EIGHTEENTH SYMPOSIUM ON BIOTECHNOLOGY FOR FUELS AND CHEMICALS

Program and Abstracts

Park Vista Resort Hotel
Gatlinburg, Tennessee, U.S.A.
May 5–9, 1996

Sponsors:
U.S. Department of Energy
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Division of Biochemical Technology

EIGHTEENTH SYMPOSIUM ON BIOTECHNOLOGY FOR FUELS AND CHEMICALS

Program and Abstracts

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Department of Energy
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Jonathan Woodward
Oak Ridge National Laboratory
Oak Ridge, Tennessee

DISCLAIMER

SOCIAL PROGRAM AND TOURS

- **Sunday, May 5**

  - **Evening reception** for attendees and their guests (light snacks, no charge).

- **Monday, May 6**

  - **Morning Guest Program** for guests of attendees (continental breakfast and information on appropriate excursions will be provided). The Guest Program fee includes participation on Tuesday and Wednesday mornings as well.

  - **Evening banquet** at the Park Vista Hotel (preceded by a social hour). The symposium fee includes the social hour and banquet, but guests of attendees must purchase tickets.

- **Tuesday, May 7**

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

  - An afternoon at **Dollywood**, showcasing the rich culture and heritage of Southern Appalachia and including six live musical shows, exhilarating rides, craft shops, demonstrations, and attractions such as the Eagle Mountain Sanctuary, an operating 1923 Dentzel carousel, and Dolly Parton's Museum. Ticket price includes admission and transportation.

  - Afternoon **tour of Oak Ridge National Laboratory (ORNL)**, with an emphasis on bioprocessing research facilities. (*Registration for the ORNL tour by non-U.S. citizens must be made by February 19, 1996*, to allow time to obtain approvals. Registration deadline for U.S. citizens is April 5, 1996.)

  - Local hiking trips can be arranged at no additional cost for any date.

- **Wednesday, May 8**

  - 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. A scenic 11-mile loop road, following many of the old wagon roads, allows you to see homesteads, other buildings typical of a pioneer community, and abundant wildlife.

  - An afternoon at the **Oconaluftee Indian Village** on the **Cherokee Indian Reservation**. Indian guides in native costumes lead you to primitive cabins and rustic arbors. Observe the making of a dugout canoe with fire and ax; the traditional methods of chipping flint into arrowheads; and the carving of wooden spoons, combs, and bowls. Watch as Cherokee women string beads, mold ropes of clay, and weave baskets. Inside the seven-sided council house, learn of Cherokee history, ritual, and culture. Ticket price includes admission and transportation.

PROGRAM

Sunday Evening, May 5, 1996

6:00–10:00 p.m. Registration (*Tennessee Ballroom Foyer*)

7:00–10:00 p.m. Reception (*Tennessee Ballroom Foyer*)

Monday Morning, May 6, 1996

8:00 a.m.–5:00 p.m. Registration (coffee) (*Tennessee Ballroom Foyer*)

9:00 a.m. Guest Program (*Gardenview Room A*)

8:20 a.m. Welcome and Introduction to the Symposium (*Tennessee Ballroom*)—**Brian H. Davison**, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Session 1—Thermal, Chemical, and Biological Processing of Feedstocks

Chair: **Mark T. Holtzapple**
Texas A&M University
College Station, Texas

Cochair: **Robert Torget**
National Renewable Energy Laboratory
Golden, Colorado

8:30 Introduction and Session Overview (*Tennessee Ballroom*)

8:40 **Paper 1. AFEX Pretreatment, Enzymatic Hydrolysis, and Fermentation of Glucose and Xylose from Corn Fiber by *Saccharomyces* Strain (1400pLNH32)**, *M. Moniruzzaman* and B. E. Dale, Texas A&M University, College Station, Texas; B. S. Dien, R. B. Hespell, and R. J. Bothast, U.S. Department of Agriculture, Peoria, Illinois; Z. D. Chen and N. W. Y. Ho, Purdue University, West Lafayette, Indiana

9:05 **Paper 2. Ammonia Recycled Percolation as a Complementary Pretreatment to the Dilute-Acid Process**, *Z. Wu* and Y. Y. Lee, Auburn University, Auburn, Alabama

9:30 **Paper 3. Enhancing Biomass Digestibility Using Lime Pretreatment**, V. Chang, B. Burr, and *M. T. Holtzapple*, Texas A&M University, College Station, Texas

9:55 Intermission

10:10 **Paper 4. Two-Phase Model of Hydrolysis Kinetics and Its Applications to Anaerobic Degradation of Particulate Organic Matter**, *V. A. Vavilin*, L. Ya. Lokshina, and S. V. Rytov, Russian Academy of Sciences, Moscow, Russia

10:35 **Paper 5. Cellulase Precipitation and Flotation**, S. Avelino, *C. C. Santana*, and E. A. Miranda, Faculdade de Engenharia Quimica, UNICAMP, Campinas, SP, Brazil

11:00 **Paper 6. Cellulosic Ethanol Production with Consolidated Bioprocessing Using Thermophilic Bacteria**, *L. R. Lynd*, S. Baskaran, P. van Walsum, J-H. Kim, and S. Casten, Dartmouth College, Hanover, New Hampshire; W. R. Lin, G. Ozcengiz, and A. L. Demain, Massachusetts Institute of Technology, Cambridge, Massachusetts

11:25 Session Adjournment

Monday Afternoon, May 6, 1996

Session 2—Biological Research

Chair: **Valerie Sarisky-Reed**
Department of Energy
Washington, D.C.

Cochair: **Jonathan Woodward**
Oak Ridge National Laboratory
Oak Ridge, Tennessee

1:00 Introduction and Session Overview (*Tennessee Ballroom*)

1:10 **Paper 7. Asparaginase II: A Model for Nitrogen Regulation of a Periplasmic Enzyme in *Saccharomyces cerevisiae***, *E. P. S. Bon*, E. Carvaial, M. Stanbrough, D. Rowen, and B. Magasanik, Universidad Federal do Rio de Janeiro, Rio de Janeiro, Brazil

1:35 **Paper 8. Hydrogen Production in a Combination of Marine Green Algae and Photosynthetic Bacteria**, *Y. Miura*, Kansai Electric Power Co., Amagasaki, Hyogo, Japan; T. Akano, K. Fukatsu, and H. Miyasaka, Kansai Electric Power Co. and Rite Amagasaki 2nd and Nankoh Laboratories; T. Mizoguchi, K. Yagi, and I. Maeda, Osaka University; Y. Ikuta and H. Matsumoto, Mitsubishi Heavy Industries, Ltd.

2:00 **Paper 9. Production of α-Terpineol from Limonene**, S. Savithiry and *P. Oriel*, Michigan State University, East Lansing, Michigan

2:25 **Paper 10. Cellulase Superfolds: Diversity of Structure and Convergence of Function**, *M. E. Himmel*, J. O. Baker, W. S. Adney, R. A. Nieves, and S. R. Thomas, National Renewable Energy Laboratory, Golden, Colorado

2:50 Intermission

3:10 **Paper 11. Study on the Production of a Xylanolytic Complex from *Penicillium canescens* 10-10c**, A. Gaspar, C. Roques, T. Cosson, and *P. Thonart*, Faculté des Sciences Agronomiques, Gembloux, Belgium

3:35 **Paper 12. Fermentation of Biomass-Derived Glucuronic Acid by *pet*-Expressing Recombinants of *E. coli* B**, *H. G. Lawford* and J. D. Rousseau, University of Toronto, Toronto, Ontario, Canada

4:00 **Paper 13. Further Improvement of Recombinant *Saccharomyces* Yeast for Xylose Fermentation**, *N. W. Y. Ho*, Z. D. Chen, and A. Brainard, Purdue University, West Lafayette, Indiana; S. Toon, C. J. Riley, and G. P. Philippidis, National Renewable Energy Laboratory, Golden, Colorado; R. E. Lumpkin, SWAN Biomass Company, Downers Grove, Illinois

4:25 **Paper 14. Genetic Engineering of the Xylose Fermenting Yeast *Pichia stipitis***, *T. W. Jeffries*, B. P. Davis, N. Q. Shi, J. Y. Cho, K. M. Dahn, P. Lu, H. K. Sreenath, and J. Hendrick, Forest Products Laboratory, U.S. Department of Agriculture Forest Service, Madison, Wisconsin

4:50 Session Adjournment

Monday Evening, May 6, 1996

6:15 Social Gathering (*Tennessee Ballroom Foyer*)

7:00 Banquet (*Tennessee Ballroom*)

8:00 After-dinner address: "The Fabulous Appalachian Heritage," **J. R. Irwin and the Museum of Appalachia Band**, Museum of Appalachia, Norris, Tennessee

Tuesday Morning, May 7, 1996

8:00–noon Registration (coffee) (*Tennessee Ballroom Foyer*)

9:00 Guest Program (*Gardenview Room A*)

Session 3—Bioprocessing Research

Chair: **Robert R. Dorsch**
DuPont
Wilmington, Delaware

Cochair: **Christos Hatzis**
National Renewable Energy Laboratory
Golden, Colorado

8:30 Introduction and Session Overview (*Tennessee Ballroom*)

8:45 **Paper 15. Cellulase Production Based on Hemicellulose Hydrolysate from Steam Pretreated Willow**, Zs. Szengyed, *K. Rèczey*, and G. Zacclei, Lund University, Lund, Sweden

9:10 **Paper 16. Effect of Impeller Geometry on Gas-Liquid Mass-Transfer Coefficient in Filamentous Suspension**, *S. Dronawat*, C. K. Svihla, and T. R. Hanley, University of Louisville, Louisville, Kentucky

9:35 **Paper 17. Investigation of Bifurcation Parameter in Ethanol Fermentation with Gas Stripping**, *H-W. Hsu* and C-W. Loh, National University of Singapore, Singapore

10:00 Intermission

10:20 **Paper 18. Performance of Immobilized *Saccharomyces cerevesiae* in a Fluidized-Bed Reactor for Fuel Ethanol Production**, *M. Y. Sun*, P. R. Bienkowski, and O. F. Webb, Oak Ridge National Laboratory, Oak Ridge, Tennessee

10:45 **Paper 19. Adsorption of Albumin on Different Polymeric Surfaces and Its Impact on Permeability of Membranes**, J. Johansson and *R. K. Bajpai*, University of Missouri, Columbia, Missouri

11:10 **Paper 20. Fumaric Acid Production in Airlift Loop Reactors with Porous Spargers**, *J. Du*, N. J. Cao, and G. T. Tsao, Purdue University, West Lafayette, Indiana

11:35 **Paper 21. Fermentation Process for the Production of Succinic Acid**, *N. P. Nghiem*, B. H. Davison, B. E. Suttle, and G. L. Richardson, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Noon Session Adjournment

Noon Wine tasting and luncheon buffet (*Tennessee Ballroom*)

Tuesday Afternoon, May 7, 1996

1:15–6 p.m. An afternoon at Dollywood

1:15–6 p.m. Tour of Oak Ridge National Laboratory

Tuesday Evening, May 7, 1996

Session 4—Industrial Needs for Commercialization

Chair: **Dale Monceaux**
Raphael Katzen Associates International, Inc.
Cincinnati, Ohio

Cochair: **James L. Gaddy**
Bioengineering Resources, Inc.
Fayetteville, Arkansas

This session features two talks, followed by a roundtable discussion of commercial applications of bioprocesses for production of fuels, chemicals, and materials.

7:00 Overview, Papers, and Roundtable discussion (*Tennessee Ballroom*)

7:10 **Paper 22. Agribusiness By-products as Potential Feedstocks for Higher-Value-Added Products**, *C. A. Abbas*, Archer Daniels Midland, Decatur, Illinois

7:35 **Paper 23. Biomass-to-Ethanol: Program Update**, *R. H. Walker*, SWAN Biomass Company, Downers Grove, Illinois

8:00 Roundtable Discussion

Panelists: D. Monceaux, Raphael Katzen Associates International, Inc.; J. L. Gaddy, Bioengineering Resources, Inc.; C. A. Abbas, Archer Daniels Midland; R. H. Walker, SWAN Biomass; R. R. Dorsch, DuPont, S. J. Gatto, BC International (invited); R. J. Salazar, Omni Interests, Inc. (invited).

9:00 Session Adjournment

Wednesday Morning, May 8, 1996

8:00–noon Registration (coffee) (*Tennessee Ballroom Foyer*)

9:00 Guest Program (*Gardenview Room A*)

Session 5—Emerging Topics in Industrial Biotechnology

Chair: **Bruce Dale**
Michigan State University
East Lansing, Michigan

Cochair: **Eric Kaufman**
Oak Ridge National Laboratory
Oak Ridge, Tennessee

8:30 Introduction and Session Overview (*Tennessee Ballroom*)

8:45 **Paper 24. Biotechnology, Energy Conversion Efficiency, and the Production of Renewable Fuels and Chemicals**, *E. Greenbaum*, J. W. Lee, C. V. Tevault, and S. L. Blankinship, Oak Ridge National Laboratory, Oak Ridge, Tennessee

9:10 **Paper 25. Principles for Mass Cultivation of Photoautotrophs**, *A. Richmond* and H. Qiang, Ben Gurion University, Sede-Boker, Israel

9:35 **Paper 26. Enzymatic Transesterification of Organophosphorous Esters in Organic Solvent with Phosphotriesterase**, *K. Sode* and S. Oh-uchi, Tokyo University of Agriculture and Technology, Tokyo, Japan

10:00 Intermission

10:20 **Paper 27. Enzymatic Catalysis in Organic Solvents: Polyethylene Glycol–Modified Hydrogenase Is Soluble in Toluene and Retains Sulfur-Reducing Activity**, *C. Kim* and M. W. W. Adams, University of Georgia, Athens, Georgia; C. A. Woodward and E. N. Kaufman, Oak Ridge National Laboratory, Oak Ridge, Tennessee

10:45 **Paper 28. Use of Microbubble Dispersions in Synthesis Gas Fermentations**, M. D. Bredwell and *R. M. Worden*, Michigan State University, East Lansing, Michigan

11:10 **Paper 29. Biodesulfurization of Dibenzothiophene and Crude Oil Using Electrospray Reactors**, *E. N. Kaufman*, J. B. Harkins, M. Rodriguez, L. A. Sy, M. A. Spurrier, P. T. Selvaraj, and C. Tsouris, Oak Ridge National Laboratory, Oak Ridge, Tennessee

11:35 **Paper 30. Amazon: Fauna, Flora, and Potential Bioprocessing Applications**, *J. D. Fontana*, Federal University of Paraná, Paraná Brazil

Noon Session Adjournment

Wednesday Afternoon, May 8, 1996

1:15–5:00 p.m. Tour of Cades Cove area of the Great Smoky Mountains National Park. Hiking trips may be arranged at no additional cost.

1:15–5:00 p.m. An afternoon at the Oconaluftee Indian Village on the Cherokee Indian Reservation

2:00–4:00 p.m. Special Topic Discussion Groups

Techno-Economic Modeling of Lignocellulosic Conversion to Ethanol. Leader: **Jack N. Saddler**, Faculty of Forestry, University of British Columbia, Vancouver, British Columbia, Canada

Biocatalysis and Bioprocessing in Nonaqueous Media. Leader: **John Sheehan**, National Renewable Energy Laboratory, Golden, Colorado

Wednesday Evening, May 8, 1996

6:30 Social Gathering (*Tennessee Ballroom Foyer*)

7:00 **Poster Session** (*Tennessee Ballroom*), Chair: **Karel Grohmann**, U.S. Citrus and Subtropical Products Research Laboratory, Winter Haven, Florida. A list of poster titles follows this agenda.

10:00 Refreshments (*Tennessee Ballroom*)

Thursday Morning, May 9, 1996

Session 6—Environmental Biotechnology

Chair: **Mary Jim Beck**
Tennessee Valley Authority
Muscle Shoals, Alabama

Cochair: **Joni M. Barnes**
Idaho National Engineering Laboratory
Idaho Falls, Idaho

8:30 Introduction and Session Overview (*Tennessee Ballroom*)

8:45 **Paper 31. A Biological Multidisciplinary Model to Predict Municipal Landfill Life—Successful Application to the Belgium Anton Site**, *R. Drion*, X. Taillieu, and P. Thonart, Faculté des Sciences Agronomiques, Gembloux, Belgium

9:10 **Paper 32. Intrinsic Bioremediation of Gas Condensate Hydrocarbon: Results of Over 2 Years of Groundwater, Soil Gas, and Soil Core Analysis and Monitoring**, *K. L. Sublette*, R. Kolhatkar, G. Trent, K. Raterman, and J. B. Fisher, University of Tulsa, Tulsa, Oklahoma

9:35 **Paper 33. Pilot-Scale Bioremediation of PAH-Contaminated Soils**, *S. Pradhan*, B. Liu, R. Kelley, J. Conrad, and V. Srivastava, Institute of Gas Technology, Des Plaines, Illinois

10:00 Intermission

10:15 **Paper 34. Role of Mass Transfer in Bioremediation of Soil**, S. K. Lotfabad, M. J. Dudas, M. A. Pickard, and *M. R. Gray*, University of Alberta, Edmonton, Alberta, Canada

10:40 **Paper 35. Development of a Membrane-Based Vapor-Phase Bioreactor**, N. Rouhana and *P. R. Bienkowski*, The University of Tennessee, Knoxville, Tennessee

11:05 **Paper 36. Enhancement of Mineralization and Degradation of PCB Congeners by an Integrated Biological-Chemical Treatment Process**, *B. K. Soni*, R. L. Kelley, and V. J. Srivastava, Institute of Gas Technology, Des Plaines, Illinois

11:30 **Paper 37. Observations of Metabolite Formation and Variable Yield in Thiodiglycol Biodegradation Processes: Impact on Reactor Design**, T-S. Lee, W. A. Weigand, and *W. E. Bentley*, University of Maryland, College Park, Maryland

Noon Symposium Adjournment and Closing Remarks—**Brian H. Davison**

Thursday Afternoon, May 9, 1996

Symposium attendees are invited to attend an open afternoon workshop of the International Energy Agency (IEA) from 1:00 to 5:00 p.m. This workshop, to be led by Dr. J. N. Saddler, focuses on IEA's efforts in conversion of lignocellulose. (The IEA meets in closed session during the morning.) Details will be available at the Registration Desk.

POSTER PRESENTATIONS

(Invited to Participate)

Thermal, Chemical, and Biological Processing of Feedstocks

Poster 38. Deactivating Effects of Fermentation Residues on the Catalytic Upgrading of Lactic Acid, *M. S. Tam*, D. J. Miller, and J. E. Jackson, Michigan State University, East Lansing, Michigan

Poster 39. The Peel of Lime (*Citrus aurantifolia*) as a Source of Acetylesterase, *B. R. Evans* and J. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 40. Solid-State Fermentation of Sorghum to Ethanol: Year Two, *L. L. Henk* and J. C. Linden, Colorado State University, Fort Collins, Colorado

Poster 41. Pyrolysis of Steam-Classified Municipal Solid Waste, *J. M. Sebghati* and M. H. Eley, University of Alabama, Huntsville, Alabama

Poster 42. Bleached Cellulose from Sugarcane Bagasse for Chemical Processing, L. F. F. Faria, J. C. S. Barboza, A. A. Serra, and *H. F. de Castro*, Faculdade de Engenharia Quimica de Lorena, Lorena, SP, Brazil

Poster 43. The Role of Pure Supplementary Enzymes on Cellulose Hydrolysis, *P. Kotiranta*, M. Tenkanen, and L. Viikari, VTT Biotechnology and Food Research, VTT, Finland

Poster 44. Cost Estimates and Sensitivity Analyses for the Ammonia Fiber Explosion (AFEX) Process, L. Wang, *B. E. Dale*, L. Yurttas, and I. Goldwasser, Michigan State University, East Lansing, Michigan

Poster 45. Solvent-Phase Thermal Cracking of Lignin for Production of Oxygenates and Other Potential Liquid Fuels, *B. H. Lee* and Y. Y. Lee, Auburn University, Auburn, Alabama

Poster 46. Preprocessed Barley, Rye, and Triticale as a Feedstock for an Integrated Fuel Ethanol–Feedlot Plant, *K. Sosulski*, Saskatchewan Research Council, Saskatoon, Saskatchewan, Canada; S. Wang, M. Ingledew, and F. Sosulski, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Poster 47. Biodiesel–Fuel Oil Blends as an Alternative Heating Oil, F. Karaosmanoğlu, *Ü. G. Beker*, and K. B. Ciğizoğlu, Istanbul Technical University, Istanbul, Turkey

Poster 48. Biodiesel Refinement Through Washing with Hot Water, F. Karaosmanoğlu, *K. B. Ciğizoğlu*, M. Tüter, and Ü. G. Beker, Istanbul Technical University, Istanbul, Turkey

Poster 49. Sugars from Biomass as a Raw Material for Renewable Chemicals, S. Schmidt, T. K. Hayward, *N. Padukone*, R. Wooley, and C. Hatzis, National Renewable Energy Laboratory, Golden, Colorado

Poster 50. Effect of Hydrothermal Treatment of Wood on Cellulose Hydrolysis, *J. Weil*, A. Sarikaya, D. Rau, C. Ladisch, M. Brewer, R. Hendrickson, and M. R. Ladisch, Purdue University, West Lafayette, Indiana

Poster 51. Environmentally Safe Biotechnological Utilization of Plant Biomass from the Areas Polluted by Heavy Metals and Radionuclides, *M. Rabinovich*, A. Jalsrain, L. Vasilchenko, and J. Kozlov, Russian Academy of Sciences, Moscow, Russia

Biological Research

Poster 52. Optimization of Seed Production for a Simultaneous Saccharification Cofermentation Biomass-to-Ethanol Process Using Recombinant *Zymomonas*, *H. G. Lawford* and J. D. Rousseau, University of Toronto, Toronto, Ontario, Canada

Poster 53. Corn Steep Liquor as a Cost-Effective Nutrition Adjunct in High-Performance *Zymomonas* Ethanol Fermentations, *H. G. Lawford* and J. D. Rousseau, University of Toronto, Toronto, Ontario, Canada

Poster 54. A New Photosynthetic Pathway, *J. W. Lee* and E. Greenbaum, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 55. Partial Biooxidation of Methane to Methanol by Methane-Assimilating Bacteria, *G. A. Kovalenko*, Institute of Catalysis, Novosibirsk, Russia

Poster 56. Production of L(+)-Lactic Acid from MSW Hydrolyzate by Immobilized *Lactobacillus pentosus*, S. D. Zhou and *T. A. McCaskey*, Auburn University, Auburn, Alabama; J. Broder, Tennessee Valley Authority, Muscle Shoals, Alabama

Poster 57. A Stable Lipase from *Candida lipolytica*: Cultivation Conditions and Crude Enzyme Characteristics, *F. V. Pereira-Meirelles*, M. H. M. Rocha-Leao, and G. L. Sant'Anna, Jr., Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Poster 58. Tailoring of Glucoamylase Isoenzymes Through Medium Composition, *J. G. Silva, Jr.*, and E. P. S. Bon, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Poster 59. Experimental Data Analysis: An Algorithm for Smoothing of Data and Determining Enzyme and Microbial Kinetic Rates, *K. T. Klasson*, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 60. Regulation of Phosphotransferase in Glucose and Xylose Fermenting Yeasts, V. W. Yang and *T. W. Jeffries*, Forest Products Laboratory, U.S. Department of Agriculture Forest Service, Madison, Wisconsin

