic hydrolysis and both dilute and concentrated sulfuric acid hydrolysis. The ethanol production step was evaluated by employing a uniform fermentation test of sugars recovered by the three hydrolysis methods. The final processing step of stillage disposal was addressed by evaluation of the stillages for animal feed from both a nutritional and health standpoint.

Results of the various processing steps will be presented, as well as predictions of success of the scheme for waste reduction, value recovery, and value addition.

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A STRUCTURED MODEL FOR VEGETATIVE GROWTH AND SPORULATION IN *BACILLUS THURINGIENSIS*

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A mathematical model has been developed for the δ-endotoxin-producing *Bacillus thuringiensis*. The structure of the model involves the processes taking place during vegetative growth, those leading to the initiation of sporulation under conditions of carbon and/or nitrogen limitation, and the sporulation events. The key features in the model are the pools of compounds such as NAD/NADH, ADP/ATP, PRPP/IMP, CDP/GTP, pyrimidine nucleotides, amino acids, nucleic acids, cell envelop proteins, and vegetative and spore proteins. These, along with sigma-factors that control the nature of RNA-polymerase during the different phases, effectively simulate the vegetative growth and sporulation. The initiation of sporulation is controlled by the intracellular concentration of GTP. Parameters of the model have been identified on the basis of balanced growth during vegetative phase and from experimental data during sporulation.

Results of simulation of vegetative growth, initiation of sporulation, spore-formation, and production of δ-endotoxin under C- or N- limitation will be presented. Batch and fed-batch modes of operations will also be simulated and compared. Based upon these, appropriate strategies of reactor operation will be discussed.

Poster 94

PREDICTION OF BED HEIGHT IN A SELF-AGGREGATING YEAST, ETHANOL TOWER FERMENTER

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The self-aggregated yeast cells in a tower fermenter were fluidized by a high, local, liquid velocity, which was caused by the bubbles of carbon dioxide generated during fermentation. Prediction of the bed height of the plug-flow portion of the fermenter was based on the terminal velocities of the yeast aggregates. They were determined by the particle size of the aggregates and the constituents of the nutrients in the fermentation juice. The particle sizes of the cell aggregates were controlled by the rate of aeration and ranged from 0.5 to 5 mm. Above the limited bed height, the fermenter is a CSTR. The cell separation was accomplished by a cell separator which was designed according to the terminal velocity of the yeast cell aggregates.

Poster 95

THEORETICAL AND EXPERIMENTAL INVESTIGATION OF AN UPFLOW SOLIDS-RETAINING BIOREACTOR FOR CELLULOSE CONVERSION

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Continuous processing holds promise for lowering the cost of ethanol production from cellulosic biomass. Reactor designs which exploit the characteristics of insoluble cellulosic biomass for continuous production could be of particular merit. Because of density differences between lignocellulosic substrates and the aqueous milieu, there exists potential for differential substrate retention due to sedimentation. Such retention can further be expected to result in retention of the enzymes and/or cells responsible for hydrolysis due to their tendency to adhere to the raw material. Modelling results will be presented indicating that simultaneous substrate and biocatalyst retention has a multiplicative effect on the volumetric productivity of the reactor. Further, multiplicative productivity increases may be expected in a quiescent columnar reactor due to the development of a nonhomogeneous cellulose distribution profile approximating plug flow.

Analysis, design, and experiments pertaining to an Upflow Solids-Retaining Bioreactor (USRB) will be presented. Pretreated mixed hardwood was used as the substrate for fermentation by the thermophilic anaerobic bacterium *Clostridium thermocellum*. Experimental results include evidence for the formation during fermentation of porous mats which enhance sedimentation relative to the non-aggregated particles present in the absence of cells. Performance data and comparisons will be made, and theoretical and applied considerations will be discussed.