Poster 61. Free Sterols Synthesized by *In Vitro* Cultures of Flax, A. Cunha and

Poster 62. Enhanced Fermentation of Sugar Mixtures by *Pichia stipitis* Mutant FPL-061, *H. K. Sreenath* and T. W. Jeffries, Forest Products Laboratory, U.S. Department of Agriculture Forest Service, Madison, Wisconsin

Poster 63. Production of Xylitol from D-Xylose by *Debaryomyces hanenii*, *J. M. Dominguez*, University of Vigo, Orense, Spain; C. S. Gong and G. T. Tsao, Purdue University, West Lafayette, Indiana

Poster 64. Production of 2,3-Butanediol from Pretreated Corn Cob by *Klebsiella oxytoca* in the Presence of Fungal Cellulase, N. Cao, Y. Xia, *C. S. Gong*, and G. T. Tsao, Purdue University, West Lafayette, Indiana

Poster 65. The Effect of Dissolved Oxygen on Xylose Fermentation by *Candida* sp., *C. S. Chen* and J. D. Juan, Da-Yeh Institute of Technology, Taichung, Taiwan, Republic of China; N. J. Cao and C. S. Gong, Purdue University, West Lafayette, Indiana

Poster 66. Oxygen Sensitivity of Algal Hydrogen Production, *M. Ghirardi*, S. Toon, and M. Seibert, National Renewable Energy Laboratory, Golden, Colorado

Poster 67. Production of Cellulases and Xylanases by *Trichoderma sp.* F09700.1b and *Aspergillus sp.* F01200.1b Under Semisolid Culture Conditions, *O. García-Kirchner*, C. M. Morales, and B. T. Robledo, Instituto Politecnico Nacional, Ticomán, Zacatenco, Mexico

Poster 68. Obtaining Microbial Pectinases from Orange Peel by Semisolid Culture of Three Different Fungi Strains, *M. M. E. Gómez*, C. M. Takaki, and O. García-Kirchner, Instituto Politecnico Nacional, Ticomán, Zacatenco, Mexico

Poster 69. Optimization of Reaction Conditions for an Enzymatic Conversion of Glucose to Hydrogen: Initial Studies of pH, Temperature, and Reactant Effects, R. J. Edmonston and *J. Woodward*, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 70. Some Biotechnological Properties of Sterol Mutants of Microalgae, *Y. Nakonechny*, St. Petersburg University, St. Petersburg, Russia

Poster 71. Effect of Carbon Dioxide on Succinate Production by *Fibrobacter succinogenes*, *R. R. Gokarn*, M. A. Eiteman, and S. A. Martin, University of Georgia, Athens, Georgia

Poster 72. *Acetobacter* Cellulosic Biofilms: Search for New Cellulogenic Modulators and Treatments for Modified Pellicles, *J. D. Fontana*, M. Baron, C. G. Joerke, M. B. Soares, and M. F. Guimarães, Federal University of Paraná, Paraná, Brazil

Poster 73. Astaxanthinogenesis in *Phaffia rhodozyma*: Optimization of Low-Cost Culture Media, Pigment Supercritical Fluid Extraction, and Yeast Cell Lysis, *J. D. Fontana*, M. B. Chociai, M. Baron, M. F. Guimarães, C. G. Joerke, C. Ulhoa, and T. M. B. Bonfim, Federal University of Paraná, Paraná, Brazil

Poster 74. Immobilization of Inulinase and Glucose Dehydrogenase for Production of Hydrogen, *M. Baron*, J. D. Fontana, J. A. Florêncio, and M. F. Guimarães, Federal University of Paraná, Paraná, Brazil; R. J. Edmonston and J. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 75. Expression of Malic Enzyme Allows Production of Succinic Acid from Glucose in a Mutant *Escherichia coli*, L. Stols, G. Kulkarni, B. G. Harris, and *M. I. Donnelly*, Argonne National Laboratory, Argonne, Illinois

Poster 76. Metabolic Engineering of an Arabinose-Fermenting *Zymomonas mobilis*, K. Deanda, M. Zhang, C. Eddy, and *S. Picataggio*, National Renewable Energy Laboratory, Golden, Colorado

Poster 77. Fermentation of Pectin and Orange Peel by Ethanologenic Soft-Rot Bacteria, *K. Grohmann*, R. G. Cameron, and J. Manthey, U.S. Citrus and Subtropical Products Laboratory, Winter Haven, Florida; B. S. Buslig, Florida Department of Citrus, Winter Haven, Florida

Poster 78. Atomic Force Microscope Measurements of Substrate-Enzyme Interactions in Cellulase Systems, *I. Lee*, B. R. Evans, and J. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Poster 79. Microbial Phosphorylases for the Conversion of Maltodextrin to Glucose-1-Phosphate, *B. Nidetzky*, R. H. Griessler, D. Haltrich, and K. D. Kulbe, BOKU University of Agriculture, Vienna, Austria

Poster 80. Enzymatic Synthesis of Sorbitol and Gluconic Acid—Process Considerations Employing Isolated Glucose-Fructose Oxidoreductase from *Zymomonas mobilis*, *B. Nidetzky*, M. Fürlinger, D. Haltrich, and K. D. Kulbe, BOKU University of Agriculture, Vienna, Austria

Poster 81. Induction of Xylose Reductase and Xylitol Dehydrogenase Activities in *Candida tenuis*, *D. Haltrich*, M. Kern, B. Nidetzky, and K. D. Kulbe, BOKU University of Agriculture, Vienna, Austria

Poster 82. Production of Hemicellulose- and Cellulose-Degrading Enzymes by Various Strains of *Sclerotium rolfsii*, *D. Haltrich*, A. Sachslehner, C. Kirschner, B. Nidetzky, and K. D. Kulbe, BOKU University of Agriculture, Vienna, Austria

Poster 83. Production of D- or L-Lactate in *Escherichia coli*, H-C. Jung and *J-G. Pan*, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea; D-E. Chang and J-S. Rhee, Korea Advanced Institute of Science and Technology, Taejon, Korea

Bioprocessing Research

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Abstracts for Oral Sessions

Paper 1

AFEX PRETREATMENT, ENZYMATIC HYDROLYSIS, AND FERMENTATION OF GLUCOSE AND XYLOSE FROM CORN FIBER BY *Saccharomyces* STRAIN (1400pLNH32)

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Corn fiber is a grain-processing residue containing significant amounts of cellulose, hemicellulose, and starch, which is collected in corn wet-milling facilities where fuel ethanol is currently manufactured. Pretreatment of high-moisture corn fiber by AFEX and fermentation of an enzyme hydrolyzate of mixed sugars by a recombinant *Saccharomyces* strain (1400pLNH32) were investigated. Approximate optimal pretreatment conditions for unground corn fiber containing 150% moisture on a dry weight basis were temperature, 90°C; mass ratio of ammonia to dry corn fiber, 1:1; and residence time, 30 min. More than 85% of the theoretical sugar yield was obtained during enzymatic hydrolysis (48 h) after pretreatment of corn fiber under optimized conditions. The recombinant *Saccharomyces* strain (1400pLNH32) efficiently (91% theoretical yield) fermented both glucose and xylose contained in the corn fiber enzyme hydrolyzate and produced about 22 g/L ethanol.

Paper 2

AMMONIA RECYCLED PERCOLATION AS A COMPLEMENTARY PRETREATMENT TO THE DILUTE-ACID PROCESS

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Dilute-acid pretreatment (DA) has proven to be highly effective in removing the hemicellulose fraction from biomass, thus producing solid residues high in glucan and lignin content. The treated biomass is easily hydrolyzed by cellulose enzymes. The high lignin content in the solid residues, however, may cause high enzyme consumption due to irreversible adsorption of cellulose enzymes onto lignin. This problem may be amplified in the simultaneous saccharification and fermentation process, which is usually run in fed-batch mode, and the residual lignin is expected to accumulate in large amounts. Because of its effectiveness in delignification, the ammonia recycled percolation (ARP) can be applied as a complementary pretreatment to DA. We have investigated this combination of these two processes, ARP followed by DA. The net result was near-complete fractionation of the biomass into the three main components. The treated solids contained primarily glucan. In converting such materials to fermentable sugars, the rate and extent of hydrolysis are increased. A higher ethanol concentration is therefore achieved. The adsorption of cellulose enzymes on lignin was also investigated by using lignins generated from DA, ARP, and the combined processes. The data on cellulose enzyme adsorption on lignin showed that lower lignin content in the substrate resulted in lower enzyme loading and thus increased enzyme efficiency.

Paper 3

ENHANCING BIOMASS DIGESTIBILITY USING LIME PRETREATMENT

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Alkaline pretreatment is known to render biomass highly digestible to enzymatic hydrolysis. Sodium hydroxide and ammonia have traditionally been used. Sodium hydroxide is a very strong base and therefore effective at low loadings; unfortunately, it is very expensive and difficult to recover. Ammonia is a weaker base and requires higher loadings; fortunately, it is easily recovered due to its volatility. Because lime is the least expensive alkali, it is surprising that little attention has been paid to the use of lime as a pretreatment agent.

The scant literature on lime pretreatment has focused on enhancing the ruminant digestibility of agricultural residues. Given the desire of animal scientists to develop simple animal feed processes, they have used low lime loadings at room temperature over extended time periods (e.g., weeks). In general, this approach has yielded very poor results, so lime has gained the reputation of being ineffective.

In our investigations of lime pretreatment, we have used more aggressive treatment conditions employing higher temperatures. As a consequence, the treatment can be shortened to a few hours. Nearly quantitative sugar yields from lime-treated biomass have been achieved using low enzyme loadings. Because we employed higher lime loadings, it was imperative to recover the lime and recycle it. This was achieved by carbonating the wash water with carbon dioxide, which precipitates lime as calcium carbonate. A lime kiln can be used to regenerate the recovered calcium carbonate.

A preliminary investigation of the process economics reveals it to be potentially very economical, with a cost of only $8 to $20/tonne depending on specific circumstances.

Paper 4

TWO-PHASE MODEL OF HYDROLYSIS KINETICS AND ITS APPLICATIONS TO ANAEROBIC DEGRADATION OF PARTICULATE ORGANIC MATTER

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Hydrolysis is normally rate limiting if the organic substrate is in particulate form. Monod, Contois, and first-order kinetic equations, traditionally considered with dissolved substrate, have been tested more or less successfully on the anaerobic degradation of suspended solids. However, for the hydrolysis process, a heterogeneous reaction system, such as particulate substrate contacted with microbial cells and related enzymes, must be taken into consideration.

A two-phase dynamic model describing the particulate substrate degradation as a heterogeneous reaction has been developed. Following the model, an adsorption process of enzymes released by hydrolytic microorganisms is considered for the first phase of the process. Enzymes can spread until an available surface of solids is covered. In the second phase, the degradation rate of suspended solids concentration depends on the surface area, which, in turn, is a function of the current concentration of suspended solids. The particles were assumed to be disklike, cylindrical, or spherical in form, and relative rate constants were evaluated. It was also taken into account that some part of the particulate substrate is not degraded at all. During the second phase, the solids removal rate depends on influent and effluent solids concentrations for the complete-mixing continuous-flow reactor.

The model was tested on the experimental data of anaerobic digestion of cellulose [Noike et al., 1985] and of sludge [O'Rourke, 1968; Shimizu et al., 1993]. Good agreement between model and data was obtained over a large variation of retention time. The washout phenomenon of hydrolytic microorganisms was described at low retention time.

It can be shown that the known Contois model as well as the first-order model are simplifying approximations of this two-phase model.

Paper 5

CELLULASE PRECIPITATION AND FLOTATION

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The production of commercial enzymes requires downstream processing technology based on unit operations of low fixed and operational costs. High purification is not a primary requirement, but it is always desirable. In this work, we describe the precipitation of *Trichoderma reesei* cellulase by precipitation with cellulose ethers in the presence of ammonium sulfate. Particle recovery from the liquid phase was accomplished by continuous-column flotation, exploiting the hydrophobicity of the salted-out cellulose ether particles. Activity recovery with the precipitate was a function of salt and cellulose ether concentrations. The mass ratio between cellulose ether and protein also controlled the precipitation activity recovery due to inhibition of the enzyme by the ethers (up to 40% at some conditions). Activity recovery by flotation had high efficiency regarding the fraction of activity precipitated: around 100% of the activity present in the precipitate was recovered as column overflow (concentrate). The use of modified substrate as precipitant and flotation agent suggested that this technique is an affinity separation.

Paper 6

CELLULOSIC ETHANOL PRODUCTION WITH CONSOLIDATED BIOPROCESSING USING THERMOPHILIC BACTERIA

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Consolidated bioprocessing, whereby production of cellulases and fermentative product formation are accomplished simultaneously via a single microbial system, has a potentially revolutionary impact on the economics of processes converting cellulosic biomass to biocommodity products. Development of consolidated bioprocessing systems can be approached either by conferring the ability to produce cellulases to organisms that are excellent ethanol producers but do not naturally produce hydrolytic enzymes or by improving the ethanol production characteristics of organisms that are naturally excellent producers of cellulases. Thermophilic bacteria are leading candidates for the latter approach. To utilize thermophilic bacteria for ethanol production, one must show that these organisms can (1) produce high concentrations of ethanol, (2) be "robust" in relation to conditions likely to be encountered in an industrial environment, and (3) produce ethanol with satisfactory selectivity. Relevant results are presented from recent work on the thermophiles *Clostridium thermosaccharolyticum* and *Clostridium thermocellum.*

Although considerable data support the proposition that the tolerance of *C. thermosaccharolyticum* to added ethanol is comparable with that of more-conventional ethanol-producing organisms, reported ethanol production by this and other thermophiles is generally limited to ≤30 g/L. This limitation has generally been attributed to ethanol inhibition; however, we have been unable to demonstrate inhibition by produced ethanol. Instead, our results indicate that inhibition/limitation is explainable in terms of either insufficient nutrient supply or accumulation of ions resulting from addition of base to maintain pH. These data also have a bearing on the issue of "robustness," since they establish that the tolerance of *C. thermosaccharolyticum* is comparable with that of other industrial microorganisms with respect not only to ethanol but also to potassium.

Ethanol selectivity is logically approached using the tools of molecular biology, which are in general not developed for ethanol-producing thermophiles. Recent results provide most of the molecular tools necessary to perform pathway engineering of thermophiles. These include methods to protect foreign DNA from the restriction endonuclease systems of thermophilic hosts and to transform thermophilic hosts using electroporation. In addition, we report progress in cloning catabolic genes using PCR.

Paper 7

ASPARAGINASE II: A MODEL FOR NITROGEN REGULATION OF A PERIPLASMIC ENZYME IN *Saccharomyces cerevisiae*

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The production of several extracellular enzymes is known to be negatively affected by readily metabolized nitrogen sources, such as NH_4^+, although there is no consensus regarding the involved mechanisms. Asparaginase II is a periplasmic enzyme of *Saccharomyces cerevisiae* coded by the ASP3 gene. Asparaginase II activity is found after nitrogen starvation or in cells which have been grown in a poor nitrogen source such as proline. We show here that the level of asparaginase II is dependent upon the functional GLN3 gene and that the response to nitrogen availability is under the control of the URE2 gene product. In this respect the expression of ASP3 is similar to the system which regulates glutamine synthetase and NAD-linked glutamate dehydrogenase. Interestingly, attempts to express ASP3 as a result of fusing the coding sequence of the gene to either CYC1 or ADC1 promoters have failed so far. One possible explanation would be that the formation of the fully extracellular active asparaginase II is under the control of a regulatory system that is more complex than the known system for the two intracellular enzymes noted above.

Paper 8

HYDROGEN PRODUCTION IN A COMBINATION OF MARINE GREEN ALGAE AND PHOTOSYNTHETIC BACTERIA

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Oxygen produced photosynthetically inhibits or inactivates the key enzyme of biophotolysis, hydrogenase or nitrogenase. This is the main cause of instability in photosynthetic hydrogen production. We have proposed a unique biophotolysis, in which hydrogen production is temporally separated from oxygen production in alternating light/dark cycles. Our biophotolysis system consists of three main steps for energy conversion: (1) photosynthetic starch accumulation in microalgae, (2) algal fermentation to produce hydrogen and organic compounds, and (3) further conversion of organic compounds to produce hydrogen by photosynthetic bacteria. In our biophotolysis system, stably sustained hydrogen production with high molar yield has been achieved in a combination of *Chlamydomonas* MGA 161 and *Rhodovulum sulfidophilus* W-1S in bench-scale experiments. In this work, our biophotolysis system was scaled up to pilot-plant scale. The optimum conditions for photosynthesis and degradation of starch in green algae and hydrogen production from organic substrate by photosynthetic bacteria were investigated. The stable hydrogen production on pilot-plant scale was achieved in a combination of *Chlamydomonas* MGA 161 and *Rhodovulum sulfidophilus* W-1S in natural day/night cycles under sunlight.

Paper 9

PRODUCTION OF α-TERPINEOL FROM LIMONENE

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The monoterpene R-(+) limonene is the major constituent of citrus oil recovered from waste citrus peels, providing an attractive opportunity for biotransformation to higher-value monoterpenes. As part of an investigation directed toward metabolic engineering of monoterpene pathways in bacteria, we have isolated a *Bacillus* thermophile capable of growth on limonene and have transferred this capability to *Escherichia coli* using a 9.8-kB segment of the thermophile chromosomal DNA. This report describes the utilization of the thermostable limonene hydratase from the pathway to produce the specialty chemical α-terpineol, using whole cells of recombinant *E. coli*. In this system, the recombinant cells catalyze limonene conversion at elevated temperature in a two-phase bioreactor, with neat limonene constituting the organic phase.

Paper 10

CELLULASE SUPERFOLDS: DIVERSITY OF STRUCTURE AND CONVERGENCE OF FUNCTION

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In nature, the enzymatic degradation of cellulose is a fundamental, if not universal, mechanism of biomass conversion. For the production of alcohol fuels from lignocellulosic biomass, however, this process must be understood at the molecular level to develop highly efficient and thus cost-effective catalysts. One important step toward this goal is the determination of high-resolution structures for cellulases based on x-ray and NMR studies. Proteins are classified to reflect both structural and evolutionary relatedness. The principal levels recognized include the family, superfamily, and fold. Folds are grouped into the all-alpha, all-beta, alpha/beta, and alpha + beta categories. Many proteins cannot be easily classified and are known as structural singlets. A recent survey of the 3000+ protein structures logged in the Brookhaven Data Base (PDB) revealed that only 12 cellulases or related enzymes (i.e., those that hydrolyze the beta-glycosyl bond) have been determined. These are 2AYH, 1CBH, 3CBH, 1CEL, 1CLC, 1ENG, 2EXO, 1EXG, 1GHR, 1MAC, 1TML, and 1XYS (PDB code). This paper discusses the similarities and differences in key structural features among this group and attempts to draw correlations to function.

Paper 11

STUDY ON THE PRODUCTION OF A XYLANOLYTIC COMPLEX FROM *Penicillium canescens* 10-10c

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Xylanases (E.C.3.2.1.8) belong to the hemicellulolytic enzymes (hydrolases). Their natural substrate is xylan, which is a heteropolysaccharide constituent of the secondary plant cell wall. The primary and most often studied microorganisms producing these enzymes are *Trichoderma reesei, Aspergillus niger, Clostridium* sp., *Bacillus* sp., and *Streptomyces* sp.

Xylanases have potential uses in many industrial processes and in agrofood or other industries; development of these enzyme applications is well advanced in bread making, in brewery (filtration), in the fruit juice industry (extraction, filtration, clarifying), in animal feeding (digestibility), in compost, and in the paper industry.

We have screened about 100 microorganisms (including nonidentified yeasts, fungi, and bacteria) for their ability to produce a xylanolytic complex. About 40 of them were hemicellulolytic; among these, we studied *Penicillium canescens* 10-10c, considering its ability to produce the enzymic complex in quantity and considering its particularities. The complex is FPase free and has its optimum activity at pH 4.6–5.0 and 55–60°C on birchwood xylan. The best inducers of the complex are soja meal and wheat straw; expression of the xylanases is repressed by glucose, xylose, and lactose. The optimization of culture medium and technology (fed batch) permits us to obtain productions reaching up to ±1000 IU/mL.

Paper 12

FERMENTATION OF BIOMASS-DERIVED GLUCURONIC ACID BY *pet*-EXPRESSING RECOMBINANTS OF *E. coli* B

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The economics of large-scale production of fuel ethanol from biomass and wastes requires the efficient utilization of all the sugars from the hydrolysis of the heteropolymeric hemicellulose component of lignocellulosic feedstocks. Glucuronic and 4-O-methyl-glucuronic acids are major side chains in xylans of the grasses and hardwoods that have been targeted as potential feedstocks for the production of cellulosic ethanol. The amount of these acids is similar to that of arabinose, which is now being viewed as another potential substrate in the production of biomass-derived ethanol; however, these acidic glucose derivatives are seldom reported, because they are often released as glucuronoxylo- disaccharides and trisaccharides and are absorbed by the high-performance liquid chromatography column commonly used for separation of monosaccharides [F. Alterthum et al., *Appl. Environ. Microbiol.* **55**, 1943–48 (1989); L. O. Ingram, T. Conway, and F. Alterthum, U. S. Patent 5,000,000].

Wild-type *E. coli* is heterofermentative, but transformation with genes for ethanol production (pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis*—collectively called the "*pet* operon") results in a redirection of pyruvate metabolism to increase ethanol selectivity.

The objective of this study was to compare the end-product distribution associated with the metabolism of glucuronic acid by *E. coli* B (ATCC 11303) and two different ethanologenic recombinants—a strain in which *pet* expression was via a multicopy plasmid (pLOI297) and a chromosomally integrated construct (KO11) [K. Ohta et al., *Appl. Environ. Microbial.* **57**, 893–900 (1988)]. Batch fermentations were conducted in pH-controlled 2-L bioreactors using a modified LB medium with 2% glucuronate. The set point for pH control was found to influence the ethanol yield, but, in all cases, acetic acid was a significant coproduct. The results offered insights into metabolic fluxes and the regulation of pyruvate catabolism in the wild type and engineered strains.

Paper 13

FURTHER IMPROVEMENT OF RECOMBINANT *Saccharomyces* YEAST FOR XYLOSE FERMENTATION

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Cellulosic biomass is an ideal feedstock for producing renewable fuel ethanol. Most cellulosic biomass contains substantial amounts of both glucose and xylose polymers, but the natural *Saccharomyces* yeasts traditionally used for ethanol production ferment only glucose. Recently, the Molecular Genetics Group at LORRE achieved a breakthrough by developing recombinant *Saccharomyces* yeasts that effectively ferment both sugars. One of the yeasts, LNH32, ferments 8% glucose and 4% xylose to ethanol in 2 days. We now report the development of an improved, stable xylose-fermenting *Saccharomyces*, designated LNH-ST, that is capable of effectively fermenting both glucose and xylose after being cultured in a nonselective medium (e.g., glucose) for an unlimited number of generations.