THE USE OF AN AQUEOUS TWO-PHASE SYSTEM FOR THE ABE FERMENTATION

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An aqueous two-phase system was applied to the in situ extractive ABE fermentation as a novel technique which combines fermentation and separation. Cells were confined in one phase, while products were extracted to the other phase in order to overcome product inhibition and low product concentration. This is a problem which is encountered in many fermentations. The characteristics of a particular fermentation, e.g., aerobic or anaerobic and/or product inhibition or not product inhibition, should be considered for the selection of an optimal two-phase system. The ABE fermentation is anaerobic fermentation with significant product inhibition. The optimal two-phase system was selected by considering both the partition coefficients of products and the interfacial kinetics, i.e., cell partitioning behavior which is a key to increasing the fermentation efficiency by reducing product inhibition. The effects of molecular weight and concentration of polymers on the yields of products and the cell growth behavior were investigated. The two-phase system was compared to the performance of an ordinary fermentation.

CONTINUOUS FERMENTATION OF D-XYLOSE BY IMMOBILIZED *PICHIA STIPITIS*: COMPARISON BETWEEN CSTR AND CPFR

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The main purpose of this work was to compare the performances of two different kinds of reactors (CSTR and CPFR) in order to enhance the ethanol productivity in the fermentation of D-xylose by *Pichia stipitis* immobilized in κ-carrageenan. Immobilization was carried out in a 4% aqueous suspension of κ-carrageenan, which was mixed with the inoculum. The bioparticles were treated with $Al(NO_3)_3$ as hardener agent.[1] The fermenters operated during a long period of time (about 30 d). Best results were obtained in the packed-bed reactor (CPFR) that allows one to operate at higher final ethanol concentrations. This fact was explained because of the observed strong product inhibition.[2] The overall productivities reached values >3.8 g/(L•h). However, the specific productivities of yeast in the continuous-stirred-tank reactor (CSTR) were always greater because the bioparticles were kept in close contact with the broth, while in the CPFR, there are at least two problems: (1) the produced gas can avoid the intimate contact between the substrate and the particles and (2) the possible existence of preferential paths.

1. Chamy, R., Nuñez, M. J., and Lema, J. M., *Enzyme Microbiol. Technol.* (in press).
2. Chamy, R., Nuñez, M. J. Sanroman, A., and Lema, J. M. *Mededelingen van Fakulteit Landbouwwetenschappen* (in press).

USE OF A NOVEL IMMOBILIZED β-GALACTOSIDASE REACTOR TO HYDROLYZE THE LACTOSE CONSTITUENT OF SKIM MILK

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A novel chemical reactor, consisting of β-galactosidase from *Aspergillus oryzae* immobilized onto a ribbed membrane made from a 50:50 ratio of polyvinylchloride and silica, was used to hydrolyze the lactose constituent of skim milk. The ribbed membrane is rolled so that the reactant fluid flow is along the axis of the cylindrical reactor, through the annular openings formed by the ribs. This axial-annular flow reactor can be used to hydrolyze the lactose in skim milk without the problems observed with other reactor configurations, namely, plugging due to particulates, microbial contamination, and large pressure drop.

Multi-response nonlinear regression methods were employed to determine the kinetic parameters of the rate expressions, based on a proposed enzymatic mechanism that includes the formation of oligosaccharides. HPLC methods were employed to monitor the concentrations of all species present in the effluent stream.

For the reactor conditions used in this research, a rate expression which includes the separate inhibition effects of α- and β-galactose (and the associated mutarotation reaction) is sufficient to model the hydrolysis of lactose in skim milk. At 30°C and a space time of 9 min, 70% of the lactose present in skim milk can be hydrolyzed with the axial-annular reactor.

A SIMPLE, STRUCTURED MODEL FOR THE GROWTH OF WILD CARROT CELLS

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Simple models for product formation by plant cells require knowledge of the growth and/or growth rate. The growth curve during batch cell suspension culture is relatively complex, with the exponential phase frequently preceded by an appreciable lag, and followed by an idiophase, during which growth may be by cell enlargement rather than by division, if growth is limited by phosphate or nitrogen source. An unstructured model which accounts for these important features of the growth curve is unknown. We have therefore undertaken the development of a simple, structured model in an effort to describe the cell growth process. Such a model should adequately account for the uptake of the major nutrients as well as the cell growth. In the case of our experiments on wild carrot cells grown on ammonium as sole nitrogen source and buffered by succinate, the nutrients accounted for are sugars (sucrose or selected mixtures of glucose and fructose), ammonium, phosphate, and the succinate.