The LNH-ST yeast has been tested extensively by National Renewable Energy Laboratory (NREL) researchers at both bench-top and pilot-plant scale as part of the NREL/SWAN Biomass CRADA. Results confirm that the recombinant organism is stable and capable of effectively cofermenting glucose and xylose to ethanol. Models using parameters based on these data show that the organism can carry out simultaneous saccharification and cofermentation of pretreated corn fiber feedstock to ethanol within 3 days. The performance of this yeast brings biomass conversion technology closer to commercialization than ever before.

Paper 14

GENETIC ENGINEERING OF THE XYLOSE FERMENTING YEAST *Pichia stipitis*

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Pichia stipitis is one of the best xylose fermenting yeasts known. In order to better understand the mechanisms for xylose fermentation and to obtain improved strains, we have developed a genetic system based on auxotrophic markers and native autonomous replication sequences (ARS). Expression of genes on multicopy ARS-based vectors results in elevated levels of enzymatic activities. Targeted disruption enables the selective inactivation of cloned sequences. Mating can be accomplished through the use of auxotrophic markers. To determine which genes are critical for xylose metabolism in this organism, we have cloned and overexpressed several genes for xylose assimilation and fermentation. Some of the genes involved in fermentation exhibit unusual structural and kinetic properties. Various combinations of fermentative and assimilative enzymes enhance the fermentation rate. We have also cloned genes essential to respiratory and growth processes. Heterologous expression of genes from *S. cerevisiae* or homologous overexpression of genes from *P. stipitis* enhances fermentative properties. This paper presents an overview of the *P. stipitis* genetic system and the effects of genetic manipulation in this host.

Paper 15

CELLULASE PRODUCTION BASED ON HEMICELLULOSE HYDROLYSATE FROM STEAM PRETREATED WILLOW

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The high cost of ethanol production from lignocellulosics using enzymatic hydrolysis is due to the high production cost of cellulolytic enzymes. The aim of the present study was to investigate the cellulolytic enzyme production of *Trichoderma reesei* Rut C 30, which is known as a good cellulase-secreting microorganism, using willow as the carbon source. The willow, which is a fast-growing energy crop in Sweden, was impregnated with 1–4% SO_2 and steam pretreated at 207°C for 5 min. The pretreated material was washed and the wash water, which contains several soluble sugars from the hemicellulose, was supplemented with fibrous pretreated willow and used for enzyme production. In addition to sugars, the liquid contains degradation products, such as acetic acid and furfural, which are inhibitory. The problems were solved by evaporation of the washing water and by using only the nonvolatile fraction. Some of the inhibitors were selected (i.e., acetic acid, furfural), and their single effect on the enzyme fermentation was studied. The preliminary data look very promising, and the study is ongoing. Results will be presented at the meeting.

Paper 16

EFFECT OF IMPELLER GEOMETRY ON GAS-LIQUID MASS-TRANSFER COEFFICIENT IN FILAMENTOUS SUSPENSION

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Rheological measurements of filamentous suspensions can be difficult to obtain using conventional instruments. The impeller method, a successful alternative, requires the use of Newtonian and non-Newtonian calibration fluids to calculate constants necessary to apply the method to filamentous suspensions. Experiments were conducted with three different types of impeller geometries. The Newtonian constant, c, was found to be a linear function of Reynolds number, rather than a constant as previous research suggests. Many previous workers have assumed that the applicable shear rate in such devices is related to the impeller speed by a fluid-independent constant, but results indicate that the shear-rate constant is fluid dependent if either a turbine impeller or a vane impeller is used. A properly designed helical ribbon impeller could overcome this problem. Experiments are being conducted with a helical ribbon impeller on suspensions of cellulose fibers and *Aspergillus niger* broths.

The consistency and reproducibility of data obtained using turbine, vane, and helical impellers are examined and critically assessed. The results will have application in design of rheometers for use in process control and product quality assessment in the fermentation and pulp and paper industries.

Paper 17

INVESTIGATION OF BIFURCATION PARAMETER IN ETHANOL FERMENTATION WITH GAS STRIPPING

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Ethanol fermentation with *in situ* gas stripping (GSEF) alleviates the ethanol toxicity to the microorganism, resulting in higher productivity and reduced costs of product purification and concentration. It may also provide a gentle method of mixing, and cleaning equipment is not needed. However, with the stripping of volatile inhibitory products from a fermenter, the substrate and nonvolatile fermentation inhibitors, which may be either fermentation by-products or salts from the feed, accumulate. Thus, a substrate-inhibition effect will appear in a GSEF system.

A modification of the Ghose-Tyagi specific-growth-rate model and the Luedeking-Piret production model are used to investigate the GSEF system. When substrate inhibition is present together with product inhibition, the system exhibits multiple steady states (bifurcation). In this presentation, the range of bifurcation effects on the critical substrate-inhibition parameter with other GSEF parameters—the stripping factor, feed concentration, dilution rate, etc.—is numerically investigated and the results are presented in dimensionless forms.

Paper 18

PERFORMANCE OF IMMOBILIZED *Saccharomyces cerevisiae* IN A FLUIDIZED-BED REACTOR FOR FUEL ETHANOL PRODUCTION

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The performance of immobilized *Saccharomyces cerevisiae* was evaluated in a 2.5-cm-ID fluidized-bed reactor. *S. cerevisiae* is predominantly used for ethanol fermentations because it is operationally very robust. Corn maltodextrin and yeast extract were used as feedstocks. Experimental conversion and productivity were measured as a function of residence time, dextrose feed concentration, and ethanol concentrations. Percentages of theoretical yields are also presented for these data. Immobilized-cell fluidized-bed reactors have demonstrated significant advantages for fuel ethanol production. During previous long-term experiments (8 weeks), very high volumetric productivities (up to 250 g ethanol $\cdot L^{-1} \cdot h^{-1}$) and excellent yields (96 to 97% of theoretical) were demonstrated using immobilized *Zymomonas mobilis*. Implications for industrial production of ethanol are discussed.

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Paper 19

ADSORPTION OF ALBUMIN ON DIFFERENT POLYMERIC SURFACES AND ITS IMPACT ON PERMEABILITY OF MEMBRANES

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The need for frequent and drastic cleaning procedures for fouled membranes has impeded a widespread use of membranes in bioprocessing. As a result, the phenomenon of membrane fouling has received considerable attention lately. In this work, it has been hypothesized that the process of membrane fouling is initiated by interactions between membrane surfaces and biomolecules, primarily proteins, in the solutions around the membranes. This hypothesis has been investigated by studying the adsorption of a common hydrophobic protein (albumin) on different membrane surfaces and the effect protein adsorption itself has on solvent flux across the membrane. Several microporous membranes with different pore sizes and surface hydrophobicity were selected for this work. The membrane surfaces were additionally modified by subjecting them to a low-temperature plasma polymerization and were characterized by surface area, contact-angle analysis, and electron microscopy. Adsorption isotherms over a large range of solution phase concentrations were determined by use of ^{14}C-albumin. Water permeability of the membranes was measured by dead-end filtration before and after protein adsorption. The adsorption isotherms and water permeability data will be presented and discussed with respect to the surface characteristics of the membranes.

Paper 20

FUMARIC ACID PRODUCTION IN AIRLIFT LOOP REACTORS WITH POROUS SPARGERS

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Airlift loop reactors with porous spargers were investigated and used in the process of fumaric acid production by *Rhizopus* strains. In order to enhance the oxygen mass transfer, which is very important for organic acid fermentation, two kinds of porous spargers (10 μm and 100 μm) were employed. Gas holdup, liquid circulation velocity, mixing time, bubble size, bubble rise velocity, and axial distribution of bubbles in the riser and the downcomer were measured in a 50-L rectangular airlift loop reactor. Local volumetric mass-transfer coefficients (k_La) were also measured in the gas sparger zone with different spargers. The results indicate that the k_La is a strong function of both the superficial gas velocities and the spargers. High k_La values are obtained for the porous spargers. Fumaric acid can be produced by *Rhizopus* strains from the oxidative utilization of glucose, with calcium carbonate being added to neutralize the acid to prevent pH-inhibition effect. Due to the formation of calcium fumarate and the tendency of mycelial fungi to form mycelial pellets or clumps, the oxygen mass-transfer coefficient decreases and encourages ethanol instead of acid production. A 10-L laboratory airlift loop reactor with a porous sparger was employed for the fumaric acid fermentation. The results show that the turbulence of two-phase flow in airlift loop reactors not only produces favorable conditions for mass transfer but can also be useful for forming small well-distributed mycelial pellets and suspending them. The dissolved oxygen concentration during the fermentation is also measured to determine the optimum operating gas velocity. The results show that the airlift loop reactor has a higher capacity for producing fumaric acid than the stirred tank with a 6.5-L working volume.

Paper 21

FERMENTATION PROCESS FOR THE PRODUCTION OF SUCCINIC ACID

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A fermentation process is being developed for the production of succinic acid using the anaerobic organism *Anaerobiospirillum succiniciproducens*. In rich media with glucose as the main carbon source and yeast extract and peptone as the organic nitrogen sources, it was found that pH 6 was optimum for succinic acid production. At pH values below and above 6, the rates of succinic acid production were significantly decreased. When 1.5 *M* sodium carbonate was used to maintain the pH at 6, it was found that carbon dioxide sparging was not needed to maintain succinic acid production at the optimum rates. Both yeast extract and peptone could be replaced by corn steep liquor to render the fermentation media more suitable for commercial applications. In such media and at pH 6 and 39°C, 33 g/L succinic acid was produced after 25 h, which gave an average productivity of 1.3 g/(L·h). These results are discussed.

Paper 22

AGRIBUSINESS BY-PRODUCTS AS POTENTIAL FEEDSTOCKS FOR HIGHER-VALUE-ADDED PRODUCTS

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The continued expansion in agricultural processing represents new challenges and opportunities for U.S. agribusiness as it meets increasing world demands for food and feed. Improved process economics can be realized in a number of ways: for example, continuous improvements in overall process control via automation, reduction of waste streams from plant operations, and additional processing of by-products to higher-value-added commodities. Therefore, research and development in agribusiness plays a crucial role in maintaining and improving the competitiveness of this U.S. industry in the global economy.

The corn wet milling industry is a good example of an area where some of the above concepts apply. Overall, this industry is highly automated, with extensive waste stream recycling and good by-product recovery and utilization. Nevertheless, opportunities still exist for further economic gains by the conversion of corn processing by-products into higher-value-added commodities. The focus of this presentation is to describe the current ongoing research at Archer Daniels Midland Company to convert corn hull fiber to a number of products of possibly higher value or higher profit margin. Major process considerations are outlined and briefly discussed.

Paper 23

BIOMASS-TO-ETHANOL: PROGRAM UPDATE

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An up-to-the-minute status report of the progress made by SWAN Biomass Company in commercializing its biomass-to-ethanol technology is provided. In addition, some of the mistakes made, lessons learned, and successes achieved as the program proceeded are reviewed.

SWAN was formed in 1995 to commercialize the biomass-to-ethanol technology developed by Amoco Corporation, Purdue University, Iogen Corporation, National Renewable Energy Laboratory, and others. Although SWAN incorporates technology components from a variety of sources, the program under which the technology was developed, refined, and prepared for market was funded primarily by the U.S. Department of Energy, Amoco, and Stone & Webster Engineering Corporation. This collaboration provides an excellent model for government-industry collaboration in future projects that have high risks of development but that can benefit both society as a whole and industrial partners as individual entities if the initial hurdles to successful commercialization are overcome.

Paper 24

BIOTECHNOLOGY, ENERGY CONVERSION EFFICIENCY, AND THE PRODUCTION OF RENEWABLE FUELS AND CHEMICALS

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A key factor in the production of renewable fuels and chemicals utilizing biomass energy resources is the question of net thermodynamic conversion efficiency of solar energy into stored chemical energy. In the Chemical Technology Division at Oak Ridge National Laboratory, it has recently been discovered that certain unicellular green algae that are deficient in the Photosystem I reaction center are capable of performing complete photosynthesis. This discovery, reported in a recent article by the authors of this presentation [*Nature* **376**, 438–41 (1995)], has important implications for the analysis of energy conversion efficiency in biomass production.

The standard model of how photosynthesis works, the Z-scheme, is inextricably linked to the question of energy conversion efficiency and biomass production. This model employs two serial light reactions that work cooperatively in the conversion of light energy into chemical energy. A simple analysis of the Z-scheme model indicates that the maximum thermodynamic conversion efficiency of light energy into chemical energy is approximately 10%. However, our new discovery of photosynthesis in mutant B4 of *Chlamydomonas reinhardtii*, an alga that possesses only the Photosystem II light reaction center, suggests that conversion efficiencies of approximately 20% are, at least in principle, possible. This presentation focuses on a discussion of this new advance in photosynthesis research and how it relates to the question of biotechnology for the production of renewable fuels and chemicals.

Paper 25

PRINCIPLES FOR MASS CULTIVATION OF PHOTOAUTOTROPHS

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Mass cultivation of microalgae outdoors as part of an industrial enterprise focused on production of various natural products is based on efficient utilization of the abundant, high-intensity solar energy. The yields obtained in the "open raceway," the dominant reactor used by the microalgal industry throughout the world, are, however, very small compared with those that are theoretically achievable. This phenomenon reflects low photosynthetic efficiency that culminates in high production costs. We have developed a novel reactor that has a particularly narrow (1.3- to 2.6-cm) light path and a very effective mode of vigorous stirring. Unprecedented cell densities are readily supported by this reactor, resulting in very high daily output rates (60 g dry wt/m^2 or 2 g/L). The photic zone of the reactor is only 1 or 2 mm deep, but the turbulent flow induces a high frequency of light-dark cycles, ~50 m/s per cycle. A high and constant rate of light utilization is thereby facilitated, without the commonly observed decline in photosynthetic efficiency occurring as the light source becomes more intense. The principles to ensure high efficiencies in production of photoautotrophic mass have been elucidated as follows: a temperature-controlled enclosed reactor with a narrow light path and superhigh cell densities, which is extremely light limited and coupled with vigorous stirring to benefit from the flashing-light effect.

Paper 26

ENZYMATIC TRANSESTERIFICATION OF ORGANOPHOSPHORUS ESTERS IN ORGANIC SOLVENT WITH PHOSPHOTRIESTERASE

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Enzymes have great potential for use as catalysts in synthetic organic chemistry and occupy a key position because of the regiospecificity and stereospecificity that they generally allow. It is known that the phosphotriesterase from *Flavobacterium* sp. has a wide range of substrate specificity and that it is used to degrade various kinds of organophosphorus pesticide. From these characteristics, it is expected that one could apply the phosphotriesterase to synthesize the organophosphorus compounds as biologically active molecules. In this study, we demonstrated the novel transesterification and the optical resolution in organic solvent by use of the phosphotriesterase.

The transesterification of trialkyl phosphate to alcohol in various kinds of organic solvents was developed using the phosphotriesterase. The ^{31}P-NMR spectra were used for reaction analysis performed with diethyl *p*-nitrophenyl phosphate (paraoxon) and benzyl alcohol as model compounds. The reaction concluded after 48 h, and the formation of benzylester of phosphoric acid was confirmed. As another application of phosphotriesterase, the optical resolution of racemic alcohol, phosphoric acid, and phosphoric acid compounds was also performed with hydrolyzation by phosphotriesterase. The confirmation of optical yield was analyzed with capillary electrophoresis which contained cyclodextrin in the electrophoresis buffer.

Paper 27

ENZYMATIC CATALYSIS IN ORGANIC SOLVENTS: POLYETHYLENE GLYCOL–MODIFIED HYDROGENASE IS SOLUBLE IN TOLUENE AND RETAINS SULFUR-REDUCING ACTIVITY

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Naturally occurring enzymes may be modified by covalently attaching hydrophobic groups that render the enzyme soluble and active in organic solvents. Solvent-soluble enzymes have the potential to greatly expand applications of biocatalysis. We have utilized as model systems the redox protein ferredoxin and the enzyme hydrogenase, both purified from the hyperthermophile *Pyrococcus furiosus*, an organism that grows optimally at 100°C. The hydrogenase catalyzes the reversible activation of hydrogen gas and the reduction of elemental sulfur to hydrogen sulfide in aqueous solution. Neither protein is soluble in toluene. Both proteins were modified by polyethylene glycol *p*-nitrophenyl carbonate. The polyethylene glycol (PEG)–modified ferredoxin was not soluble in toluene and converted to the native form upon heat treatment, indicating that the PEG was noncovalently attached to the protein. On the other hand, the PEG-modified hydrogenase retained all of its hydrogen evolution and sulfur reduction activity in aqueous solution. Moreover, the pure modified enzyme was completely soluble in toluene at high concentrations (≥5 mg/mL). The enzyme also catalyzed hydrogen oxidation in toluene using the dye benzyl viologen as the electron donor. Neither benzyl viologen nor PEG *p*-nitrophenyl carbonate alone demonstrated hydrogen oxidation activity. The modified hydrogenase also catalyzed sulfide production from sulfur in toluene. The activity was tenfold higher than that produced in an aqueous system with equal enzyme activity, demonstrating the advantages of organic biocatalysis. Applications of bioprocessing in nonaqueous media are expected to provide significant advances in the areas of fossil fuels, renewable feedstocks, organic synthesis, and environmental control technology. The availability of high concentrations of modified hydrogenase in toluene permits a detailed study of its molecular, redox, and catalytic properties. The results of such analyses are discussed.

Paper 28

USE OF MICROBUBBLE DISPERSIONS IN SYNTHESIS GAS FERMENTATIONS

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In recent years, fermentations have been developed to convert synthesis gas, which consists primarily of carbon monoxide and hydrogen gases, into two- and four-carbon organic alcohols and acids. Synthesis gas is readily obtained from the gasification of coal, thereby allowing the use of abundant U.S. coal as the raw material. The microbial biocatalysts are orders of magnitude less sensitive to sulfur poisoning than conventional metallic catalysts, reducing the cost of sulfur removal. However, because the aqueous solubility of carbon monoxide and hydrogen gases is low, even less than that of oxygen, interphase mass transfer is the rate-limiting step in synthesis gas fermentations.

The use of microbubbles, surfactant-stabilized bubbles approximately 50 μm in diameter, to enhance gas-to-liquid mass transfer is being investigated. Experimental results will be analyzed with both steady-state and unsteady-state mathematical models, and correlations for the mass-transfer coefficient and interfacial area will be presented. The use of microbubbles in synthesis gas fermentations will also be presented.

Paper 29

BIODESULFURIZATION OF DIBENZOTHIOPHENE AND CRUDE OIL USING ELECTROSPRAY REACTORS

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Biological removal of organic sulfur from crude oil offers an attractive alternative to conventional thermochemical treatment due to the mild operating conditions afforded by the biocatalyst. In order for biodesulfurization to realize commercial success, reactors must be designed which allow for sufficient liquid-liquid and gas-liquid mass transfer while simultaneously reducing operating costs. To this end, we have investigated the use of electrospray reactors for the desulfurization of the model compound dibenzothiophene as well as actual crude oil. The electrospray reactor creates an emulsion of aqueous biocatalyst (5- to 20-μm-diameter droplets) in the organic phase by concentrating forces at the liquid-liquid interface rather than imparting energy to the bulk solution as is done in impeller-mixed reactors. The residence time of the biocatalyst emulsion within the reactor as well as further breakage and coalescence may be controlled by the application of additional electric fields within the apparatus. Desulfurization experiments in batch-stirred reactors are compared with those performed in electrospray reactors to demonstrate the advantages of this processing system. Electrospray reactors are expected to yield faster desulfurization, with lower energy and biomass requirements than conventional stirred-reactor systems.

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Paper 30

AMAZON: FAUNA, FLORA, AND POTENTIAL BIOPROCESSING APPLICATIONS

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The biodiversity and peculiarities of the fauna and flora of the world's largest rain forest are described. Examples of current foods, beverages, and phytopharmaceuticals such as the multiuse palm *Bactrys gasipaes* ("pupunha"), the caffeine-containing *Paullinia cupana* ("guarana"), and the ascorbic acid–rich *Myrciaria dubia* ("cacari") are discussed, along with less familiar sources such as the multivitamin-rich palm *Astrocaryum aculeatum* ("tucumã"), the olive oil–like coconut *Jessenia bataua* ("pataua"), and the fast-sprouting nut *Pachira aquatica* ("mamorana"). Seed endosperm, a by-product of *Schizolobium amazonicum* ("Cuiba pine") logging, affords 50% dry wt of a very viscous galactomannan resembling guargum (LQP-UFPR). When moved from its habitat to the Amazon, the costly timber provider *Dalbergia nigra* ("jacaranda-da-Bahia") reaches a 30-cm trunk diameter in only 8 years. When transferred from firm to flooded land, *Corchorus capsularis* (jute) experiences an increase in growth rate from 0.2 to 0.9 m/month. The native pseudococoa *Theobroma grandiflorum* ("cupuacu") seeds produce an alternative chocolate. A strain of *Picnoporus sanguineus* (orange mold) displays a 68% efficiency in the clarification of pulp/paper plant wastewater (UTAN-AM/UNICAMP-SP). Through natural or induced photochemistry, a *Chromobacterium violaceum* strain from the Negro river generates colored antibiotic derivatives besides violacein; one of them, once hydroxymethylated, resulted in 100% immobilization and growth inhibition of the Tulahuen strain of *Trypanosoma cruzi* (UNICAMP-SP).

A filamentous mold collection was assembled from decayed wood, and several species are being selected for novel amylolytic, pectolytic, lignocellulolytic, and hemicellulolytic enzymes. The related steps of hydrolysis/fermentation in the Indian alcoholic beverage "taruba" from cassava are being reproduced to elucidate the efficient *Rhizopus/Hansenula* consortium (ICB/FUAM).

Among the described 1400 species of fish, promising results are being obtained through the aquaculture of high-value fisheries such as *Arapaima gigas* ("pirarucu") and *Trichechus inunguis* (Amazon manatee). Surprisingly, other fish species can provide oil (liver) enriched in w-3 polyunsaturated fatty acids (EPA, DHA), previously found only in marine species.

Alternative strategies for the Amazon basin (INPA-AM and MPEG-PA) are also briefly reviewed.

[Funding provided by CAPES, CNPq/PADCT-SBIO (Project 82.0124/95.4)]

Paper 31

A BIOLOGICAL MULTIDISCIPLINARY MODEL TO PREDICT MUNICIPAL LANDFILL LIFE—SUCCESSFUL APPLICATION TO THE BELGIUM ANTON SITE

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The degradation processes within landfills are the key to understanding and controlling the environmental impacts. This degradation results from different microbial activities, which interact throughout the life of the landfill and are submitted to several abiotic factors.

Approaching these complex biological and chemical processes requires a multidisciplinary technique: we have developed, thanks to Walloon Region support, a pattern or model joining the evolutions of key biological parameters. Such parameters qualify biogas (composition, production rate); leachate (pH, BOD_5, COD, volatile fatty acids, redox potential, nitrogen balance, humic acids, etc.); or solid waste (moisture, settling, cellulose percentage, etc.).

This paper presents relationships, including those explained by our model. We first show the mathematic form of the model and apply it with success to the case of the Anton Solayn municipal landfill.