Our approach to the model development is to lump the amino acids, the NMPs, the NDPs, and the NTPs, each into groups, and to write a single kinetic model for each group and for the production of RNA and protein. This approach accounts for the gross behavior of the major ammonium and phosphate derivatives and yields a form which can account for the growth lag we observe with wild carrot cells.

There are, however, major questions remaining, including that of the mechanism by which NTP production is regulated and that of the details of the mechanism of phosphate uptake.

ENZYME ADSORPTION/DESORPTION DURING THE HYDROLYSIS OF PRETREATED POPLAR

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The hydrolysis of pretreated poplar wood was carried out with *Trichoderma reesei* GC123 cellulase. Varying cellulase concentrations (1.26, 2.52, 5.04 mg-protein/mL) were used on substrate concentrations of 2.5% w/v, 5% w/v, and 10% w/v at pH 4.8 and 40°C. The concentration of enzyme protein remaining in solution, the glucose concentration, and the total sugar concentration were measured as a function of time during the hydrolysis. The enzyme rapidly adsorbed initially, reaching a maximum in about 30 minutes. It remained at this maximum until near the end of hydrolysis. After this, 55 to 75% of the cellulase returned to solution as the remaining cellulose was hydrolyzed. The time required for this maximum adsorption before enzyme returned to solution was found to be inversely proportional to the initial enzyme concentration. However, a fraction of the cellulase remained on the lignin at the end of hydrolysis. At lower initial enzyme concentrations, the ability to convert cellobiose to glucose was reduced. Dilution of the solution of unhydrolyzed substrate (lignin) with buffer did not cause the cellulase to desorb over the range of concentrations studied. Furthermore, the addition of enzyme to the solution at the end of hydrolysis did not result in further adsorption on the lignin. Hence, it appears that the lignin was saturated with enzyme over the range of concentrations studied.

THE SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF PRETREATED WOODY CROPS TO ETHANOL

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Four promising woody crops: (Populus *maximowiczii* x *nigra* (NE388), P. *trichocarpa* x *deltoides* (N11), P. *tremuloides*, and Sweetgum *Liquidambar styraciflua*) were pretreated by dilute sulfuric acid and evaluated in the simultaneous saccharification and fermentation (SSF) process for ethanol production. The yeast, *Saccharomyces cerevisiae*, was used in the fermentations, alone and in mixed culture with β-glucosidase. All SSFs were run at 37°C for eight days and compared to saccharifications at 45°C under the same enzyme loadings. *S. cerevisiae* alone achieved the highest ethanol yields and rates of hydrolysis at the higher enzyme loadings, while the mixed culture performed better at the lower enzyme loadings without β-glucosidase supplementation. The best overall rates of fermentation and final yields were achieved with P. *maximowiczii* x *nigra* (NE388) and Sweetgum *Liquidambar styraciflua* followed by P. *tremuloides* and P. *trichocarpa* x *deltoides* (N11). Although there were some differences in SSF performance, all of these pretreated woody crops show promise as substrates for ethanol production.

COS DEGRADATION BY SELECTED CO-UTILIZING BACTERIA

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Carbonyl sulfide (COS) is a major organic sulfur contaminant present in synthesis gas. Traditional methods of removal include absorption with amine-based systems, hot carbonate systems, and physical-solvent systems. Recently, several CO-utilizing bacteria have shown the ability to degrade COS in the presence of CO. This paper summarizes the results of degradation studies by four CO-utilizing bacteria as a function of bacterial inoculum size. Rates of degradation and product formation are compared and discussed.