We then present results of our 1-ton laboratory-scale simulated landfill. Several abiotic factors affect microbial metabolism in the landfill. Some of them are chosen because they can facilitate a landfill management method. We show that waste moisture, well known as an enhancer of methanogenesis, has to be lowered during the acidogenesis phase to avoid a high-acidity inhibitor of methanogen flora growth.

Paper 32

INTRINSIC BIOREMEDIATION OF GAS CONDENSATE HYDROCARBON: RESULTS OF OVER 2 YEARS OF GROUNDWATER, SOIL GAS, AND SOIL CORE ANALYSIS AND MONITORING

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Condensate liquids have been found to contaminate soil and groundwater at two gas production sites in the Denver Basin operated by Amoco Production Company. These sites have been closely monitored since July of 1993 to determine whether intrinsic aerobic or anaerobic bioremediation of hydrocarbons occurs at a sufficient rate and to an adequate endpoint to support a no-intervention decision. Groundwater monitoring, soil gas analysis, and analysis of soil cores suggest that bioremediation is occurring at these sites by multiple pathways including aerobic oxidation, sulfate reduction, and methanogenesis. Results of over 2 years of monitoring of the chemistry and microbiology of these sites will be presented to support this conclusion.

Paper 33

PILOT-SCALE BIOREMEDIATION OF PAH-CONTAMINATED SOILS

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A pilot study was conducted in Charleston, South Carolina, to evaluate the slurry-phase bioremediation of Manufactured Gas Plant soils contaminated with polynuclear aromatic hydrocarbons (PAHs). The technologies tested were the Institute of Gas Technology's Manufactured Gas Plant–Remediation (MGP-REM) Process and conventional bioremediation.

The MGP-REM Process combines two interchangeable and cyclic steps, biological treatment and chemical treatment, that can be applied as needed depending on the nature and degree of contamination. The biological treatment exploits the ability of microorganisms to break down pollutants into less hazardous forms. The chemical treatment modifies the recalcitrant/carcinogenic PAHs to produce intermediates that are more susceptible to subsequent biodegradation.

Four test runs were conducted with 30% total solids slurry for approximately 4 weeks under appropriate biological and chemical treatment conditions. The study showed that the MG-REM Process obtained 90% reduction of total PAHs and 60% reduction of carcinogenic PAHs. The total PAHs were reduced from 1020 to 110 mg/kg, and the carcinogenic PAHs were reduced from 137 to 57 mg/kg. In contrast, the two test runs employing conventional bioremediation achieved only 60 and 37% removal of total PAHs, and no reduction of carcinogenic PAHs was observed.

Paper 34

ROLE OF MASS TRANSFER IN BIOREMEDIATION OF SOIL

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Although microorganisms have the potential to remove a variety of organic contaminants from soils, many of the contaminants of interest have very low solubility in water. This paper reviews our work on the chemical behavior and transport processes that limit the bioavailability of polynuclear aromatic hydrocarbons (PAHs). Soil is a multiphase system of mineral and organic solids, liquids, and gases, all of which can influence directly or indirectly the sorption or uptake of PAHs. PAHs are deposited onto soil from a carrier solvent, which determines their distribution in soil aggregates and subsequent availability. In actual contaminated soils, PAHs are deposited as part of a complex mixture of coal-derived tar components. The behavior of PAHs in this multicomponent mixture in soil differs significantly from pure compounds deposited in an equivalent pristine soil. These studies show that the physical-chemical interactions of contaminants with soil components are a crucial factor in determining the potential for bioremediation processes to achieve low residual concentrations.

Paper 35

DEVELOPMENT OF A MEMBRANE-BASED VAPOR-PHASE BIOREACTOR

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A vapor-phase bioreactor has been developed, utilizing porous metal membranes with a cylindrical design that employs radial flow as opposed to the traditional axial flow of the vapor. The system was evaluated for the biodegradation of *p*-xylene from an airstream by *Pseudomonas putida* ATCC 23973 immobilized onto sand. The biocatalyst was placed in the annular space between two cylindrical porous metal membranes. Details of the reactor system are presented, along with abiotic and biological experimental results verifying system performance.

Paper 36

ENHANCEMENT OF MINERALIZATION AND DEGRADATION OF PCB CONGENERS BY AN INTEGRATED BIOLOGICAL-CHEMICAL TREATMENT PROCESS

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Several bacterial cultures were isolated from PCB-contaminated soil using PCB, biphenyl, and chlorobenzoic acid as the carbon source. These isolates were identified as *Pseudomonas aurofaciens*, *Bacillus macerens*, *Bacillus cereus*, and *Arthobacter uratoxydens*. The performance of these new isolates was compared with that of the best-known aerobic cultures, namely, *Alcaligenes eutrophus* (H850) and *Pseudomonas* sp. (LB 400). Resting cells showed a better rate of mineralization of 2-chlorobiphenyl and 2,2',4,4'-tetrachlorobiphenyl than did the growing cultures for new isolates. The mineralization of PCBs by resting cells was also shown to be greater than that of growing cells, even with yeast extract supplementation. Chemical treatment was started by using H_2O_2(4 v/v %) and $FeSO_4$ (10 m*M*). The results obtained showed >90% mineralization by the integrated approach for 2-chlorobiphenyl and >65% for 2,2',4,4'-tetrachlorobiphenyl.

Paper 37

OBSERVATIONS OF METABOLITE FORMATION AND VARIABLE YIELD IN THIODIGLYCOL BIODEGRADATION PROCESSES: IMPACT ON REACTOR DESIGN

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The complete microbial degradation of thiodiglycol (TDG), the primary hydrolysis product of sulfur mustard, by *Alcaligenes xylosoxydans* ssp. *xylosoxidans* (SH91) was accomplished in laboratory stirred-tank reactors. An Andrews-type substrate-inhibition model was used to describe cell growth and TDG consumption at low initial TDG concentration. At high initial TDG concentration, we observed significant changes in by-product formation, presumably owing to alterations in metabolic flux due to capacity constraints in the TDG-utilization pathways. A variable-yield function has improved model predictability. A hypothesis for explaining variable yield and by-product formation will be presented. A novel repeated semibatch operating mode was then developed, based on the by-product observations. This operating mode circumvents the substrate-inhibition problem so that 500 m*M* TDG feed (~60 g/L) is degraded within 4 to 5 days.

Abstracts for Poster Sessions

Poster 38

DEACTIVATING EFFECTS OF FERMENTATION RESIDUES ON THE CATALYTIC UPGRADING OF LACTIC ACID

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Lactic acid is becoming the focus of increased study because of its promising future as an inexpensive, renewable resource. Early studies with lactic acid in our laboratory led to the discovery of a condensation pathway to 2,3-pentanedione; a 60% theoretical yield with 80% selectivity toward 2,3-pentanedione was obtained upon optimization using CsOH on silica as a catalyst with highly refined lactic acid feed. Economic considerations of implementing this technology demand the use of less refined lactic acid feedstocks. Initial reaction studies with crude lactic acid fermentation broth in the form of ammonium lactate showed no activity toward the formation of 2,3-pentanedione. Similar results were also observed when an equimolar solution of NH_3OH and refined lactic acid was used as feed. The extent of deactivation by ammonia is studied through reaction experiments using refined lactic acid feeds containing different concentrations of ammonium hydroxide: 15 mol % of ammonium lactate is found to be sufficient to cause a permanent 50% decline in the theoretical yield of 2,3-pentanedione. Initial results suggest that deactivation occurs after the catalyst is subjected to a molar quantity of ammonium lactate equal to that of the alkali metal present.

Poster 39

THE PEEL OF LIME *(Citrus aurantifolia)* AS A SOURCE OF ACETYLESTERASE

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The peel of lime, *Citrus aurantifolia*, was examined for the presence of acetylesterase activity. Preliminary experiments indicate that the lime peel contains higher acetylesterase activity per milligram total protein than navel orange peel. The preparation of extract from lime peel is facilitated by the nature of the peel, which does not have as thick an albedo as oranges, grapefruit, or lemons. When orange and lime peel extracts were applied to a Superdex 75 gel filtration column, the Superdex acetylesterase pool from lime peel had a specific activity twice that of the comparable acetylesterase-containing pool from navel orange peel for the substrate B-D-glucose pentaacetate. The optimal temperature and pH for acetylesterase activity on the substrates B-D-glucose pentaacetate and triacetin were compared for the citrus acetylesterases. Optimal conditions for acetylesterase activity were found to differ for the two substrates.

Poster 40

SOLID-STATE FERMENTATION OF SORGHUM TO ETHANOL: YEAR TWO

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A low-cost method of producing ethanol from sorghum has been developed using a modified ensiling method. After a 28-day fermentation period, pressed red juice of the fermented sorghum contained 5.2 w/v % ethanol. Percent fermentable carbohydrate (°Brix) for freshly harvested sorghum was 15.6, for an ethanol yield of 0.33.

Temperature changes were monitored in a pilot-scale silo containing 450 kg sorghum, Montrachet wine yeast, and formic acid. The temperature increased during the initial 7 days to a maximum of 27°C in the silo center. A gradual decline was then seen in the temperature over the next 21 days to 13.6°C. Providing anaerobic conditions are maintained, this ethanolic silage should remain stable for processing over a long period of time.

Processing methods are discussed. Estimates of heat buildup in large-scale silos are projected from these temperature studies.

Poster 41

PYROLYSIS OF STEAM-CLASSIFIED MUNICIPAL SOLID WASTE

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Steam-classified municipal solid waste (MSW) has been studied for use as a combustion fuel and feedstock for composting and for cellulytic enzyme hydrolysis. A preliminary study has been conducted using a prototype plasma-arc pyrolysis system to convert the steam-classified MSW into a pyrolysis gas and vitrified material. Using a feed rate of 50 lb/h, 300 lb of the material was pyrolyzed. The major components of this pyrolysis gas were H_2, CO, and CH_4. A detailed presentation of the emission data along with information about the system used will be included.

[Work conducted in cooperation with Plasma Energy Applied Technology, Inc., Huntsville, Alabama]

Poster 42

BLEACHED CELLULOSE FROM SUGARCANE BAGASSE FOR CHEMICAL PROCESSING

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With a constantly increasing use of the purest cellulose for several industrial applications, it will be necessary to rely on raw materials other than wood and cotton linters to maintain the balance between supply and demand. Cellulose is produced most inexpensively from agroindustrial residues by using well-established technologies. In this work, sugarcane bagasse was fractionated into cellulose, hemicellulose, and lignin by a steam explosion treatment. The cellulose fraction was then submitted to a short bleaching sequence (PEH–hydrogen peroxide, alkaline extraction, and hypochlorite) to remove residual levels of lignin. The chemical and physical analysis revealed that the product has high α-cellulose contents (91.4%); alkali solubility values of 92.35% (R10), 6.59% (S18), and 3.68% (S10-18); ash contents of 0.4%; ISO brightness of 75.4%; and low residual lignin contents (2.03%). Therefore, the product proves to be chemically reactive enough to make an ideal starting material for all cellulose derivatives and modifications, particularly where demands are made on purity. Thus, it can be considered as a potential alternative to supplement cellulose supplies produced by wood and linters for use in chemical industries.

Poster 43

THE ROLE OF PURE SUPPLEMENTARY ENZYMES ON CELLULOSE HYDROLYSIS

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Hydrolysis of lignocellulosic materials to monomeric sugars can be achieved by acid or enzymatic hydrolysis. The main drawback of using enzymes in the total hydrolysis of cellulosic substrates to monosaccharides is the cost.

The efficiency of the enzyme preparation critically depends on the type and the chemical composition of the substrate. Cellulose is highly crystalline, which makes it difficult to degrade. In addition to cellulose, plant materials contain hemicellulose, xylans, and mananas. The components of the substrate (i.e., cellulose and hemicellulose) may be physically and/or chemically interlinked. Therefore, several enzymes active on cellulose as well as on hemicellulose are needed. The amount of limiting enzyme can today be easily elevated by gene technology.

Trichoderma reesei is one of the most efficient producers of cellulases and hemicellulases. At least two different cellobiohydrolases (CBH I and CBH II), four different endoglucanases (EG I, EG II, EG III, and EG IV), and one β-glucosidase are produced by most strains.

Most commercial enzyme preparations available for total hydrolysis are produced by *T. reesei.* These mixtures may, however, not have optimal ratios of different enzymes for most efficient hydrolysis.

In this study enzymes limiting the hydrolysis were identified and enzyme mixtures optimized by enrichment of commercial preparations with purified enzymes. The role and importance of CBH I and β-glucosidase in the total hydrolysis of microcrystalline cellulose were evaluated.

Poster 44

COST ESTIMATES AND SENSITIVITY ANALYSES FOR THE AMMONIA FIBER EXPLOSION (AFEX) PROCESS

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The ammonia fiber explosion (AFEX) process is a promising new pretreatment procedure for enhancing the reactivity of lignocellulosic materials. AFEX has many advantages over existing processes. The laboratory phase of AFEX development is nearing completion, but the process is not yet commercially developed. This study was undertaken in an effort to support and assist AFEX commercialization through process cost modeling.

The paper reports on a computer simulation program which was developed especially for the AFEX process. Through the energy and mass balances, process designs have been conducted for each projected unit operation of the AFEX process. Cost estimates were then obtained, including Fixed Capital Investment, Operating Costs, and Return on Investment. The computer process simulation package is able to model different possible processing scenarios. The effects of selected scenarios on the projected costs of a large-scale AFEX processing plant will be described. A sensitivity analysis has also been performed, which shows the effects of key system variables on the overall processing costs.

Poster 45

SOLVENT-PHASE THERMAL CRACKING OF LIGNIN FOR PRODUCTION OF OXYGENATES AND OTHER POTENTIAL LIQUID FUELS

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The amendment of the Clean Air Act in 1990, which mandated addition of oxygenates to gasoline in certain areas of the United States, has brought about a keen interest in production of oxygenates from renewable resources. In this study, we have explored the concept of converting lignin into fuel materials, including oxygenates. Lignin is the least-utilized biomass component. Unlike those from the pulping process, the lignins generated from the biomass conversion process are relatively clean, free of sulfur or sodium. It is a feedstock very much amenable to further conversion. The primary goal of this research is to broaden the existing data base pertaining to this concept. We have limited the conversion methodology to noncatalytic thermal cracking in the solvent phase without supplementation of hydrogen or hydrogen donor solvent. Two different solvents were employed: acetone and butanol. Although these solvents are miscible with water, they were used on a water-free basis. The cracking experiments were done in externally agitated tubing bomb reactors placed inside a sand bath. The reaction temperatures ranged from 250 to 450°C. The overall conversion was determined by the weight of the residual solid. The liquid products were analyzed by GC/MS. The overall conversion generally increased with reaction temperature up to 400°C and leveled off thereafter. The highest conversions observed were in the vicinity of 60% for both acetone and butanol cracking. In the case of cracking in the acetone phase, hydrocarbons as well as oxygenates were found. The identified primary products included branched alkenes (dimethyhexene), aromatics (trimethyl benzene), side-chain oxychemicals (methylpentanone, tri-methyl-cyclohexanone), ketones (methyl-isobutyl ketone), and phenolics (methyl, ethylphenol). The products from butanol phase cracking included esters (methyl-propyl, butyl esters), ethers (*N*-butyl, isobutyl ethers), and phenolics (methoxy, di-butoxy phenols). The liquid side product composition at various reaction conditions, the boiling point distribution, and the overall miscibility with hexane, gasoline, and diesel are among the main items reported in this paper.

Poster 46

PREPROCESSED BARLEY, RYE, AND TRITICALE AS A FEEDSTOCK FOR AN INTEGRATED FUEL ETHANOL–FEEDLOT PLANT

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The fuel ethanol industry in western Canada operates mainly on wheat feedstock. Rising wheat prices, which today are 50% higher than a year ago, have forced the industry to look into other cereal grains. Because other cereals are deficient in starch content as compared with wheat, increased processing cost is accompanied by reduced ethanol yield.

Preprocessing of grain by abrasion on a Sataki dehuller, to remove bran in rye and triticale or hulls and bran in barley, was evaluated as a mean of increasing the starch contents in feedstock for fermentation. The influence of grain preprocessing on ethanol production and energy consumption was evaluated.

Removal of 7 to 19% of dry matter from cereal grains resulted in increased concentrations of starch from 58–64% to 68–70% in preprocessed material. Plant throughput was increased by 8–20%, depending on grain. Preprocessing of grain decreased gas consumption by 3–11% and energy requirements by 4% per 1 L of ethanol.

Poster 47

BIODIESEL–FUEL OIL BLENDS AS AN ALTERNATIVE HEATING OIL

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Among the new energy sources, biomass is particularly attractive compared with fossil resources since it has the advantage of being renewable. In case of an emergency fuel shortage, a dependable and readily available source of liquid fuel is essential to keep all energy-intensive operations on track. Vegetable oils, one of the most important biomass sources, seem to be a convenient solution since they can be used as a fuel oil or a diesel fuel alternative provided that some modifications are performed on their structure. More than 350 oil-bearing crops have been identified, and because of their origin, these crops are environmentally friendly. In this field one of the popular energy plants is rapeseed. Rapeseed oil methyl ester (biodiesel) can be used in diesel engines and heating systems as diesel fuel and fuel oil. The aim of this study was to evaluate the possibilities of biodiesel and its fuel oil blends as an alternative to heating oil. Alternative fuel blends were prepared by adding 25, 50, and 75 vol % biodiesel to commercial fuel oil. The fuel properties of biodiesel, blend fuels, and reference fuel oil supplied by TÜPRAS Petroleum Refinery (Izmit, Turkey) were determined according to the ASTM standard methods for fuel oil. An overall evaluation of the experimental data indicates that biodiesel and its blends can be proposed as a possible substitute for fuel oil.

Poster 48

BIODIESEL REFINEMENT THROUGH WASHING WITH HOT WATER

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The ester product of the vegetable oil–alcohol transesterification reaction (biodiesel) is the best and most popular diesel fuel alternative. The refinement of the transesterification reaction product is technically difficult and brings an extra cost to the total expense of the whole process. Also, the purity level of the biodiesel must conform to the standards. The aim of this study was to investigate the refinement step of the esterification product mixture (by washing with hot distilled water) obtained by the rapeseed oil–methanol transesterification reaction (vegetable oil/alcohol molar ratio: 1:6; catalyst: NaOH, 1.6% by the weight of the oil; temperature: 65 ± 1°C). The method was applied at three different temperatures (50, 65, and 80°C). A general evaluation of the process indicates that best results were those resulting from the washing the mixture at 50°C, where the refinement yield (percent), water content (weight percent), and acid number (milligrams of KOH per gram) were found to be 84.2, 0.07, and 0.47, respectively.

Poster 49

SUGARS FROM BIOMASS AS A RAW MATERIAL FOR RENEWABLE CHEMICALS

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The availability of inexpensive sugars as raw material continues to be a critical factor in the cost-effective production of renewable chemicals. The pretreatment of biomass consisting of chemical and enzymatic hydrolysis can produce hexose and pentose sugars at high yields. However, these sugars are often present in a complex mixture with acids, phenolics, and other hydrolysis by-products. These by-products can severely inhibit downstream fermentation and increase purification costs of the final product. A separation process has been investigated that can not only produce sugars as a "clean" fermentable stream but also potentially make the by-products available as coproducts. The process is based on ion-exclusion principles and offers ease of scaleup for commercial production of sugars from biomass. Experimental results of separation of sugars from sawdust hydrolyzates in bench-scale batch runs and pilot-scale continuous runs are presented. Economic analyses are discussed to identify future research directions that meet cost targets. The availability of such a process that produces fermentable sugars from biomass can have a significant impact on the future of the renewable chemical industry.

Poster 50

EFFECT OF HYDROTHERMAL TREATMENT OF WOOD ON CELLULOSE HYDROLYSIS

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This research examines pretreatment of lignocellulosic material using water at a controlled temperature and pH. A small of KOH (relative to the total reaction volume) is added in order to control the pH. The reactor in which the lignocellulosic slurry was treated is a 2-L Parr 304 stainless steel reactor with three turbine-propeller agitators and a PID temperature controller. The controller is accurate to ±1.0°C. A serpentine cooling coil, through which water is circulated, was located inside the reactor to provide the needed temperature control. A bottom port, as well as two inlet ports, is built into the reactor to allow sampling and/or collection of the pretreated material as needed. This poster describes the effects of pH-controlled cooking of wood sawdust at temperatures ranging from 200 to 240°C. The pretreatments were effective. Enzyme hydrolysis and simultaneous saccharification and fermentation showed dramatic improvements in cellulose hydrolysis and utilization relative to untreated materials. These results are discussed and presented in the context of pretreatment research on aqueous and steam methods by Bobletter, Haw et al., Brownell and Saddler, and Mok and Antal. Factors that impact the use of water as a pretreatment medium are also discussed.

Poster 51

ENVIRONMENTALLY SAFE BIOTECHNOLOGICAL UTILIZATION OF PLANT BIOMASS FROM THE AREAS POLLUTED BY HEAVY METALS AND RADIONUCLIDES

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Although bioconversion of plant renewables for transportation fuels or chemical feedstock is considered one of the most attractive alternatives for a petroleum-oriented society, the production costs of biofuels still remain too high to compete with fossil fuels.

Evaluations, however, show the process to be quite feasible for the utilization of hazardous wastes or biomass resources having negative value [M. L. Rabinovich et al., "Complex Bioconversion of Secondary Biomass Resources," pp. 1090–95 in *Biomass for Energy and Industry*, ed. D. O. Hall et al., Herndon, Va., Ponte Press, 1994; L. M. Norenko et al., "Cyclic Utilization of Lignocellulosics," pp. 1337–42 in *Biomass for Energy and Industry, Agriculture and Environment, vol. 2*, ed. Ph. Chartier et al., Pergamon, Tarrytown, N.Y., 1995]. One of the techniques proposed for this particular case includes partial enzymatic hydrolysis of pretreated waste, separation of sugar syrup from lignaceous spent solids, further consumption of solids by a fungal enzyme producer to obtain both hydrolytic enzymes and a high-absorption-capacity natural byproduct consisting of highly dispersed plant lignin and fungal cell walls enriched by chitin, treatment of sugar syrup by the absorbent so produced, and subsequent fermentation to yield ethanol or other organic feedstock separated by distillation. This process provides efficient separation of biofuels from contaminated trace pollutants, which are, in turn, accumulated as "heavy fractions" of the "biocracking" process.

Poster 52

OPTIMIZATION OF SEED PRODUCTION FOR A SIMULTANEOUS SACCHARIFICATION COFERMENTATION BIOMASS-TO-ETHANOL PROCESS USING RECOMBINANT *Zymomonas*

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The efficient utilization of nonglucose sugars represents an opportunity to significantly reduce the cost of producing fuel from biomass and wastes. The five-carbon sugar D-xylose is a major component of hemicellulose, which represents about one-third of the carbohydrate content of lignocellulosic biomass. Numerous potential industrial ethanol fermentation biocatalysts have been surveyed by the National Renewable Energy Laboratory (NREL) in a comprehensive study that compared known metabolic characteristics with a weighted list of fermentation performance criteria, including yield, ethanol tolerance, specific productivity, GRAS status, and sensitivity to inhibitory compounds typically present in biomass hydrolyzates [S. K. Picataggio, M. Zhang, and M. Finkelstein, *ACS Symp. Ser.* **566**, 342–62 (1994)]. Using a nutrient-supplemented, dilute-acid hardwood prehydrolyzate as a screening medium, several strains of *Zymomonas* were selected as targets for improvement by metabolic engineering that was specifically directed at creating recombinants with the ability to conferment glucose and xylose [M. Zhang et al., *Appl. Biochem. Biotechnol.* **51/51**, 527–36 (1995); M. Zhang et al., *Science* **267**, 240–43 (1995)].

At this meeting last year, NREL scientists described a simultaneous saccharification cofermentation (SSCF) process for ethanol production from cellulose and xylose by one of their genetically engineered *Zymomonas* cultures [S. K. Picataggio et al., *Appl. Biochem. Biotechnol.* (Proc. 17th Symp.), in press]. In extending this work, our objective was to establish optimal conditions for the cost-effective production of seed culture compatible with the SSCF process design. For this purpose we employed a factorial design series of pH-controlled standard thermal reactor batch fermentations to examine the interactive effects of several operational variables on cell density—the medium consisted of a synthetic hardwood hemicellulose hydrolyzate with a xylose-to-glucose mass ratio of 5:1. The variables that were tested included (1) pH, (2) acetic acid concentration, (3) sugar loading, and (4) level of nutrient supplementation using corn steep liquor. The experimental design permitted a statistical analysis with respect to the significance of the level of each variable selected for optimization in this unit operation of the overall SSCF process.

[Research conducted under the terms of a subcontract (AAP-4-11195-03) between the National Renewable Energy Laboratory and the University of Toronto]

Poster 53

CORN STEEP LIQUOR AS A COST-EFFECTIVE NUTRITION ADJUNCT IN HIGH-PERFORMANCE *Zymomonas* ETHANOL FERMENTATIONS

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The ethanologenic bacterium *Zymomonas* has been demonstrated to possess fermentation performance characteristics that are superior to those of yeast [H. G. Lawford, *Appl. Biochem. Biotechnol.* **17**, 203–11 (1988); M. Beavan et al., *Appl. Biochem. Biotechnol.* **20/21**, 319–26 (1989)]. In a recent National Renewable Energy Laboratory survey of several microorganisms, *Zymomonas* was selected as the most promising host for improvement by genetic engineering directed to pentose metabolism [S. K. Picataggio, M. Zhang, and M. Finkelstein, *ACS Symp. Ser.* **566**, 342–62 (1994); M. Zhang et al., *Appl. Biochem. Biotechnol.* **51/52**, 527–36 (1995)]. Minimization of costs associated with nutritional supplements and seed production is essential for economic large-scale production of fuel ethanol [L. R. Lynd, R. T. Elander, and C. E. Wyman, *Appl. Biochem. Biotechnol.* (Proc. 17th Symp.), in press]. Previous work conducted in our laboratory showed that light and heavy steep waters from corn wet milling were effective nutritional supplements for *Zymomonas* fermentation [M. M. Newman and K. L. Kadam, *Appl. Biochem. Biotechnol.* (Proc. 17th Symp.), in press].

The purpose of this study was to determine the minimum amount of CSL that was compatible with high-performance fermentation by *Zymomonas*. Different wild-type strains of *Z. mobilis* were used, and fermentations were conducted in pH-controlled bioreactors in batch and continuous modes with CSL as the sole nutritional source. The results were interpreted in terms of the economic impact of using CSL relative to alternative nutritional resources. The results provide useful information for the future development of a biomass-to-ethanol process that would use *Zymomonas* as the biocatalyst.

Poster 54

A NEW PHOTOSYNTHETIC PATHWAY

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The discovery of a new photosynthetic pathway, driven exclusively by the Photosystem II (PSII) light reaction, is reported. Experiments performed with specific inhibitors of electron transport and mutants of *Chlamydomonas reinhardtii* indicate that the flow of photogenerated electrons proceeds from PSII to ferredoxin/$NADP^+$ reduction through the plastoquinone pool and cytochrome b/f complex. An analysis of the energetics of PSII-only photosynthesis reveals that the maximum thermodynamic conversion efficiency of light energy into chemical energy can, in principle, be approximately twice that of conventional Z-scheme photosynthesis.

Poster 55

PARTIAL BIOOXIDATION OF METHANE TO METHANOL BY METHANE-ASSIMILATING BACTERIA

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Methane-assimilating bacteria *Methylococcus capsulatus* performed partial biooxidation of methane to methanol. Methane monooxygenase and methanol oxidase enzymes involved in the oxidative biotransformation were investigated in order to optimize the conditions for methanol generation. The biocatalytic activity of bacterial cells depended on the presence of enzyme cofactors and inhibitors in the reaction medium. Formate (sodium salt) in concentrations of more than 10 m*M* was found to be the most efficient cofactor for methane monooxygenase of *Methylococcus capsulatus*. Ethylenediaminetetraacetic acid (EDTA) in concentrations of ~20 m*M* inhibited further bioconversion of produced methanol. The high reaction rate of methanol accumulation (up to 200 $nmol \cdot min^{-1} \cdot mg^{-1}$ of dry cells) was observed under the optimal conditions in the presence of cofactor (formate) and inhibitor (EDTA).

Immobilization of methane-utilizing bacteria was carried out by adsorption on honeycomb monoliths composed of alumina, titanium dioxide, ceramics, and carbon. The adsorption ability, biocatalytic activity, and stability of immobilized bacteria were studied. The titanium dioxide–based monolith was found to be the best adsorbent for bacterial cells, and immobilized microorganisms retained more than 50% of the activity of suspended cells.

Poster 56

PRODUCTION OF L(+)-LACTIC ACID FROM MSW HYDROLYZATE BY IMMOBILIZED *Lactobacillus pentosus*

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In recent years there has been an increased interest in the biotechnological production of L(+)-lactic acid for manufacture of environmentally benign polylactic acid polymers (PLP). These polymers are 100% biodegradable and have been approved for use by the U.S. Food and Drug Administration.

Eight *Lactobacillus* strains have been screened for potential industrial production of L(+)-lactic acid from municipal solid waste (MSW) hydrolyzate. Of these, *L. pentosus* B-227 showed the most promise for production of L(+)-lactic acid. Under static batch fermentation conditions, 57.4 mg/mL of lactic acid was produced from MSW hydrolyzate (82.8 mg/mL fermentable carbohydrate). Ninety-one percent of the acid produced was L(+)-lactic acid, which is the preferred isomer for production of PLP. Further experiments are being explored for continuous production of L(+)-lactic acid by immobilization of *L. pentosus* B-227 culture in calcium alginate. Fermentation parameters with immobilized culture to maximize L(+)-lactic acid production from MSW hydrolyzate will be presented.

Poster 57

A STABLE LIPASE FROM *Candida lipolytica*: CULTIVATION CONDITIONS AND CRUDE ENZYME CHARACTERISTICS

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Physiological and biochemical aspects of lipase production by a selected strain of *Candida lipolytica* are discussed. Concerning media composition, it was observed that peptone was the best nitrogen source and that oleic acid (OA) or triglycerides (TAG), like olive oil, were essential carbon sources. Repression by glucose and stimulation by OA or TAG were observed. Experiments carried out with the best medium showed a fairly constant level of intracellular lipase activity during cultivation. Extracellular lipase activity was significant only at the early stationary growth phase, when the main carbon source (olive oil) was practically exhausted. Several olive oil concentrations were tested, and the variation of substrate content was monitored during cultivation. Variations in extracellular lipase activity during fermentation, measured by the three currently used techniques (spectrophotometric, titrimetric, and agar plate diffusion methods), showed similar profiles. Crude lipase produced showed the following optimal conditions: pH of 7.0 to 8.5 and temperature of 55°C. The enzyme was very stable at 5°C for at least 366 days without any additives. Activation and stabilization of the lipase by polysaccharides were also investigated and showed promising results.

Poster 58

TAILORING OF GLUCOAMYLASE ISOENZYMES THROUGH MEDIUM COMPOSITION

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Two major glucoamylase isoenzymes (GAI and GAII) have been identified in culture supernatants of *Aspergillus awamori*. It has been suggested that stepwise degradation of a native enzyme in the course of fungal cultivation by proteases and/or glucosidases may result in the formation of isoenzymes which have different characteristics concerning substrate specificity and stability to pH and temperature. In this study, the glucoamylase produced by *Aspergillus awamori* using liquid media with C/N 10 [2.0% starch, 0.45% $(NH_4)_2SO_4$] and C/N 26 [5.2% starch, 0.45% $(NH_4)_2SO_4$] was analyzed. In both cases GAI and GAII were characterized in terms of hydrolytic activities, molecular weight, and isoeletric point (pI). Through the use of HPLC gel filtration and FPLC chromatofocusing, a molecular weight of 110 kDa and a pI of 3.45 and a molecular weight of 86 kDa and a pI of 3.65 were obtained for GAI and GAII respectively. A different isoenzyme proportion was observed through the use of two C/N ratios. In the lower carbohydrate content fermentation (C/N 10), the GAI form predominates (85%), whereas in the C/N 26 medium, 80% GAII was observed. Isoenzymes from C/N 10 showed equivalent activities towards maltose (1.9 U/mg protein) and starch (16.0 U/mg protein). A different pattern was, however, observed for C/N 26 glucoamylases. The activities of the isoenzymes towards maltose and starch, respectively, were 4.18 and 23.6 U/mg for GAI and 8.14 and 64.0 U/mg for GAII. Gel electrophoresis, amino acid analysis, and structural data confirm that both preparations are glucoamylases with a high degree of homogeneity.

Poster 59

EXPERIMENTAL DATA ANALYSIS: AN ALGORITHM FOR SMOOTHING OF DATA AND DETERMINING ENZYME AND MICROBIAL KINETIC RATES

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Reaction rate determination from experimental data is generally an essential part of evaluating enzyme and microbial kinetics or factors influencing kinetic rates. Commonly used methods include simple forward, centered, or backward finite-divided-difference equations using two or more data points. A geometrical approach was presented by LeDuy and Zajic [*Biotechnol. Bioeng.* **15**, 805 (1973)] and involves the construction of a circle with three neighboring points on the perimeter. The center coordinates are calculated, and a straight-line equation between the center coordinates and the second (middle) point is constructed; the normal to this equation will then yield the "derivative" at the second point.

The advantage in using the method described in this paper is that experimental error may be largely accounted for by incorporation of a smoothing step for the experimental data. The proposed method is built on the construction of a "best" curve through the data points; the curve is then the basis for determining derivatives (dX/dt). A typical manual method and the suggested numerical method are shown below:

Manual Method
1. Plot experimental data (X as a function of t).
2. Draw a smooth curve through or close to the points.
3. Collect readings from the curve and find derivatives at each of the original values of t.

Numerical Method
1. Construct cubic spline with a "smoothness factor."
2. Use spline functions to find derivatives at original t values.

The construction of cubic splines is uncomplicated with today's personal computers and readily available programming languages. This paper presents the development of an algorithm and computer code for smoothing experimental data and derivative determination.

Poster 60

REGULATION OF PHOSPHOTRANSFERASE IN GLUCOSE AND XYLOSE FERMENTING YEASTS

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In *Saccharomyces cerevisiae*, glycolytic enzymes are induced by glucose. Hexokinase (HK) mediates carbon catabolite repression. Much less is known about the regulation of glycolysis in xylose fermenting yeasts. This research examined the titers of HK and other phosphotransferases in *S. cerevisiae* and two xylose fermenting yeasts, *Pachysolen tannophilus* and *Candida shehatae*, as a function of the carbon source and aeration. HK, D-xylulokinase (XUK), and 6-phosphofructokinase (PFK) varied with aeration and starvation, the yeast species, and the carbon source.

Glucose-grown *S. cerevisiae*, glucose-grown *P. tannophilus*, and xylose-grown *C. shehatae* had the highest specific activities of PFK, HK, and XUK, respectively. The PFK activities in *S. cerevisiae* were 1.5 to 2 times higher than in the other two yeasts. HK activities in *P. tannophilus* and *C. shehatae* were twofold to threefold higher when cells were grown on glucose than on xylose, but induction was not observed during short-term aerated or anaerobic incubations. Transfer from glucose to xylose rapidly inactivated HK in *P. tannophilus*. Xylose induced XUK activity in both *P. tannophilus* and *C. shehatae*, and glucose repressed and inactivated XUK in *C. shehatae*. This indicated that the basic regulatory mechanisms differ in the two xylose fermenting yeasts. This is the first report of phosphotransferase regulation in xylose fermenting yeasts.

Poster 61

FREE STEROLS SYNTHESIZED BY *IN VITRO* CULTURES OF FLAX

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Seedlings of flax (*Linum usitatissimum*) were established and calli induced from hypocotyl explants on MS medium supplemented with phytohormones. The effects of auxins and citokinins on the production of biomass and sterols were determined. Campesterol, stigmasterol, and β-sitosterol were the main sterols accumulated in *in vitro* growing seedlings and calli as well as in seeds. No significant differences on composition and production of sterols were found between nonmorphogenetic calli and organogenic or embryogenic calli when the MS medium was supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and zeatin (ZEA). Nonorganogenic flax calli maintained with these phytohormones accumulated contents of sterols significantly higher than those developed on MS medium supplemented with indole-3-butyric acid (IBA) and kinetin (KIN). The specific sterols content, namely that of β-sitosterol, varied along with the biomass growth cycle of flax calli developed either with 2,4-D and ZEA or with IBA and ZEA. In both cases, the highest sterol contents were found at the end of the exponential phase. As these sterols are used as precursors of therapeutic steroids, *in vitro* cultures of plant species like this can be envisaged as a way to obtain, in a controlled way, the raw chemicals used in the steroid industry.

Poster 62

ENHANCED FERMENTATION OF SUGAR MIXTURES BY *Pichia stipitis* MUTANT FPL-061

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We studied the effects on *Pichia stipitis* fermentations of xylose, glucose, and arabinose mixtures; oxygen levels; and nutrients. The fermentation rate of the mutant strain *P. stipitis* FPL-061 was not significantly different from that of the parental strain *P. stipitis* CBS 6054 during fermentation of pure glucose or xylose. But during fermentation of a mixture of glucose and xylose, the mutant strain FPL-061 produced more ethanol. FPL-061 attained a maximum ethanol concentration of 29.4 $g \cdot L^{-1}$ with a yield of 0.42 $g \cdot g^{-1}$ sugar consumed. By comparison, under the same conditions CBS 6054 produced 25.7 $g \cdot L^{-1}$ of ethanol with a yield of 0.35 $g \cdot g^{-1}$ sugar consumed. Both the parental and mutant strains fermented xylose rapidly in the presence of L-arabinose; however, neither strain fermented L-arabinose. During fermentation of mixed sugars, FPL-061 consumed glucose more rapidly than xylose and produced more ethanol at 8 and 12% sugar concentrations, whereas at 16 and 20% mixed sugars, glucose was consumed slowly by FPL-061, and xylose was mostly unutilized. During xylose fermentation, both the parent and mutant strains exhibited high cell growth and rapid rates of ethanol respiration at high oxygen levels. In contrast, at low oxygen levels, the ethanol formation rate was slow, and xylitol accumulation was observed in both the strains. The ethanol production rate by FPL-061 was higher than its parental strain at an aeration rate of 5.4 mol $O_2 \cdot L^{-1} \cdot h^{-1}$ during xylose fermentation. Doubling the amount of yeast nitrogen base, urea, and peptone did not improve ethanol production during fermentation of mixed sugars by FPL-061. Addition of corn steep liquor to the mixed sugar fermentation by FPL-061 increased glucose consumption rate but reduced xylose utilization and lowered ethanol production.

Poster 63

PRODUCTION OF XYLITOL FROM D-XYLOSE BY *Debaryomyces hansenii*

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Xylitol, a naturally occurring five-carbon sugar alcohol, can be produced from D-xylose through microbial hydrogenation. Xylitol has found increasing use in the food industries, especially in confectionery. In this study, we examined the effect of cell density on the xylitol production by the yeast *Debaryomyces hansenii* NRRL Y-7426 from D-xylose under microaerobic conditions. The rate of xylitol production increased with increasing yeast cell density to 3 g/L. Beyond this amount there was no increase in the xylitol production with increasing cell density. The optimal temperature was between 28 and 37°C, and the optimal shaking speed was 300 rpm. The rate of xylitol production increased linearly with increasing initial xylose concentration. A high concentration of xylose (120 g/L) was consumed rapidly and produced about 100 g/L of xylitol.

Poster 64

PRODUCTION OF 2,3-BUTANEDIOL FROM PRETREATED CORN COB BY *Klebsiella oxytoca* IN THE PRESENCE OF FUNGAL CELLULASE

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2,3-Butanediol is valuable as a chemical feedstock or as a liquid fuel. It can be converted into various chemicals such as methyl ethyl ketone or butadiene. Many species of bacteria have the ability to convert simple carbohydrates into butanediol. For example, *Klebsiella oxytoca* has the ability to utilize all of the major carbohydrates present in hemicellulose and cellulose including pentoses to produce 2,3-butanediol with relatively good yield. In this study, we examine the ability of *K. oxytoca* to produce butanediol from ground corn cob in the presence of a fungal cellulase preparation. We also examined the effect of various pretreatments of corn cob before it was subjected to fermentation. The highest yield of butanediol was obtained after the corn cob was first subjected to ammonia steeping followed by dilute acid pretreatment. A final butanediol concentration of 25 g/L was obtained from 88 g/L corn cob cellulose after 72 h of incubation.

Poster 65

THE EFFECT OF DISSOLVED OXYGEN ON XYLOSE FERMENTATION BY *Candida* sp.

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The yeast *Candida* sp. was used to ferment xylose into ethanol. The effect of dissolved oxygen on fermentation was studied. The specific range of dissolved oxygen in which the yeast gives the best fermentation performance was quantitatively located. Fermentation was carried out with various initial sugar concentrations. Dissolved oxygen (0, 1.5, 2, 3, 8, and 10 ppm) was controlled by manipulating the oxygen concentration in the aeration gas mixture (nitrogen plus oxygen) while maintaining constant total gas flow rate (400 mL/min). The dissolved oxygen concentration in the fermentation broth was monitored by a DO meter. The temperature effect was also studied. Results showed that 30°C was the optimum temperature, and the fermentation ceased at 35°C. Small concentrations of oxygen (2 ppm) did enhance the fermentation, in terms of both production rate and ethanol concentration. However, higher oxygen concentrations adversely affected the fermentation.

Poster 66

OXYGEN SENSITIVITY OF ALGAL HYDROGEN PRODUCTION

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Photoproduction of H_2 by green algae is theoretically more efficient than that by bacteria or cyanobacteria, since the process utilizes electrons originating from photosynthetic oxidation of water and does not require the accumulation of metabolic intermediates. This makes green algae an attractive alternative for future biological H_2-producing systems. However, green algae photoproduce H_2 only under anaerobic conditions, due to the extreme sensitivity of the activated algal hydrogenase to O_2. In this study, we have characterized the O_2 sensitivity of the hydrogenase in WT and cw15 (cell wall–less) *Chlamydomonas reinhardtii* cells and isolated variants of cw15 with higher O_2 tolerance to H_2 production. These variants were obtained by a selection procedure based on the ability of the cells to survive in the presence of 2.8% O_2, following induction of the hydrogenase enzyme. We will also report on the results of selective pressure applied to mutagenized cells, in order to obtain mutants that are tolerant to higher concentrations of O_2. The ultimate goal of this work is to develop mutants that can be used in a photobioreactor for continuous H_2 production from water under aerobic conditions.

Poster 67

PRODUCTION OF CELLULASES AND XYLANASES BY *Trichoderma* sp. F09700.1b AND *Aspergillus* sp. F01200.1b UNDER SEMISOLID CULTURE CONDITIONS

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Solid substrate fermentations offer an interesting potential for the industrial production of enzymes by micelial fungi, which can be cultivated on suitable natural materials. Such methods seem to be particularly attractive for the production of cellulases, which can be employed to degrade lignocellulosic wastes as a source of carbon and to produce food, pharmaceuticals, and chemical products at lower costs. Cellulose-containing organic wastes that are available in large quantities include a variety of agricultural processing residues.

Considering that the semisolid fermentation offers one alternative, in the present study we have investigated the use of sugarcane bagasse pith for improved production of cellulolytic enzymes with two different filamentous fungi (*Trichoderma* sp. and *Aspergillus* sp.) isolated in a simplified medium containing untreated bagasse sugarcane pith as support and an inductor with simple minerals, CSL, and tap water until obtaining a relative moisture of 78%. Thus, after 5 days of growing in trays under aseptic conditions in a controlled chamber at 29°C with constant humidity, acceptable levels of enzymatic activities were produced. *Trichoderma* sp. F09700.1b produced the highest activity of FPase, and *Aspergillus* sp. F01200.1b, the highest activities of β-glucosidase and xylanases.

Yields for *Trichoderma* sp. in units per milliliter were as high as 0.30 for FPase, 0.44 for CMCase, 0.45 for β-glucosidase, and 2.26 for xylanase; those for *Aspergillus* sp. were 0.21 for FPase, 0.57 for CMCase, 1.10 for β-glucosidase, and 3.37 for xylanase—in both cases after three successive washes with distilled water from bagasse pith. These were obtained at optimum growth and enzyme assay conditions.

Poster 68

OBTAINING MICROBIAL PECTINASES FROM ORANGE PEEL BY SEMISOLID CULTURE OF THREE DIFFERENT FUNGI STRAINS

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Microbial enzymes catalyzing the degradation of pectic polysaccharides play an important role in foods and food processing and also in the biological degradation of plant materials. Pectic enzymes are used and have many industrial applications. In Mexico there are different agroindustrial wastes, such as the orange peel, with appreciable quantities of pectin. This pectin may be utilized to induce extracellular pectinolytic enzymes.

Our principal interest was centered on the use of these by-products as a source of enzymes at low cost. We felt that it was important to study some of the variables that affect the production of pectinases with different strains of fungi isolated from semisolid culture.

Investigations were conducted at the flask level in order to demonstrate that milled and without-terpene orange peel could be used as a raw material for the production of fungal pectinases. The strains of *Aspergillus* sp. F01200.4b, *Rhizopus* sp. F08500.1b, and *Penicillium* sp. F07500.5b were grown in a culture medium prepared with treated orange peel, mineral salts, and tap water until 65% relative moisture in semisolid culture was obtained. At the same time, a submerged fermentation under similar environmental conditions and using the same culture medium was evaluated as a reference or control.

Results obtained after 4 days of growing in flasks under aseptic conditions in a controlled chamber at 28°C with constant humidity showed that *Penicillium* sp. are the best producer using this technique. With *Penicillium* sp., it was possible to obtain filtrates with 11.2 units of pectinase activity (determined as mg reducing sugars $\cdot mL^{-1} \cdot h^{-1}$, which represents a 35% increase with respect to the submerged fermentation using the same strain.