PARAMETERS AFFECTING THE KINETICS OF ETHANOL PRODUCTION FROM CO, CO_2 AND H_2 BY *CLOSTRIDIUM LJUNGDAHLII*

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Clostridium ljungdahlii, strain PETC, is capable of converting CO, CO_2, and H_2 in synthesis gas to ethanol and acetate. Significant efforts have been directed toward forcing the bacterium to produce ethanol instead of the favored product, acetate. This paper presents the results of a kinetic study using CO and CO_2/H_2 as substrates. The effects of nutrients concentration, sulfur gas toxicity, and choice of substrate on intrinsic kinetics are addressed.

A COMPARISON OF INHIBITORY EFFECTS ON *S. CEREVISIAE* AND *Z. MOBILIS* IN BATCH AND CONTINUOUS CULTURE

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Based upon volumetric ethanol productivity and start-up time in the immobilized cell reactor, *Z. mobilis* has been found to be preferred over *S. cerevisiae*. However, *S. cerevisiae* was found to be more tolerant of pH fluctuations than *Z. mobilis*. This paper examines the effects of the presence of small quantities of impurities, such as acid hydrolyzate by-products or acid recovery solvents, on the performance of the two organisms. The kinetics, productivities, start-up time, and longevity of *S. cerevisiae* and *Z. mobilis* were compared with varying quantities of impurities in batch culture and the immobilized cell column.

STUDIES ON WHOLE CELL IMMOBILIZATION OF YEAST *SACCHAROMYCES CEREVISIAE* NSI 113 ON A PACKED-BED CALCIUM-ALGINATE SUPPORT ON CANE MOLASSES

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The studies on immobilization of the yeast, *Saccharomyces cerevisiae* NSI 113, on a packed-bed of Ca-alginate support were done on Indian cane molasses. The effect of calcium chloride concentration on the rate of ethanol production and cell leakage, the effect of Na-alginate concentration, and the effect of sand on fermentation were studied. Cell leakage from the gel decreased with increases in calcium chloride concentration to about 0.4%, but there was no appreciable change in cell leakage. The ethanol production drastically decreased by increasing Ca-chloride concentration. By increasing the concentration of Na-alginate, the cell loss decreased, but there is no appreciable change beyond 7% of Na-alginate. Fermentation efficiency increased by 1% on the addition of sand into the gel (one-half of the wet weight of gel). The standard method was used for cell entrapment to the extent of 4% of Na-alginate solution, in which cells had been mixed to give a 20% (w/v) slurry. The fermentation system was prepared by using 0.2% $CaCl_2$ solution. It was observed that fermentation efficiency was improved from 81 to 93% with an ethanol yield of 8.68%.

COMPARATIVE EVALUATION OF CATALASE IMMOBILIZATION METHODS ON DIFFERENT SUPPORTS

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The catalase was immobilized on different supports using the following three methods: (1) adsorption on "Ponilex" anionite, (2) covalent binding on the same support, and (3) reticulation by means of glutaraldehyde on cellulose fibers. The kinetic characteristics of the immobilized catalase preparations differ from each other and depend on the nature of the support and on the immobilization method. Thus, the immobilization capacity has a value of 0.65 mg enzyme/mL support for adsorption. The value of this parameter is much higher if the immobilization of catalase is performed by covalent binding (e.g., 6.0 mg/mL). In the case of enzyme reticulation on cellulose fibers by glutaraldehyde, the immobilization capacity is 6.5 mg/mL. The optimum pH values are different. When the enzyme is adsorbed on the macromolecular support, the pH is 6.5, while the optimum pH is 7.0. The specific activity of the free enzyme has a mean value of 18640 U/mg of protein. If the enzyme is immobilized by adsorption, this value decreases to 7850 U/mg (yield 42.12%). The catalase immobilization through covalent binding leads to an enzymatic preparation with an average specific activity of 6170 U/mg (yield 33%). Through the catalase reticulation on cellulose fibers by glutaraldehyde, one obtains a yield of 31%, corresponding to a catalytical activity of 5778 U/mg protein (mean value).