Poster 69

OPTIMIZATION OF REACTION CONDITIONS FOR AN ENZYMATIC CONVERSION TO HYDROGEN: INITIAL STUDIES OF pH, TEMPERATURE, AND REACTANT EFFECTS

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Hydrogen was successfully produced by an enzymatic method using glucose as a substrate. The process involved the oxidation of glucose by glucose dehydrogenase, with the concomitant reduction of the cofactor $NADP^+$, which was reoxidized by hydrogenase, resulting in the evolution of molecular hydrogen. Kinetic effects of enzyme activity at different pH values in relation to hydrogen evolution were studied. Using glucose dehydrogenase from *Thermoplasma acidophilum*, two pH optima were observed. Highest rates of production were obtained at pH 6.5, whereas near 100% yields of hydrogen were obtained at pH 8.0. The pH optimum for the peak rate of hydrogen evolution was found to correspond to the stability of *Pyrococcus furiosus* hydrogenase. A significant role for this hydrogenase was discovered in relation to determinations of both rate and yield of hydrogen evolution for this method.

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Poster 70

SOME BIOTECHNOLOGICAL PROPERTIES OF STEROL MUTANTS OF MICROALGAE

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The collection of mutant strains of *Chlamydomonas* and *Chlorella* with altered sterol contents was previously obtained in various ways. Research concerning multiple effects of these mutations has been conducted for groups of *Chlamydomonas* strains distinguished by high levels of resistance to the polyene antibiotic nystatin. Some phenotypic behavioral patterns may be of essential importance for industrial use.

No cases of spontaneous reversions were noted during investigations. Fast and complete settling of suspensions is similar to properties of pf-mutants (paralyzed flagella). When nys^+ mutants were cultured in the closed cultural volume (without any additions of fresh media), the cultures kept the ability to form colonies for a long time after inoculation, while the irreversible degradation phase usually came in 4–5 weeks. (At the present time the age of one such culture exceeds 1.5 years.) The dry weight of biomass determined at growth on solid media reached unusually high levels (25–30%), while for wild-type strains, values of 8–12% were noted. Especially it should emphasize common increased resistance of these mutants to a number of tested chemical and physical agents—streptomycin, erythromycin, chloroform vapors, and others.

The specified features—stability, viscosity, mobility, viability, and resistance—facilitate both maintenance of active and clean culture and biomass harvest. At the same time, a rigid cell wall caused certain difficulties in its processing. So the standard pigment extraction procedures gave only 20% of output against more than 95% in the control. In this connection some strains were constructed with the help of cell-wall mutants and are now being tested. Another direction of current investigations is connected with the scaling of the cultivation of sterol mutants, and 5-L volumes are developed already.

Poster 71

EFFECT OF CARBON DIOXIDE ON SUCCINATE PRODUCTION BY *Fibrobacter succinogenes*

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Fibrobacter succinogenes, a ruminal cellulolytic bacterium, produces succinate, formate, and acetate as major end products of cellulose fermentation. Intermediate glucose produced from hydrolysis of cellulose is metabolized via the Embden-Meyerhof-Parnas pathway and reductive tricarboxylic acid cycle. A key enzyme in the succinate branch, phosphoenolpyruvate carboxykinase, is involved in catabolic carbon dioxide fixation; hence, the availability of carbon dioxide is a key parameter in succinate production by this organism. The results of a study elucidating the role of dissolved carbon dioxide concentration on succinate production are described.

Poster 72

Acetobacter CELLULOSIC BIOFILMS: SEARCH FOR NEW CELLULOGENIC MODULATORS AND TREATMENTS FOR MODIFIED PELLICLES

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Biotechnological uses for *Acetobacter xylinum* cellulosic pellicles are now firmly established in the world market. BioFill/Bioprocess (Brazil) wound dressing and Cellulon fibers (United States) are examples.

New prospects in biofilm production and uses may be concerned with the improvement of bacterial growth and cellulogenesis and with the post-treatment of pellicles in order to alter physical, chemical, and biological properties. For this purpose, we are examining the following aspects: (1) novel positive modulators for the cellulogenic complex other than the already reported pseudopurines present in tea infusions; (2) lipophylic derivatives of the precursor for the "second messenger" of cellulose synthase activation; (3) pellicle treatment with acidic and alkaline reagents and the resulting alterations in the physical, chemical, and biological properties (e.g., the binding of specific dyes) and in the cellulolytic analysis; and (4) pellicle interaction with native plant and microbial polymers. Data from this experimental approach are presented.

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Poster 73

ASTAXANTHINOGENESIS IN *Phaffia rhodozyma*: OPTIMIZATION OF LOW-COST CULTURE MEDIA, PIGMENT SUPERCRITICAL FLUID EXTRACTION, AND YEAST CELL LYSIS

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The production of astaxanthin, a highly oxygenated pigment, was previously attained with the sucrolytic/ureolytic yeast *Phaffia rhodozyma* using low-cost media such as diluted raw sugarcane juice or cellulolyzed cane bagasse and amylolyzed crude starch, all carbon sources added as limited amounts (0.1%) of simple (urea) or complex (soya meal, leather shavings from tannery) inexpensive nitrogen sources either in polymeric or in proteolyzed forms.

Aiming for carotenoid bioproduction improvement, new approaches were undertaken: (1) addition of strategic trace elements (e.g., Ni^{2+} for the metalloenzyme urease) or terpenoid precursors; (2) mild preinversion of cane free sugars with hot diluted phosphoric acid further neutralized to sodium or ammonium phosphate and the subsequent effect in the yeast growth lag phase; (3) yeast cell lysis by enzymes from *Trichoderma* sp. ("Cerrados" from Brazil, Novosym) as compared with gastrointestinal enzymes from selected species of fish; and (4) CO_2 supercritical fluid extraction of the whole yeast cell, looking for selective astaxanthin removal. Data on these prospects will be presented.

[Support provided by World Bank/CNPq-PADCT-SBIO]

Poster 74

IMMOBILIZATION OF INULINASE AND GLUCOSE DEHYDROGENASE FOR PRODUCTION OF HYDROGEN

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Inulinase I (Novozym 230) catalyzes the conversion of inulin, a β-(2 ⇒ 1)-polyfructan, to fructose, which is a sweetener and a potential substrate for oxidoreductive enzymes in hydrogen production. When aminopropyl controlled-pore silica (CPS, average pore size of 375 Å) was used as a support and glutaraldehyde as a bifunctional cross-linking agent, the immobilization yield was 36.4%. The enzyme also was linked to partially *N*-deacetylated crab chitin using 4,4'-biphenyl-bis-diazonium fluoborate as coreagent, and the performance of the immobilized inulinase I upon recycling still showed 51% conversion of inulin to fructose after ten recycles.

Glucose dehydrogenase from calf liver and *Bacillus megaterium*, which catalyzes the oxidation of glucose to gluconic acid with concomitant reduction of $NADP^+$, was also tested in the immobilization protocol. Preliminary studies using two types of aminopropyl CPS (average pore sizes of 170 and 500 Å) and glutaraldehyde showed the possibility of up to 12 repeated uses of the immobilized enzyme with a good substrate conversion. The best results were obtained using aminopropyl CPS-500, and the effect of bovine serum albumine on stabilizing the protein layer was observed.

[Research sponsored by CNPq (Brazil) and the U.S. Department of Energy]

Poster 75

EXPRESSION OF MALIC ENZYME ALLOWS PRODUCTION OF SUCCINIC ACID FROM GLUCOSE IN A MUTANT *Escherichia coli*

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Malic enzyme functions physiologically to convert malic acid and NAD to pyruvic acid, NADH, and CO_2. However, the reverse nonphysiological reaction is favored thermodynamically; genetic regulation and the kinetic properties of the enzyme favor its physiological role. We have evaluated the potential of malic enzyme to function metabolically in the nonphysiological direction to catalyze the fixation of CO_2 and production of C4 dicarboxylic acids. The previously cloned malic enzyme gene from *Ascaris suum* was recloned into the vector pTRC99a to allow its expression in the presence of glucose. The new expression vector, pMEA1, was introduced into *E. coli* NZN111. This strain lacks pyruvate formate lyase and the fermentative lactate dehydrogenase. It is unable to grow fermentatively on glucose but accumulates pyruvate to greater than 1 m*M* concentration before growth ceases. Overexpression of the *A. suum* malic enzyme at 37°C was toxic; at 30°C, however, when lower concentrations of inducer were used, moderate overexpression was obtained, with the apparent production of inclusion bodies. Nonetheless, the expression of malic enzyme was sufficient to restore the ability to ferment glucose. Succinic acid was formed as the major fermentation product.

Poster 76

METABOLIC ENGINEERING OF AN ARABINOSE-FERMENTING *Zymomonas mobilis*

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An economical process for conversion of lignocellulosic biomass to ethanol depends on the rapid and efficient conversion of its cellulose and hemicellulose components. While many microorganisms can ferment the glucose in the cellulose fraction to ethanol, conversion of the xylose and arabinose in the hemicellulose fraction has proven more difficult. *Zymomonas mobilis* has many favorable characteristics for fuel ethanol production including high ethanol yield, tolerance, and specific productivity. We previously developed a strain of *Z. mobilis* for simultaneous cofermentation of the glucose and xylose prominent in many lignocellulosic feedstocks [*Science* **267**, 240–43]. We report here on a new strain of *Z. mobilis* recently developed for fermentation of the arabinose commonly found in corn fiber, agricultural residues, and herbaceous energy crops. Five genes encoding the enzymes necessary for converting arabinose to common intermediates of the Entner-Doudoroff pathway were simultaneously introduced into *Z. mobilis* under the control of strong constitutive promoters that direct their expression even in the presence of glucose. The engineered strain grows on arabinose as a sole carbon source and produces ethanol at near-theoretical yield.

Poster 77

FERMENTATION OF PECTIN AND ORANGE PEEL BY ETHANOLOGENIC SOFT-ROT BACTERIA

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The processing of citrus fruit generates large amounts of pectin-rich residues, mainly in the form of peel and segment membranes. These residues contain both soluble sugars and insoluble cell wall polysaccharides. The high carbohydrate content and susceptibility to enzymatic hydrolysis make these residues an attractive feedstock for enzymatic saccharification. The resulting sugars can be fermented to ethanol or other products. Further development of this technology is hampered by the high cost of commercial pectinolytic and cellulolytic enzymes, which are required for efficient hydrolysis of citrus peel tissues. Therefore, we have initiated an investigation of saccharification and fermentation of orange peel by the ethanologenic soft-rot bacteria of the genus *Erwinia*, constructed by Professor L. O'Neal Ingram at the University of Florida. These bacteria are pectinolytic and also exhibit weak cellulolytic and hemicellulolytic activities. Our preliminary investigations indicate that at least two strains of these bacteria ferment galacturonic and polygalacturonic acids, citrus pectin, and pectin in orange peel to a mixture of ethanol and acetate at 30°C and pH = 6.5 or 7.0. These investigations also indicate that the efficiency of saccharification and fermentation of concentrated orange peel slurries is decreased by a catabolic repression of pectinolytic enzyme production.

Poster 78

ATOMIC FORCE MICROSCOPE MEASUREMENTS OF SUBSTRATE-ENZYME INTERACTIONS IN CELLULASE SYSTEMS

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The tapping-mode atomic force microscope (AFM) has been used to study the interaction between catalytically active and inactive cellobiohydrolase I (CBH I) and cotton fibers. Images of both enzyme and microfibril molecules can be resolved from the AFM. CBH I was catalytically inactivated by treating with ammonium hexachloropalladate but still retained the ability to bind to the cotton fiber. We have observed inactivated CBH I on cotton fiber under the AFM. The images from AFM suggest that the catalytic activity of CBH I was required for fiber disruption. However, at high magnification, nanometer-size defects were observed throughout the surface of the microfibrillar surface of cotton fibers treated with inactivated CBH I. These defects possibly came from the washing process since we did not observe any defect on the unwashed surface.

Poster 79

MICROBIAL PHOSPHORYLASES FOR THE CONVERSION OF MALTODEXTRIN TO GLUCOSE-1-PHOSPHATE

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In the presence of a large excess of inorganic phosphate, α-glucan phosphorylases catalyze the degradation of α-1,4-D-glucans into glucose-1-phosphate. The latter compound is a useful product for medical applications or for further chemoenzymatic syntheses. By employing a nonregulated bacterial phosphorylase from *Corynebacterium callunae*, several process parameters have been evaluated that are indispensable for a partial optimization of the enzymatic production of glucose-1-phosphate in continuous mode of operation.

The conversion of maltodextrin and phosphate by *C. callunae* phosphorylase immobilized in ultrafiltration membrane reactors was studied under various reaction conditions—such as different temperatures, residence times, and substrate concentrations—to evaluate the attainable degrees of conversion, the reactor productivities, and the operational stability of the respective phosphorylase preparations. Especially in cross-flow ultrafiltration systems, shear sensitivity is a major factor governing enzyme stability and reactor performance. Employing dead-end ultrafiltration, the continuous reaction was carried out for more than 3 weeks without appreciable decrease in productivity, although retention of the polymeric substrate limits the range of applicable flow rates.

Poster 80

ENZYMATIC SYNTHESIS OF SORBITOL AND GLUCONIC ACID—PROCESS CONSIDERATIONS EMPLOYING ISOLATED GLUCOSE-FRUCTOSE OXIDOREDUCTASE FROM *Zymomonas mobilis*

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Glucose-fructose oxidoreductase (GFOR) from *Zymomonas mobilis* simultaneously catalyzes the reduction of fructose and the oxidation of glucose, while tightly enzyme bound NADP(H) functions as the hydrogen carrier. Consequently, by using only a single enzyme, the conversion of mixtures of fructose and glucose into sorbitol and glucono-δ-lactone (which subsequently hydrolyzes to gluconic acid) is possible, and both the oxidized and the reduced forms of the enzyme-bound cofactor are recycled.

A continuous process employing isolated GFOR immobilized in an ultrafiltration membrane reactor is presented, and its partial optimization to efficiently convert concentrated substrate solutions (up to 2 mol/L) is reported. In the absence of stabilizing additives, enzyme inactivation limits the space-time yields of reaction. Furthermore, the implications of the reaction kinetics for a probable process configuration are discussed.

Poster 81

INDUCTION OF XYLOSE REDUCTASE AND XYLITOL DEHYDROGENASE ACTIVITIES IN *Candida tenuis*

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Xylose reductase (XR)—which is currently being investigated in our laboratory for the production of xylitol from xylose, employing the isolated enzyme in a membrane reactor together with xylitol dehydrogenase (XDH)—plays an important role in the catabolism of xylose in yeasts. These two enzymes catalyze the first two steps in xylose utilization, and their synthesis is efficiently controlled by induction and repression.

In the yeast *Candida tenuis,* both enzymes were induced when the organism was grown on D-xylose; lower activities were found after growth on L-arabinose or D-lyxose; and only constitutive enzyme levels could be detected when hexoses such as D-glucose, D-galactose, or D-mannose were used as substrates. Whereas mixtures of xylose/galactose or xylose/mannose resulted in a significant decrease of both XR and XDH activities compared with the results obtained with only xylose, xylose/arabinose mixtures showed a positive synergism with respect to enzyme synthesis. Levels of XR were slightly increased, while XDH activities were found to be more than twofold higher when using the mixed sugars. From these results it is concluded that XR and XDH synthesis is not under coordinate control in *C. tenuis*.

Poster 82

PRODUCTION OF HEMICELLULOSE- AND CELLULOSE-DEGRADING ENZYMES BY VARIOUS STRAINS OF *Sclerotium rolfsii*

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Sclerotium rolfsii, a plant pathogen basidiomycete, is known as a good producer of various lignocellulolytic enzymes. To further investigate the production of these enzymes by this fungus, a number of different *S. rolfsii* isolates were compared with respect to their ability to produce mannan-, xylan-, and cellulose-degrading enzyme activities. Although a great variability exists in the levels of enzymes formed by different strains, *S. rolfsii* proved to be an efficient producer of hemicellulolytic enzymes. The levels of mannanase (620 IU/mL), xylanase (440 IU/mL), and filter paper cellulase activity (9.2 FPU/mL) determined for the best-producing strain are remarkable. In addition, this strain produced high levels of accessory enzyme activities that are necessary for cleaving side-group substituents frequently found in various xylans or mananas.

A crude culture filtrate of *S. rolfsii* was used for the hydrolysis of various lignocellulosic material including agricultural residues. Corncobs and steam-treated wheat straw yielded especially high amounts of glucose and xylose with very low levels of cellobiose, indicating that the amount of β-glucosidase formed by *S. rolfsii* is sufficient to avoid accumulation of this disaccharide, which is known to inhibit cellulases.

Poster 83

PRODUCTION OF D- OR L-LACTATE IN *Escherichia coli*

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In order to produce D- or L-lactic acid in *Escherichia coli*, the acetate synthesis pathway was blocked by P1 transduction mutation of phosphotransacetylase (pta) and the L-lactate dehydrogenase (L-ldh) gene from *Lactobacillus casei* was introduced. We have previously reported that in anaerobic conditions, a pta mutant of *E. coli* RR1 catabolized glucose to D-lactate similar to homofermentative bacteria. More than 60 g/L of D-lactate was accumulated by intermittent feeding of glucose under anaerobic as well as microaerobic conditions. In an attempt to make this strain produce L-lactic acid, the L-ldh gene from *L. casei* was introduced, which resulted in no production of L-lactic acid. The redirection of carbon flux to L-lactate was possible only in a pta/D-ldh double mutant in which both the acetate and the D-lactate synthesis pathways were blocked. When L-ldh gene was introduced to this double mutant, a simple fed-batch cultivation of the mutant resulted in the accumulation of 45 g/L of L-lactate. Redirected pyruvate carbon flow to D- or L-lactate may be explained by a perturbed balance in intracellular cofactor fluxes in pta or pta/D-ldh mutants.

Poster 84

SUBSTRATE REACTIVITY AS A FUNCTION OF THE EXTENT OF REACTION IN THE ENZYMATIC HYDROLYSIS OF CELLULOSE

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In an effort to understand the rapid falloff in the rate of enzymatic hydrolysis of cellulose, direct measurements of substrate reactivity as a function of conversion have been made. These measurements are made by interrupting the hydrolysis at various degrees of conversion and, after washing and boiling, by restarting the hydrolysis in fresh buffer with fresh enzyme. Thus the comparison of the restart rate with the initial rate provides a direct measurement of substrate reactivity. While the falloff in the rate has long been attributed, at least in part, to a change in the nature of the substrate, such direct measurements have not previously been reported. The results indicate that the substrate is more reactive than is indicated by the uninterrupted hydrolysis rate, even after correction for product inhibition. This is an indication that the adsorbed enzyme temporarily loses much of its activity before desorbing.

Poster 85

STEADY SHEAR CHARACTERISTICS OF FILAMENTOUS SUSPENSIONS USING THE RUSHTON TURBINE, VANE IMPELLER, AND HELICAL RIBBON IMPELLER

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Aerobic fermentations of filamentous microorganisms present unique mixing problems as a result of the non-Newtonian rheology of fermentation broths and its effect on gas-liquid mass transfer. The combination of high oxygen demand and problematic broth rheology in such fermentations makes agitated tanks a popular reactor configuration. Although a considerable amount of data on gas-liquid mass transfer in non-Newtonian systems exists in the literature, much of the past work has been performed using polymer solutions in a homogeneous liquid phase. The non-Newtonian rheology of filamentous suspensions results from solid-phase interactions, and there is some question as to whether homogeneous polymer solutions can adequately model this behavior.

Gas-liquid mass-transfer coefficients are measured in suspensions of cellulose fibers with concentrations ranging from 0 to 20 gm/L. The mass-transfer coefficients are measured using the dynamic method by rapidly switching from nitrogen to oxygen. Results are presented for three different combinations of impellers at a variety of gassing rates and agitation speeds. Rheology of the cellulose fibers was also measured using the impeller method.

Poster 86

A MATHEMATICAL MODEL OF ETHANOL FERMENTATION FROM CHEESE WHEY: PART I. MODEL DEVELOPMENT AND PARAMETER ESTIMATION

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The cybernetic approach to modeling of microbial kinetics in a mixed-substrate environment has been used in this work for the fermentative production of ethanol from cheese whey. In this system, the cells grow on multiple substrates and generate metabolic energy during product formation. This paper deals with the development of a mathematical model in which the concept of cell maintenance was modified in the light of the specific nature of product formation. Continuous culture data for anaerobic production of ethanol by *Kluyveromyces marxianus* CBS 397 on glucose and lactose were used to estimate the kinetic parameters for subsequent use in predicting the behavior of microbial growth and product formation in new situations.

Poster 87

A MATHEMATICAL MODEL OF ETHANOL FERMENTATION FROM CHEESE WHEY: PART II. SIMULATIONS AND COMPARISON WITH EXPERIMENTAL DATA

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A cybernetic model for microbial growth on mixed substrates, presented in Part I, was used to simulate anaerobic fermentation of cheese whey and multiple sugars in semisynthetic media by *Kluyveromyces marxianus* CBS 397. The model simulations quite satisfactorily predicted the observed behavior in batch operation and during transition to continuous operation, in single-substrate systems as well as in media involving multiple substrates, and in semisynthetic and reconstituted cheese-whey solutions. The results of simulations and their comparison with the experimental data will be presented.

Poster 88

PRODUCTION OF FUMARIC ACID BY IMMOBILIZED *Rhizopus* USING A ROTARY BIOFILM CONTACTOR

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During the oxidative utilization of glucose, some species of mycelial fungi fix carbon dioxide and combine it with the metabolic intermediate to produce fumaric acid as the major metabolic product, with a weight yield of up to 93%. In a typical fumaric acid fermentation, calcium carbonate was added to neutralize the acid produced and to precipitate the product. Often the formation of calcium fumarate caused a drastic change in hydrodynamic conditions of the fermenter and resulted in the premature termination of operation due to the failure of agitators. Another difficulty encountered was the tendency of mycelial fungi to form mycelial pellets or clumps. This resulted in the interference of oxygen and mass transfers and encouraged ethanol instead of acid production. To overcome the problems encountered and to improve acid production, a rotary biofilm contractor (RBC) was used to carry out fermentation. Under growth conditions with nitrogen source (0.1% yeast extract), *Rhizopus* spores were able to grow onto plastic disks of RBC and form the biofilms. Biofilms were capable of producing fumaric acid with an average yield of 70 g/L from 100 g/L glucose under continuous fed-batch cycles. On a weight-yield basis, an indicated value of 75% of theoretical was obtained.

Poster 89

THE EFFECT OF PECTINASE ON THE BUBBLE FRACTIONATION OF INVERTASE FROM α-AMYLASE

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Fermentation broth normally contains many extracellular enzymes of industrial interest. To separate such enzymes on-line could be useful in reducing the cost of recovery as well as in keeping their yield at a maximum level by minimizing enzyme degradation from residue broth proteases. Several separation methods are candidates for on-line recovery such as ultrafiltration, precipitation, and two-phase partitioning. Another promising technique for on-line recovery is adsorptive bubble fractionation because it requires no contaminating additives which must be removed in a subsequent purification step. A mixture of enzymes in this aqueous bubble solution can, in principle, be separated by adjusting the pH of that solution to the isoelectric point (pI) of each enzyme, as long as the enzymes have different pIs. This study investigated a three-enzyme separation problem—the effect of pectinase on the bubble fractionation of invertase from α-amylase—by varying the pH in a batch bubble fractionation column. Air was used as the primary carrier gas. This prototype mixture exemplifies a fungal fermentation process for producing enzymes, in keeping with the presence of air in an aerobic fermentation system. The enzyme concentrations here were measured as a function of time and column position for each batch run. It was found that the invertase and α-amylase were separated at two different pHs with greater than 90% recovery and high separation ratios. The depleted residue solution was rich in pectinase.

Poster 90

LIPASE PRODUCTION BY *Penicillium restrictum* IN A LABORATORY-SCALE FERMENTER: MEDIA COMPOSITION, AGITATION, AND AERATION

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A preliminary screening work selected *P. restrictum* as a promising microorganism for lipase production. The physiological response of the fungus in regard to cell growth and enzyme production was evaluated by applying variable carbon and nitrogen nutrition, aeration (Qa), and agitation (N) in a 5000-mL bench-scale fermenter. In optimized conditions for lipase production, peptone at 2 w/v % and olive oil at 1 w/v % were used in a growth medium with a C/N ratio of 9.9. Higher C/N ratios favored cell growth to the detriment of enzyme production. Low extracellular lipase activities were observed using glucose as the carbon source, suggesting glucose regulation. Final lipase accumulation of 13,000 IU/L was obtained using an optimized specific airflow rate (Qa) of 0.25 v/v/min and an impeller speed (N) of 200 rpm. Agitation proved to be of paramount importance in ensuring nutrient availability in an aqueous medium containing olive oil as the carbon source. Proteolytic activity related to cell lysis was observed at the latter stages of fermentation. A crude lipase preparation was shown to be stable for 8 days at 37°C and pH 7.0.

Poster 91

POTASSIUM ACETATE BY FERMENTATION WITH *C. thermoaceticum*

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Potassium acetate is used for deicing of airport runways and aircraft (replacing urea and glycol), as a heat-transfer fluid, in antifreeze formulations, and in heat pumps. Currently, potassium acetate is made by reacting petroleum-based acetic acid with potassium hydroxide. An alternate process could be used: fermentation of dextrose with *C. thermoaceticum*. In this study, the fermentation was optimized in terms of productivity and acetate concentration, and the effect of pH as well as type and concentrations of nutrients and reducing agents was evaluated. Corn steep liquor and stillage from an ethanol plant were effective substitutes for yeast extract. Preconcentrating the cells by ultrafiltration improved productivity, resulting in an acetic acid concentration of 53.6 g/L at pH 6.5 using corn steep liquor. Sodium sulfide could be substituted for cysteine as the reducing agent, resulting in a yield of 0.9 or greater. The economics of fermentation-derived potassium acetate appears to be favorable.

Poster 92

MODELING FIXED- AND FLUIDIZED-BED REACTORS FOR CASSAVA STARCH SACCHARIFICATION WITH IMMOBILIZED ENZYMES

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In a previous paper, cassava starch saccharification in fixed- and fluidized-bed reactors using immobilized enzyme was modeled using a simple model in which all dextrins were grouped in a single substrate. In that case, although good fitting of the model to experimental data was obtained, physical inconsistency appeared as negative kinetic constants. In this work, a multisubstrate model, developed earlier for saccharification with free enzymes, is adapted for immobilized enzymes. This latter model takes into account the formation of intermediate substrates, which are dextrins competing for the catalytic site of the enzyme; reversibility of some reactions; inhibition by substrate and product; and formation of isomaltose. Kinetic parameters to be used with this model were obtained from initial-velocity saccharification tests using the immobilized enzymes and different concentrations of liquefied starch. The new model was successful for modeling both fixed- and fluidized-bed reactors. It did not present inconsistencies as the earlier one had and showed that glucose inhibition is about seven times higher in the fixed bed than in the fluidized bed.

Poster 93

MAXIMIZING THE XYLITOL PRODUCTION FROM SUGARCANE BAGASSE HYDROLYSATE BY CONTROLLING THE AERATION RATE

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Lignocellulosic residues are an abundant source of energy that can be used in several biotechnological processes for the generation of high-economical-value products. Sugarcane bagasse is the most important residue in Brazil, and a large amount of this residue is generated by the Brazilian sugar-alcohol industry. Xylitol, a valuable product with anticariogenic and clinical applications is one substance that can be produced by fermentation processes using this biomass as substrate. Microbial production of xylitol from agroindustrial residues is more economical since it does not require pure xylose. Furthermore, this bioprocess occurs at lower temperatures and pressures than does the chemical one. In our investigation we selected the yeast *Candida guilliermondii* FTI20037 and determined the influence of the aeration rate on xylitol formation. The hemicellulosic hydrolysate obtained after acid hydrolysis of sugarcane bagasse (H_2SO_4, 121°C) was treated with CaO, supplemented with nutrients, and inoculated with cells of *C. guilliermondii*. The experiments were performed in a 1-L fermenter at 30°C under different oxygen conditions according to a previous statistical factorial plan.

The results showed that the yeast was able to grow, consume the sugars, and produce xylitol in different tests. The maximal xylitol concentration (33.0 g/L) and xylitol production rates (0.87 g/L·h; 0.57 g/g) were attained using an aeration rate of 0.45 v/v/min (k_La 20·h^{-1}). The increase in the dissolved oxygen concentration promoted a decrease in xylitol formation, possibly due to the effect of the oxygen on the xylose reductase activity and on the regeneration of cofactors.

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Poster 94

FUEL ETHANOL PRODUCTION USING GENETICALLY ENGINEERED YEASTS: MODELING AND EXPERIMENTAL STUDIES

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Lignocellulosic biomass has been identified as an economical feedstock for large-scale fuel ethanol production. Xylose fermentation has been a bottleneck in the ethanol production process over the past few years. This problem has been overcome in recent years by advances in genetic engineering. In our laboratory, a genetically engineered yeast 1400 (pLNH33) has been developed which can ferment glucose and xylose simultaneously to ethanol. Moreover, the high ethanol tolerance of this yeast allows downstream processing costs to be reduced significantly.

Process development work has been performed on glucose, xylose, and their mixtures. The effects of substrate inhibition, product inhibition, and plasmid stability have been characterized. An overall model for growth and fermentation using the genetically engineered yeast has been developed. Simulations of the fermentation process (batch and continuous mode) have been run using this model, and good agreements have been obtained with experimental results. By using the results on purified sugars as a basis, experiments are currently being performed using lignocellulosic hydrolysates.

Poster 95

MODELING PARAMETERS OF A REACTOR RUNNING ON SUGARCANE JUICE FOR CONVERSION INTO ETHANOL BY FLOCCULENT YEAST

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The flow pattern and the kinetic parameters of a single-stage reactor incorporating an external cell recycle for sugar substrate conversion into ethanol were evaluated. The feasibility of this fermentation system using flocculating yeast has been already demonstrated for different sucrose feedstocks. Since many weak points were identified, we decided to extend our work to obtain a quantitative understanding of the dynamic behavior and the degree of perfect mixing for the reactor running on sugarcane juice. These were established by stimulus-response techniques using different tracers such as methylene blue, crystal violet, and blue dextran. Each tracer was injected in the feed medium to give a pulse change in the material concentration of approximately 100 mg. Samples taken from the product stream were analyzed by spectrophotometry technique at 645 nm. Of all the tracers used, blue dextran gave the best response, as no significant alteration in the fermentation performance and yeast flocculating properties was observed. The reactor was run under several operational conditions of varying dilution rate, yeast recycle rate, and aeration. Changes in the sedimenter capacity were also made to verify the best configuration which allows high yeast settling.

Poster 96

THE CONTINUOUS PRODUCTION OF HYDROGEN GAS FROM THE PHOTOSYNTHETIC BACTERIUM *Rhodospseudomonas capsulata* HLK-29

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A photosynthetic bacterium was isolated from the mud in the Soyang Dam in Korea and proved to be *Rhodospseudomonas capsulata*. This bacterium was mutated by UV radiation to increase hydrogen productivity and named *R. capsulata* HLK-29. The production of hydrogen from this bacterium was 155 $\mu L \cdot mg\ dry\ wt^{-1} \cdot h^{-1}$ in batch cultivation, which is higher than the productivities from other photosynthetic organisms. The optimal culture condition was also determined to be 30 m*M* of lactate and 9 m*M* of glutamine at 0.0035 $kcal \cdot cm^2 \cdot h^{-1}$ of light intensity. For continuous production of hydrogen gas, a coil-type photoreactor with a working volume of 1 L was employed. An optimal hydrogen production was observed at a dilution rate of 0.035 L/h, and the wash-out point was estimated as 0.08 L/h for this process. Overall cultivation time was lengthened to 250–300 h, compared with 60 h in batch cultivation. It was also shown that the hydrogen production was closely related to growth rate. It is concluded that the continuous production process from these photosynthetic bacteria can be economically feasible by successfully scaling up the system.

Poster 97

BIOREACTORS FOR HYDROGEN PRODUCTION: DESIGN AND OPERATION

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Continuous H_2 production at rates up to 30 mL $H_2 \cdot$L culture suspension$^{-1} \cdot h^{-1}$ was observed using a *hup*$^-$ cyanobacterial mutant of *Anabaena variabilis* in a 2-L photobioreactor (0.22-m^2 surface area). The photobioreactor was bubbled with a mixture of CO_2 and air to supply the cells with carbon and to remove H_2. The photobioreactor was run for over 1 month with maximum efficiency of light energy conversion to H_2 of 1–2%. The results of this study show that good H_2 production rates and efficiencies are achievable in a relatively simple system. Another approach employed a unique type of H_2-producing activity found in a strain of photosynthetic bacteria that shifts CO (and H_2O) into H_2 (and CO_2) in darkness. A hollow-fiber bioreactor was assembled for shifting CO into H_2. Continuous H_2 production from CO (or from thermally generated synthesis gas) at rates of up to 300 mL $H_2 \cdot$g cdw$^{-1} \cdot h^{-1}$ was observed for more than 6 months. Effluent concentrations of the remaining CO were below detectable limits (<18 ppm), and increased H_2 concentration in the effluent gas was nearly stoichiometric with CO disappearance.

Poster 98

A MEMBRANE-REACTOR SACCHARIFICATION ASSAY TO EVALUATE THE PERFORMANCE OF CELLULASES AND SUBSTRATE PRETREATMENTS UNDER SIMULATED SSF CONDITIONS

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A new saccharification assay has been devised, in which a continuously buffer-swept membrane reactor is used to remove the solubilized saccharification products, thus allowing the achievement of high substrate conversion without significant inhibitory effects from the buildup of either cellobiose or glucose. This assay serves as a reliable predictor of the performance of combinations of cellulase and substrate under simultaneous saccharification and fermentation (SSF) conditions, while retaining the analytically more direct nature of a saccharification reaction. This assay has been used to compare the effectiveness of different native and recombinant cellulase systems in the saccharification of standardized (Sigmacell) and pretreated cellulosic substrates. Cellulase systems tested include novel enzyme combinations as well as commercial preparations.

Poster 99

MEMBRANE-COMPARTED EXTRACTIVE FERMENTATION FOR LACTIC ACID PRODUCTION FROM CELLULOSIC BIOMASS

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Lactic acid production from cellulosic biomass by cellulose and *Lactobacillus delbrueckii* was studied in a fermenter-extractor employing microporous hollow-fiber membrane (HFM). This bioreactor system was operated under a fed-batch mode with continuous removal of lactic acid via extraction. The pH of the fermentation was controlled by extraction alone, without addition of alkali. Alamine 336 (a tertiary amine) was used as an extractant for lactic acid. Addition of modifier (alcohol) and diluent (paraffin) to the extractant was necessary in HFM-mediated extraction since it affects the cell activity, distribution of lactic acid, and viscosity of the solvent. Alamine 336 diluted in oleyl alcohol-kerosene was selected as a complexing agent for enhancing lactic acid separation from fermentation broth at pH $>pK_a$. The optimum pH for the simultaneous saccharification and fermentation is about 5.0. However, lower pH is favored for the extraction. To overcome the problem, a step change of pH was applied (initially set at 5.0 for cell growth and shifting down to 4.3 for extraction and fermentation). The optimum process conditions and other performance data based on various biomass species are reported, including the effect of in situ product removal on the fermentation productivity.

Poster 100

ENZYME-SUPPORTED OIL EXTRACTION FROM *Jatropha curcas* SEEDS FOR THE PRODUCTION OF BIODIESEL

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Jatropha curcas is a tropical plant widely distributed in arid areas. The seeds contain about 55% oil, which is used mainly for the production of soap and as a fuel (after transesterification as biodiesel). Various methods for recovering oil from the seeds, including extraction with organic solvents and water, have been investigated. Compared with hexane extraction (98% recovery), oil extraction using water yielded only 52% of the total oil content of the seeds. By using several cell wall–degrading enzymes during aqueous extraction, a maximum yield of 81% was obtained. The influence of cellulolytic and hemicellulolytic enzymes as well as proteases was studied. The experiments were carried out at different pH values and temperatures to determine the optimum for oil recovery using enzymes. Surprisingly, the best results (81% recovery) were obtained using an alkaline protease. The enzyme-supported aqueous extraction offers a nontoxic alternative to common extraction methods using organic solvents and affords reasonable yields.

Poster 101

CONVERSION OF RESIDUES FROM *Jatropha curcas* SEEDS TO BIOGAS

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The seeds of the tropical plant *Jatropha curcas* are used for the production of oil. Several methods have been developed for extraction of the oil. In all the processes about 50% of the seeds remain as press cake, containing mainly protein and carbohydrates. Investigations determined that the cake contains toxic compounds and cannot be used as animal feed without further processing. Preliminary experiments proved the cake to be a good substrate for biogas production. Biogas formation was studied using a semicontinuous-operation sludge-bed reactor and an anaerobic filter with a total volume of 120 L each. A maximum productivity of 6.0 $m^3 \cdot m^{-3} \cdot day^{-1}$ was obtained in the anaerobic filter with a loading of 12.7 kg $COD \cdot m^{-3} \cdot day^{-1}$, while the sludge-bed reactor turned out to be unsuitable for this substrate. Under these conditions 76% of the COD was degraded, and 1 kg COD yielded 355 L of biogas containing 61% methane.

Poster 102

PERVAPORATION FOR ENHANCED PRODUCTIVITY IN ETHANOL FERMENTATIONS

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The use of membrane processes for the recovery of fermentation products has been gaining increased acceptance in recent years. Pervaporation has been studied in the past as a process for simultaneous fermentation and recovery of volatile products such as ethanol and butanol. However, membrane fouling and low permeate fluxes have imposed limitations on the effectiveness of the process. In this study, we characterize the performance of a substituted polyacetylene membrane, poly(1-trimethylsilyl-1-propyne), or PTMSP, in the recovery of ethanol from aqueous mixtures and fermentation broths. We compare the effectiveness of PTMSP with that of a conventional membrane system based on polydimethylsiloxane, or PDMS, membranes. Our results indicate that PTMSP is a novel membrane that promises higher fluxes, higher product selectivity, and greater resistance to fouling. The impact of a PTMSP system in a large-scale ethanol fermentation for simultaneous ethanol recovery is also discussed.

Poster 103

ANALYSIS OF BIOMASS-TO-ETHANOL PROCESS SAMPLES USING HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION

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Analysis of process samples obtained from the conversion of biomass to ethanol presents many analytical challenges as a result of the complex nature of the samples. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has proven to be a useful technique for quantifying carbohydrates in samples generated during various steps of the conversion process. Coelution and baseline problems typically encountered with conventional HPLC approaches are avoided because of the selectivity of the PAD detector for carbohydrates. The sensitivity of the detector permits detection of minor components often missed by other approaches. A comparison of several analytical techniques commonly used with biomass analysis illustrates the usefulness of the HAPAEC-PAD approach.

Poster 104

STRATEGIC APPROACHES TO A BALANCED R&D PORTFOLIO IN THE RENEWABLE CHEMICAL INDUSTRY

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A technology-driven organization places considerable importance on the product of its research and development (R&D) activities. The viability and the sustained profitability of the business are linked to successful product introduction into the market. The design for success must combine technology development, market potential, and financial viability with a sound strategic business philosophy. Thus, the key factor in building a profitable, multiproduct R&D-based company is *strategic management*. This paper presents the application of a variety of strategic tools for the evaluation of diverse R&D projects leading to the selection of a balanced R&D portfolio. The demonstration of concepts is based on examples of products in the renewable chemical industry. The evaluation ranges from the use of simple screening techniques to elaborate strategic mapping of projects and industries along dimensions such as commercial readiness, market potential, competitive advantage, and profitability. Sound strategic management combines these analyses with a solid company philosophy to mange growth and risk. The results can facilitate the pivotal decision making to allocate resources, build strategic alliances, and launch new products.

Poster 105

USE OF INVESTMENT ANALYSIS TO SELECT PUBLICLY FUNDED RESEARCH AND DEVELOPMENT PROJECTS

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One of the main functions of government is to invest taxpayer dollars in projects, programs, and properties that will result in social benefits that cannot normally be purchased in the marketplace. Federal research and development programs focused on the development of biotechnologies for the production of fuels and chemicals are examples of such investment opportunities. Valuation of these programs requires the same investment analysis approaches that are necessary for private companies and individuals. Good use of investment analysis approaches to public investment opportunities will minimize our tax costs and maximize public benefit for each dollar invested.

This paper describes the use of investment analysis approaches to select publicly funded research and development programs that will yield maximum public benefit. An approach to obtaining an estimate of the free market value of technologies developed with public funds is also presented. This allows national laboratories to determine reasonable licensing arrangements for their technologies. These approaches have been incorporated into a spreadsheet model, which is also described.

Poster 106

NEW BIOREACTORS FOR THE PRODUCTION OF CELLULASES BY SOLID-STATE FERMENTATION WITH *Trichoderma reesei*

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Because it offers some advantages over liquid-state (submerged) fermentation (LSF), solid-state fermentation (SSF) is becoming popular for the production of enzymes, especially cellulases and amylase. Some of the commercial SSF bioreactors have been reported in the literature—for example, stationary trays, moving belts, rotary trays, and rotary drums—but all present some problems in cellulase production. Since continuous agitation of the solid substrate reduces the yield of cellulases, the latter two types of bioreactors cannot be employed for cellulase production. Moreover, the cellulase production peaks at about 3 weeks; thus, these bioreactors are not cost-effective if each batch runs for more than 1 week. For the production of cellulases with the mutants of *T. reesei*, we have developed two prototype pan bioreactors with a screen in the middle to support the solid substrate and to permit aeration from the bottom of the bioreactor. The yields and quality of enzyme obtained by SSF with this bioreactor were higher than those obtained with LSF. Moreover, the ratio of ß-glucosidase to filter paper cellulase in the cellulase systems using SSF was close to 1.0 in these pan bioreactors, much higher than that obtained by LSF. More than 85% of the delignified wheat straw was hydrolyzed by the enzyme system obtained by SSF in this pan bioreactor.

Poster 107

BIOLOGICAL MARKERS OF INSECT Sf9 CELLS TO ASSESS CELLULAR RESPONSES TO HYDRODYNAMIC SHEAR STRESS IN A STIRRED-TANK BIOREACTOR

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Hydrodynamic shear stress generated in a bioreactor has been linked to the loss of cell viability and the decrease of cell metabolite production. A major drawback of this approach is that the hydrodynamic phenomena are apparatus specific and the effects observed cannot be applied to other systems. In this study, three intrinsic biological markers, including (1) diacyl-glycerol (DG) levels, (2) phospholipase A_2 (PLA_2) activity, and (3) intracellular calcium ion concentrations ($[Ca^{2+}]_I$), were used to assess the effects of hydrodynamic shear stress on insect *Spodoptera frugiperda* (Sf9) cells. When the cells were subjected to 98.2 dynes/cm^2, a shear level inducing cell lysis at 6–10 h, a transient increase in PLA_2 activity was observed during initial stages of exposure to shear. The levels of DG also increased at this shear level, while a twofold increase in DG levels was observed upon exposure of cells to shear levels of 0.7–4.2 dynes/cm^2 for 45 min. Exposure of cells to nonlethal shear resulted in an increase in the basal levels of $[Ca^{2+}]_I$ and a loss of stimulation of octopamine, a biogenic neuromodulator, to Sf9 cells. Lethal shear level (98.2 dynes/cm^2) resulted in an increase in $[Ca^{2+}]_I$ after short exposure times. After longer exposure times to shear (4–8 h), $[Ca^{2+}]_I$ levels dropped to normal, concomitant to the observation of loss of cell viability. Models of shear-induced cellular response in Sf9 cells in terms of biological markers are discussed.

Poster 108

ON-LINE MONITORING OF L-LACTIC ACID BY A BIOSENSOR DURING LACTIC ACID CULTIVATIONS ON A TWO-PHASE POLYMER MEDIUM

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A biosensor is a very simple and fast analytical tool for monitoring bioprocesses. In this study, a biosensor has been used to measure L-lactic acid during lactic acid cultivations on a two-phase polymer medium. The analysis by the biosensor is based on two enzymatic reactions using L-lactic acid oxidase and peroxidase. Both L-lactic acid oxidase and peroxidase were coadsorbed onto heated graphite powder, which was used to prepare the biosensor.

The on-line monitoring system consists of a microdialysis probe as a sampling device and a flow injection analysis (FIA) system containing an L-lactic acid biosensor. The microdialysis probe was inserted through a standard port in the top plate of the bioreactor. By pumping perfusion liquid through the microdialysis probes, the sample was introduced for analysis by the FIA system. The change in current was recorded by a personal computer. The optimum operating condition, linear range, and operational stability of the L-lactic acid biosensor were investigated. Interference from cultivation medium was eliminated. The on-line measurement result was compared with that from the controlled analytical method (high-performance liquid chromatography).

Poster 109

ENZYMATIC CATALYSIS IN ORGANIC SOLVENTS: POLYETHYLENE GLYCOL–MODIFIED HYDROGENASE RETAINS SULFHYDROGENASE ACTIVITY IN TOLUENE

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Naturally occurring enzymes may be modified by covalently attaching hydrophobic groups that render the enzyme soluble and active in organic solvents and have the potential to greatly expand applications of enzymatic catalysis. The reduction of elemental sulfur to hydrogen sulfide by a hydrogenase isolated from *Pyrococcus furiosus* has been investigated as a model system for organic biocatalysis. While the native hydrogenase catalyzed the reduction of sulfur to H_2S in aqueous solution, no activity was observed when the aqueous solvent was replaced with anhydrous toluene. Hydrogenase modified with PEG *p*-nitrophenyl carbonate demonstrated its native biocatalytic ability in toluene when the reducing dye benzyl viologen was also present. Neither benzyl viologen nor PEG *p*-nitrophenyl carbonate alone demonstrated reducing capability. PEG-modified cellulase and benzyl viologen were also incapable of reducing S to H_2S, indicating that the enzyme itself and not the modification procedure is responsible for the conversion in the nonpolar organic solvent. Sulfide production in toluene was tenfold higher than that in an aqueous system with equal enzyme activity, demonstrating the advantages of organic biocatalysis. Applications of bioprocessing in nonaqueous media are expected to provide significant advances in the areas of fossil fuels, renewable feedstocks, organic synthesis, and environmental control technology.