BIOCONVERSION OF BY-PRODUCTS OF THE SUGAR INDUSTRY (MOLASSES AND SUGARBEET PULP) FOR SCP PRODUCTION

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Bioconversion experiments were performed to study different substrates, viz., sucrose, molasses, and sugarbeet pulp for utilization efficiency of four *Candida* strains (*utilis*, *tropicalis*, *solani*, and *prosilopsis*) in a 5-L Biostat. Three concentrations of sucrose and molasses were taken for fermentation, with and without nutritive supplements. *C. utilis* and *C. tropicalis* performed best, and results are discussed for the bioconversion efficiencies. In the case of sugarbeet pulp, (1) the fermentation of filtrate from saccharified pulp with a specific enzyme mixture, and (2) simultaneous saccharification and fermentation of beet pulp were carried out at 150 rpm for 48 h in a one-stage process at 40°C and in a two-stage process at 30°C. Results are discussed for biomass yield with protein recovery and bioconversion efficiencies.

ENZYMATIC SACCHARIFICATION AND FERMENTATION OF SUGAR BEET PULP FOR SCP PRODUCTION

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Single vessel experiments for the bioconversion of beet pulp were performed in 5-L fermentors (Biostat S., B. Braun, FRG). These experiments utilized two main components, viz., A-Yeast *Candida tropicalis* DSM 70151, and B- Enzyme sources (1) a Novo SP249 product (pectinase + little hemicellulase) and (2) a preparation from *Trichoderma reesei* Rut-C30 NRRL 11460 (Cellulases). In the first set of experiments, direct fermentation of saccharified filtrate of pulp with yeast was carried out at 30°C, 180 rpm for 40 h. In the second and third sets, saccharification/fermentation was performed in one stage at 37°C, 180 rpm with the difference that, in the third set, yeast was inoculated after 16 h of enzymatic reaction on beet pulp. Results are discussed for (1) biomass yield, (2) protein content, and (3) bioconversion efficiency for each set of experiments.

NEW METHODS FOR GROWING PLANT AND MAMMALIAN CELLS AND FOR DISSOCIATION OF METHANOL

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Fibroblasts on fibers produce substances for cancer and burn treatment. Mammalian and plant cells can be immobilized on fibers, and when Celite is entrapped in the fibers, a large surface area is provided. When such an RBC (rotary biological contactor) is run half full, light and air hit a thin moving film. Fast (10 to 15 min) conversion of sugar to alcohol can be done and wastewater cleaned.

In another design, cells grow on polyurethane foam, and when a stainless steel mesh is put around the foam, it protects against collisions and makes it heavier, so it can be fluidized by sparging air in the bottom.

A catalytic heat exchanger for decomposing methanol to H_2 and CO will be described where 20% more energy is saved over burning methanol because waste heat is utilized to vaporize the methanol and supply heat for the endothermic reaction. The exchanger provides for expansion of the gases for use in a car or turbine. There is no pollution, and it is expected that methanol can be produced for only 35¢ per gallon.

All these concepts are protected by U.S. patents.

STUDY ON BOD MICROBIAL SENSOR FOR WASTEWATER TREATMENT CONTROL

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A microbial sensor consisting of immobilized yeast or bacterial cells and an oxygen electrode was used for the estimation of BOD. The structure of the biosensor was delicately designed and a flow-through system was set up in this study. The response time of the biosensor is within 20 min. A linear relationship was observed between the relative current decrease and the BOD of the sample solution in the range of 1 to 45 mg/L. The operation life of the biosensor is more than one year. The reproducibility of the sensor is quite good, and the relative standard deviation is less than 6% FS at a concentration of 210 mg/L BOD. The BOD biosensor has been applied to the determination of BOD in a brewery plant and a glutamic acid plant wastewater. Satisfactory agreement was observed between the BOD_5 value (by conventional 5-d method) and the value determined by the current BOD biosensor.

COMPARATIVE MICROBIAL DEGRADATION OF ORGANIC CYANIDE

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Organic cyanides (R-CN) are extensively used in numerous industrial processes, and this extensive usage poses a substantial problem in waste disposal systems. The purpose of this study was to evaluate *Chromobacterium*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* for their ability to degrade organic cyanides. These strictly aerobic, gram-negative bacteria were isolated, identified, and biochemically characterized. The isolates utilized a variety of organic cyanides and corresponding amides as a source of carbon and nitrogen for growth. A temperature of 25 to 30°C and a pH of 6.8 to 7.0 favored the maximum utilization of cyanides. One of the metabolites of organic cyanide degradation was ammonia. Addition of readily metabolizable carbon substrates had a significant effect on the degradation of cyanides. This activity was higher in *Pseudomonas putida* than the other two organisms. It is concluded that *Pseudomonas putida* can be used to degrade organic cyanide under optimized conditions, which remain to be determined in the ecosystem.

BIOPROCESS FOR CO_2-ELIMINATION FROM POWER PLANT FLUE GAS: THE POSSIBLE USE OF MICROALGAE AND SEAWATER

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The exhaust gas of the combustion processes at power plants is considered to be a cause of impending "greenhouse warming;" but at the same time, it is a useful source of carbon dioxide for mass cultures of microalgae. In order to develop a bioprocess for both the efficient fixation of CO_2 in flue gases and the conversion of the resulting biomass to useful chemicals, we investigated the fundamental growth characteristics of microalgae under high CO_2 conditions.

More than 10 strains of microalgae, mostly from a SERI Collection, were cultured semicontinuously in a 16-h light/8-h dark cycle at an elevated CO_2 level in sparging air (15% CO_2). The effects of high CO_2 on algal growth in an artificial seawater (f/2 medium with Instant Ocean®) were as follows: half of them exhibited no growth, and the others grew with extended lag periods or decreased growth rates. *Nannochloris* sp. NANNO2 and *Nannochloropsis* sp. NANNP2 showed the most stable growth at high CO_2 levels. The high CO_2 conditions had no negative effects on lipid production of these algae.

Prospects for a CO_2-bioconversion process based on the usage of microalgae and seawater will be discussed after evaluating the effects of other environmental factors combined with the high CO_2 stress.

PRODUCTION OF OIL-DEGRADING BACTERIA AND THEIR USE IN MICROBIAL REMEDIATION OF CONTAMINATED SOILS

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In order to achieve rapid and complete microbial remediation of soils contaminated with fuel oil, it is necessary to employ microorganisms which can be established in the soil and which degrade the contaminant under the sub-optimal conditions prevailing. The aim of our investigation is to develop methods for this production of bacterial biomass with emphasis on high yields and a high efficiency in degradation of the components of mineral oils,[1,2] especially of the more persistent ones. Furthermore, practicable methods have to be developed to characterize the behavior of the bacterial population during a soil clean-up.

Results:

For growth and production of the microorganisms on fuel oil, a two-stage fermenter system was constructed in which the separate stages were operated at different dilution rates. The oil was emulsified mechanically and, with the help of biogenic surfactants,[3] formed in the first bioreactor. With influent oil concentrations ranging from 4 to 7 g/L, 80% of the oil was degraded within a retention time of 17 h in the first bioreactor. Further degradation of the oil up to 98% occurred in the second fermenter with a retention time of 85 h. The yield coefficient for the biomass, related to organic carbon, amounted to 0.1 to 0.17 depending on the inflow oil concentration.

An analysis of the bacterial population in both stages showed that five different types of bacteria mainly occur. The substances which each bacterial isolate could degrade were determined in growth experiments on single components of fuel oil.

The oil degradation rate of the microbial biomass, produced in the two-stage bioreactor, could be estimated in columns with contaminated soil and biomass. The microbial respiration rates, measured in the presence of a mixture of hydrocarbons or of individual hydrocarbons, also served as a measure of the microbial activity of the produced biomass.

1. *Ullmanns Enzyklopädie der technischen Chemie* (1978). Bd. 12. Verlag Chemie Weinheim & New York.
2. Ratledge, C. (1986). Degradation of aliphatic hydrocarbons. Chapter I, 1-46. Developements in biodegradation of hydrocarbons. Appl. Science Publishers LTD 1978. ISBN 0 85334 751 4.
3. Cooper, D. G. und Zajic, J. E. (1980). Surface active compounds from microorganisms. Academic Press, *Advances in Applied Microbiology* 26, 229-253.

METHANOL SUPPRESSION OF TRICHLOROETHYLENE DEGRADATION BY M. TRICHOSPORIUM

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Biodegradation by methylotrophs has been considered a potential method for in situ remediation, but delivery of sufficient methane could be a problem. Since methanol could be delivered more readily into soil, we examined TCE degradation under methane (0.89 M), methanol (1.187 mM), and combined methane (0.89 mM) methanol (1.187 mM) stimulated treatments using *M. trichosporium* and mixed cultures JS and DT. Degradation of TCE was determined by the summation of radiolabeled CO_2, water-soluble intermediates, and biomass transformed from ^{14}C TCE. *M. trichosporium* degraded 0.36 ± 2.08% (mean ± std dev)of the initial TCE (0.3 mg/L) with methanol stimulation, compared to 9.07 ± 1.04% with methane stimulation. JS and DT cultures degraded 4.34 ± 0.11% on methanol compared to 24.3 ± 1.38% and 34.3 ± 3.0% on methane, respectively. If methanol was added to methane-stimulated cultures, TCE degradation was reduced to 1.08 ± 1.74% for *M. trichosporium*, and 5.08 ± 0.56% for JS culture. Methanol retarded the rates of methane and oxygen utilization as well. However, methanol-stimulated cultures grew to a greater extent than methane-stimulated cultures with 14 mg/L TCE. Previous workers have shown that methanol suppresses methane monooxygenase, and we suggest this may explain the reduced amount of TCE degraded.

ISOLATION OF AMOEBIC-BACTERIAL CONSORTIA CAPABLE OF DEGRADING TRICHLOROETHYLENE

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Groundwater from a waste disposal site contaminated with chlorinated alkenes was examined for the presence of amoebic-bacterial consortia capable of degrading the suspected carcinogen, trichloroethylene (TCE). Consortia were readily isolated from all of four test wells. They contained free-living amoebae, and heterotrophic and methylotrophic bacteria. Electron microscopic examination showed bacteria localized throughout the amoebic cytoplasm and an abundance of hyphomicrobium, but not Type I methanotrophs. The presence of Type II methanotrophs was indirectly indicated by lipid analysis of one consortium. The consortia have been passaged for over two years on mineral salts media in a methane atmosphere, which would not be expected to maintain the heterotrophs or amoebae separately. The methanotrophic bacteria apparently provided a stable nutrient source, allowing the persistence of the various genera. By use of ^{14}C-radiotracer techniques, the degradation of TCE by the consortia was observed with ^{14}C eventuating predominantly in CO_2 and water-soluble products. In a more detailed examination of one consortia, the amoebae and heterotrophic components did not degrade TCE, while a mixed culture of heterotrophs and methanotrophs did degrade TCE, suggesting the latter component was the primary cause for the consortium's ability to degrade TCE. Amoebic-bacterial consortia may play a role in stabilizing and preserving methylotrophic bacteria in hostile environments.

BIOLOGICAL PRETREATMENT OF WATER HYACINTH FOR IMPROVED BIOGAS PRODUCTION

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Water hyacinth powder pretreated by ten isolated fungi was fermented anaerobically to biogas with the ultimate aim of improving gas production. Lignin-, cellulose-, and hemicellulose-degradation was recorded and showed increased biotechnological substrate value. Biogas produced from pretreated water hyacinth was more than twice the amount from untreated water hyacinth. In addition to increasing total gas production, microbial pretreatment was responsible for higher methane content in the digester gas, enriching its fuel value.

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