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Poster 110

COUPLING OF WASTEWATER TREATMENT WITH STORAGE POLYMER PRODUCTION

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Storage polymers in bacterial cells are useful biodegradable polymers that can be used as thermoplastics. However, widespread application of these polymers has been limited by the costs of production. In this study, the bacteria in the activated sludge of a sequencing batch reactor (SBR) were induced by controlling the carbon-nitrogen (C-N) ratio in the reactor liquor to accumulate storage polymers.

A 12-L SBR, treating a synthetic wastewater at a batch loading rate of 1.0 mg COD·(mg MLVSS)$^{-1}$·day^{-1} and a reaction–to–contact time ratio of 0.8, was tested with a C-N ratio that varied from 20 to 100 while the carbon concentration was kept constant.

Specific polymer production yield increased to a maximum of 0.6 g polymer/g cell with increasing C-N ratio, while specific-growth yield decreased with increasing C-N ratio. An optimum C-N ratio of around 80 provided the highest polymer production rate. Adjustment of the C-N ratio did not significantly affect the treatment efficiency.

Poster 111

NOMENCLATURE AND METHODOLOGY FOR CLASSIFICATION OF NONTRADITIONAL BIOCATALYSIS

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Because terminology associated with biocatalysis is vague and easily misinterpreted, we have developed a vocabulary with appropriate definitions and symbol representation to simplify classification of enzymatic reactions in catalysis bioprocessing. In conjunction with the derivation of this terminology, a technology road map was constructed that can be used to rank the priority of issues associated with work that has been and will be performed in this field. The usefulness of this systemization for biocatalysis becomes readily apparent when proposed technologies are to be compared with existing technologies. The approach we have taken to distinguish between systems is primarily dependent upon the phase in which each of the critical reaction components—biocatalyst, reactant(s), and product(s)—is present. Possible resident phases include aqueous (A), organic (O), vapor (V), and supercritical (Sc). With this system, a reaction scheme may be classified with a three-character identifier, such as AAO (a system in which the enzyme and substrate are present in an aqueous phase and the product is recovered from an organic phase). Special cases, such as when the biocatalyst is immobilized or the product forms an insoluble precipitate, are also discussed in the context of this nomenclature. Furthermore, the developed vocabulary allows categorization of biocatalytic bioprocessing into two distinct classes: traditional (aqueous phase only) and nontraditional (which may be further subdivided into nonaqueous, aqueous, and supercritical). Each of these reaction groups is investigated, and associated research and development issues are elucidated.

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Poster 112

ADSORPTION OF HEAVY METAL IONS BY IMMOBILIZED PHYTIC ACID

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Phytic acid (myoinositol hexaphosphate) or its calcium salt, phytate, is an important plant constituent. It accounts for up to 85% of the total phosphorus in cereals and legumes. Phytic acid has 12 replaceable protons in the phytic molecule, which gives it the ability to complex with positively charged proteins and multivalent cations. Poly (4-vinylpyridine), or PVP, and other strong-based resins have the ability to adsorb phytic acid. PVP has the highest adsorption capacity, 1.17 grams of phytic acid per gram of resin. We use PVP as the support material for the immobilization of phytic acid. The immobilized PVP can adsorb heavy metal ions such as those of copper, nickel, lead, and zinc from aqueous solutions. Adsorption isotherms of copper and lead ions to immobilized phytic acid were conducted in packed-bed columns at room temperature. Results from the adsorption-desorption tests showed that at least 0.84 mol of metal ions can be adsorbed by each mole of immobilized phytic acid. The use of immobilized phytic acid can have the potential for removing metal ions from industrial or mining wastewater.

Poster 113

MICROBIAL DEGRADATION OF CRUDE OIL

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Pollution due to oil spillage is increasing at an alarming rate. We have isolated certain oil-degrading microbes—*Alternaria* sp., *Aspergillus flavus*, *Fusarium semidectum*, *Penicillium* sp., *Aspergillus niger*, *Pseudomonas* sp., and *Candida* sp.—from the aqueous oil effluent produced by Bharat Heavy Electricals Limited, Tiruchirapalli, Tamil Nadu, India. The effects of environmental parameters (e.g., temperature, pH, nutrients, and salinity) on oil degradation and growth of microbes were also studied. The isolates were found to be most efficient in utilizing oil at 1% in mineral salts medium and achieved 90% oil degradation. At 3% oil, 65–75% biodegradation efficiency was observed. The transference of plasmid DNA from the bacterium *Pseudomonas* sp. to *E. coli* (CSH 57) Lac^+ str^r was also screened through conjugation. The conjugant (*E. coli*) did not show the ability to degrade oil but showed the parental trait of resistance to ampicillin and carbenicillin. Emulsification is a prerequisite in oil degradation, since the chemically synthesized emulsifying agent poses ecological problems. At present we are investigating the nature of the polymeric emulsifying agent (Biosurfactant), which is produced by these microbes when they are exposed to an oily surface.

Poster 114

DEPOLYMERIZATION OF LIGNINS WITH *Streptomyces lividans* AND *Pleurotus sajor-caju IN VITRO* AND *IN VIVO*

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In our previous work, the complete decolorization of dyes by *Phanerochaete chrysosporium* was correlated to the presence of a strong ligninolytic enzyme or lignin peroxidase (LiP), whereas a change of dye color in various hues by *Pleurotus sajor-caju* was correlated to the presence of a weak ligninolytic enzyme or LiP. Although data in the literature show that LiP is responsible for degradation of lignin, a few researchers have reported that high LiP activity of any organism is not necessarily correlated with degradation or depolymerization of lignin. During the present study, it was observed that although *Streptomyces lividans* and *P. sajor-caju* have low LiP activity, they were able to depolymerize various lignins into oligolignols of low molecular weights *in vivo* and *in vitro*. Depolymerization of lignin into oligolignols of low molecular weights was determined with gel permeation chromatography using Sephadex G-10. The ligninolytic enzyme for depolymerization of lignin was produced by the test organisms without any limitation of nitrogen in the medium, whereas nitrogen limitation is important for *P. chrysosporium.*

Poster 115

INTRINSIC BIOREMEDIATION OF BTEX HYDROCARBONS IN SOIL/GROUNDWATER CONTAMINATED WITH GAS CONDENSATE

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Gas condensate liquids have been found to contaminate soil and groundwater at two gas production sites in the Denver basin operated by Amoco Company. These sites have been closely monitored since July 1993 to determine whether intrinsic aerobic or anaerobic bioremediation of hydrocarbon occurs at a sufficient rate and to an adequate endpoint to support a no-intervention decision. Groundwater monitoring and analysis of soil cores suggest that intrinsic bioremediation is occurring at the sites by multiple pathways including aerobic oxidation, Fe(III) reduction, and sulfate reduction.

Laboratory investigations have been conducted to accompany field observations in order to verify hydrocarbon degradation by field microorganisms and identify the primary biodegradation mechanisms. Two types of experiments were conducted: saturated soil microcosm studies, with excess hydrocarbon and limiting amounts of electron acceptors, and slurry experiments, which were hydrocarbon limiting. The slurry experiments are described in this paper.

In the slurry experiment, 40 g of soil obtained from the field site was mixed with 80 g of an aqueous phase containing nutrients and electron acceptors in 160-mL serum bottles. The aqueous phase also contained soluble components of weathered gas condensate (mostly BTEX). The soil was either pristine or obtained from a contaminated region. Two types of electron-acceptor conditions were used, anoxic and hypoxic. The anoxic condition corresponded to zero oxygen, while the hypoxic condition corresponded to bottles which contained air. In the anoxic series nitrate, Fe(III), sulfate, or carbon dioxide was added as the electron acceptor. In the hypoxic series, each of the four electron acceptors was supplied in addition to air. Two different hydrocarbon concentrations were investigated. Benzoic acid was also used as a carbon source in a third set of experiments. The incubation temperature was 30°C. The aqueous phase was sampled for electron acceptors, hydrocarbons, and possible products of hydrocarbon degradation.

The results of this study have verified that BTEX biodegradation at the field site is possible through multiple mechanisms with utilization of sulfate, nitrate, and Fe(III) as electron acceptors in the absence of oxygen. However, benzene was degraded only in the presence of oxygen.

Poster 116

LONG-TERM EFFECTS OF CRUDE OIL CONTAMINATION AND BIOREMEDIATION IN A SOIL ECOSYSTEM

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Analyses of samples taken from three experimental soil lysimeters demonstrate marked effects on the soil chemistry and on bacterial, fungal, nematode, and plant communities 3 years after the application of crude oil. The lysimeters are located at the Amoco Production Research Environmental Test Facility in Rogers County, Oklahoma, and were originally used to evaluate the effectiveness of managed (application of fertilizer and water, one lysimeter) vs unmanaged bioremediation (one lysimeter) of Michigan Silurian crude oil compared with results from one uncontaminated control lysimeter. Five 2-ft-long soil cores were extracted from each lysimeter, each divided into three sections, and the like sections mixed together to form composited soil samples. All subsequent chemical and microbiological analyses were performed on these nine composited samples.

Substantial variation was found among the lysimeters for certain soil chemical characteristics [percentage moisture, pH, total Kjeldahl nitrogen, ammonia nitrogen (NH_4-N), phosphate phosphorus (PO_4-P), and sulfate (SO_4^{2-})]. The managed lysimeter had 10% the level of total petroleum hydrocarbons of the unmanaged lysimeter. Assessment of the microbial community was performed for heterotrophic bacteria, fungi, and aromatic hydrocarbon–degrading bacteria (toluene, naphthalene, and phenanthrene) by dilution into solid media. There was little difference in the number of heterotrophic bacteria, in contrast to counts of fungi, which were markedly higher in the contaminated lysimeters. Hydrocarbon-degrading bacteria were elevated in both oil-contaminated lysimeters. In terms of particular hydrocarbons as substrates, phenanthrene degraders were greater in number than naphthalene degraders, which outnumbered toluene degraders. Nematodes were extracted from soil samples, identified to genus, and classified according to their mode of nutrition. All vegetation and roots were removed from each lysimeter after the soil samples were taken, representative plants were pressed for identification, and the dry weight of all plants (total biomass) for each lysimeter was determined. The plant species were predominantly those found in disturbed habitats. The greatest number of species was found in the control lysimeter, while the total biomass was highest in the managed lysimeter.

Poster 117

XPS STUDIES OF URANIUM REDUCTION BY *Pseudomonas aeruginosa*

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Although a variety of microorganisms is known to exist in radioactive wastes and in natural deposits of radioactive minerals, the extent to which microbes can alter the mobility of radionuclides is not fully known. *Pseudomonas aeruginosa*, an organism under consideration for potential application in the bioremediation of uranium-contaminated effluents, has been studied with respect to speciation of uranium. For comparison, *Rhizopus arrhizus*, a fungus suspected to bind dissolved hexavalent uranium, was also investigated for uranium speciation. X-ray photoelectron spectroscopy analyses of both biomasses revealed that U(VI) was reduced to U(IV) by *P. aeruginosa* but that no reduction of uranium was observed with *Rhizopus arrhizus*. The possibility of photoreduction of uranyl nitrate by electron-donating organic materials was ruled out due to the absence of tetravalent peaks in UV/visible spectra of irradiated solutions. These results indicate that uranium can be stabilized and immobilized in biomass media.

Poster 118

THE DEGRADATION OF L-TYROSINE TO PHENOL AND BENZOATE IN PIG MANURE—THE ROLE OF 4-HYDROXYBENZOATE

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We studied the formation of odorous compounds in piggery wastes. Phenol and *p*-cresol are generally encountered in these typically anaerobic environments. They are produced from L-tyrosine by microbial metabolism. Phenol is further converted to benzoate via paracarboxylation. We investigated the role of 4-hydroxybenzoate (4HBA) in this conversion. The biochemical pathways were then studied by feeding manure with metabolites (phenol, benzoate, or 4HBA) at concentrations between 5 and 20 m*M*. Metabolites were analyzed by gas chromatography and high-performance liquid chromatography. Experiments were carried out at room temperature. We observed that phenol, when added at a concentration of 5 m*M*, was converted to benzoate without any production of 4HBA. Other experiments showed that 4HBA was decarboxylated to phenol but not dehydroxylated to benzoate. When phenol was added in the presence of benzoate (5 m*M* each) or alone at higher concentrations (10 or 20 m*M*), transient small amounts of 4HBA were observed (about 0.02 m*M*).

Our experiments show that 4HBA is not an intermediate metabolite in the conversion of phenol to benzoate. The decarboxylation of 4HBA to phenol is probably the last step of another degradation pathway. This reaction is proposed to have a weakly reversible property, which explains 4HBA production. 2-Hydroxybenzoate (2HBA) is also degraded to phenol.

In conclusion, we think that phenol is perhaps a crossroad of several pathways in anaerobic environments. It will be interesting to find the origin of 4HBA to better understand the metabolism of aromatic compounds in piggery wastes.

Poster 119

DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY INDIGENOUS MIXED AND PURE CULTURES ISOLATED FROM COASTAL SEDIMENTS

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Polycyclic aromatic hydrocarbons (PAHs) are widespread in the Gulf Coast region. Many PAHs are genotoxic and carcinogenic. The degradation of PAHs proceeds very slowly in nature. A great deal of effort has been directed toward the use of indigenous microorganisms to accomplish bioremediation of the contaminated sites. In this research, indigenous mixed microbial floras were isolated from sediment samples that had been contaminated previously with PAHs and were tested for degradation of fluorine, naphthalene, and phenanthrene as sole carbon sources. Then individual strains were isolated and examined for their ability to degrade fluorine, naphthalene, and phenanthrene. Complete disappearance of PAH compounds, as sole carbon sources, was observed in the case of mixed cultures and individual isolates that mediated degradation. The results are then discussed.

Poster 120

DEGRADATION OF AROCLOR BY CYANOBACTERIA

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Polychlorinated biphenyls (PCBs) are relatively unreactive and hydrophilic. Currently there are concerns of potential health risks due to extensive use of PCBs and their persistence in the environment. Microbial degradation is a promising mechanism to remove pollutants from soil and water. Cyanobacteria are a unique class of gram-negative, oxygen-evolving photosynthetic prokaryotes in aquatic ecosystems. The cyanobacteria *Anacystis nidulans* was treated with different concentrations of the PCB aroclor in batch cultures. The PCB disappeared from the cultures after 1 week of incubation. In addition, the aroclor enhanced the growth of the cyanobacteria. The data indicated the ability of cyanobacteria to degrade and utilize the PCB aroclor for growth. The results are then discussed.

Poster 121

MICROBIAL REDUCTIONS OF SULFUR DIOXIDE—LOWER-COST FEEDSTOCKS AND OPTIMIZED REACTOR CONFIGURATION TO IMPROVE ECONOMIC FEASIBILITY

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Removal of sulfur dioxide (SO_2) from flue gases by certain regenerable processes results in the production of a concentrated stream of SO_2. We have previously proposed a microbial process utilizing mixed cultures of sulfate-reducing bacteria (SRB) with anaerobically digested municipal sewage sludge (AD-MSS) medium as a carbon and energy source for reducing the SO_2 to hydrogen sulfide (H_2S), which can then further be reduced to elemental sulfur via a biochemical process. The cost of feedstock and the volumetric productivity of the bioreactor were the key parameters for the economic viability of the microbial process. In our previous study, we have shown that a continuous process of producing AD-MSS medium could increase the fermentable substrate concentration to greater than 5000 mg/L of chemical oxygen demand (COD). In the present work, various immobilization techniques and bioreactors were investigated to obtain maximum volumetric productivity of the SO_2-reducing bioreactor. An immobilized cell-columnar reactor with porous beads showed a higher SO_2 conversion rate of 8.1 mmol/(h·L) with 95% sulfite reduction compared with other immobilized-cell bioreactors. This reactor exhibited a higher yield of SO_2 reduction per organic carbon oxidized at 5.1 mg of COD/mmol of SO_2. An alternate feedstock such as coal synthesis gas (40–60% CO and 20–35% H_2) has been considered as another low-cost carbon and energy source. Serum bottle experiments and various reactor operations with synthesis gas as feedstock will be discussed. Initial economic analysis will be performed based on the results from laboratory bench-scale studies.

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Poster 122

AN ECONOMIC ANALYSIS OF THE BIOTREATMENT OF REFINERY SPENT SULFIDIC CAUSTIC

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Caustics are used in petroleum refining to remove hydrogen sulfide (H_2S) from various hydrocarbon streams. Once H_2S reacts with the majority of the caustic, the solution becomes known as spent sulfidic caustic. Spent caustics typically have a pH greater than 12, sulfide concentrations exceeding 2–3 wt %, and a large amount of residual alkalinity. Depending on the source, spent caustic may also contain phenols, mercaptans, amines, and other organic compounds which are soluble or emulsified in the caustic.

Spent sulfidic caustics have been successfully biotreated on the bench and pilot scales, resulting in neutralization and removal of active sulfides. Sulfides were completely oxidized to sulfate by *Thiobacillus denitrificans*. Microbial oxidation of sulfide produced acid, which was responsible for at least partially neutralizing the caustic.

An economic analysis has been conducted for a base case of biotreating 10 gal/min of spent sulfidic caustic using a suspended culture of flocculated *T. denitrificans* in a mixer/settler system consisting of a sunken concrete base. The treatment system, complete with nutrient storage, pH control, aeration, and acid storage, was designed for nine cases: three different sulfide concentrations (0.2, 0.6, and 1.0 *M*) and three different OH^- alkalinities (1.0, 2.0, and 3.0 *N*). The analysis shows that caustics can be biotreated for 4–9 cents/gal.

Poster 123

PORPHYRIN-CATALYZED OXIDATION OF TRICHLOROPHENOL

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Extensive use of chlorinated phenols, such as pentachlorophenol, as fungicides and wood preservatives has resulted in significant contamination of groundwater, surface water, and soils with these compounds. The white-rot fungus, *Phanerochaete chrysosporium*, has been shown to degrade a variety of chlorinated phenols through a system of exogenous, relatively nonspecific peroxidases or ligninases. The active site of these ligninase isozymes contains an iron-centered porphyrin which participates in one-electron oxidations of susceptible aromatic nuclei in lignin degradation. In this reaction hydrogen peroxide raises the chelated iron ion to an oxo-IV state, which then abstracts an electron from the aromatic nucleus.

We have previously shown, as have others, that porphyrin-metal complexes alone are capable of catalyzing redox reactions in the presence of bulk reducing or oxidizing agents. These porphyrin-metal complexes are potentially useful to catalyze redox reactions which convert toxic or biologically recalcitrant compounds to compounds which are less toxic and more amenable to conventional biological treatment. Porphyrins, in the absence of protein as in ligninases, are potentially more robust than microbial cultures in the treatment of inhibitory substrates.

In the work described in this paper, 2,4,6-trichlorophenol (TCP) was used as a model compound for chlorinated phenols and as a substrate for various porphyrin-metal complexes acting as oxidation catalysts. Tert-butyl hydroperoxide served as the bulk oxidizing agent. In the presence of iron-centered porphyrins, TCP was shown to be oxidized to less-chlorinated and less-toxic compounds.

Poster 124

TEMPORAL AND SPATIAL VARIATIONS OF MICROBIAL PROPERTIES IN SHALLOW SUBSURFACE SEDIMENTS

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Variations of bacterial colony forming units (CFU), anaerobic glucose mineralization rates (GMR), and bacterial functional community structure over space and time were investigated at a site near Oyster, Virginia. Samples were collected in June and August of 1994 using split-spoon coring tools and a hollow-stem auger system. In June, samples from a soybean field had higher mean log CFU values (4.56 ± 0.23) and the bacterial community exhibited greater capability to utilize multiple sole carbon sources (up to 92% of the 95 sole carbon sources were used) than samples from a corn field (log CFU = 3.43 ± 0.13, fewer than 76% of the sole carbon sources used). Using a two-way analysis of variance in which sampling date and depth served as the factors of interest, we found a significant ($P < 0.05$) time effect on the mean of log CFU for the samples above and at the water table. The means of log-transformed anaerobic GMR for the soybean field were significantly ($P < 0.05$) different below the water table for the two sampling dates. These results indicated that microbial biomass, activities, and community metabolism varied significantly in space and between these two sampling periods and were affected by water availability from recharge and rainfall.

Poster 125

NUTRIENTS WITH SURFACTANT-LIKE PROPERTIES ENHANCE BIODEGRADATION RATES OF TCE

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The effects of surfactants and surfactant-like compounds in conjunction with bacteria in enhancing rates of trichloroethylene (TCE) biodegradation were investigated. TCE degradation was assayed using *Burkholderia cepacia* G4 $PR1_{301}$, a constitutive TCE degrader (M. Shields, University of West Florida), in combination with BioTreat, a commercially available nutrient with surfactant-like properties.

A previous study performed on a soil with a high clay content indicated that BioTreat increased the biodegradation rate of TCE. Potential mechanisms for this phenomenon are (1) increased solubilization of TCE into the aqueous phase and (2) increased nutrients for the bacteria and greater numbers of colony forming units (CFUs). Currently in this study, we found that surfactants did not enhance the partitioning of TCE from the headspace into the liquid phase. This is in contrast to previous work with aromatic hydrocarbons such as toluene, which showed that surfactants enhanced partitioning of volatile organic compounds from the headspace into the liquid phase. In recent aqueous experiments, BioTreat increased TCE biodegradation rates by >50% over controls. Treatments containing BioTreat had 34% higher CFUs than treatments without BioTreat. This increase in CFUs resulted in increased TCE biodegradation rates due to the nutrient effect of BioTreat.

Poster 126

RETAINING ENZYME ACTIVITY DURING DEGRADATION OF TCE BY METHANOTROPHS

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Maintaining high rates of cometabolic degradation of trichloroethylene (TCE) by methanotrophs is difficult because of reductions in enzyme activity. Loss of enzyme activity may be the result of free radical effects caused by the epoxidation of the TCE by the soluble methane monooxygenase (sMMO) enzyme. Much of the enzyme activity can be recovered after degradation. The purpose of this study was to determine if compounds added during TCE degradation could reduce the loss of enzyme activity or increase enzyme recovery. Several types of compounds, including formate and formic acid (reducing power and a carbon source) and ascorbic acid and citric acid (free radical scavengers), were added during TCE degradation at a concentration of 2 m*M*. A saturated solution of calcium carbonate was also tested. We measured the rate and extent of enzyme recovery in the presence of formate and methane. Of the compounds tested, only calcium carbonate and formic acid had a beneficial effect. The citric acid actually had a negative effect on sMMO activity; pH in this treatment fell to 4.9. In all other treatments, the pH remained at about 7. The calcium carbonate and formic acid both reduced the loss of enzyme activity and resulted in the highest levels of enzyme activity after recovery.

Poster 127

RECYCLING OF FGD GYPSUM TO CALCIUM CARBONATE AND ELEMENTAL SULFUR USING MIXED SULFATE-REDUCING BACTERIA WITH SEWAGE DIGEST OR SYN-GAS AS A CARBON SOURCE

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A combined chemical and biological process for the recycling of flue gas desulfurization (FGD) gypsum into calcium carbonate and elemental sulfur is demonstrated. In this process, a mixed culture of sulfate-reducing bacteria (SRB) utilizes sewage digest or synthesis gas as the carbon source to reduce FGD gypsum to hydrogen sulfide. The sulfide is then oxidized to elemental sulfur via reaction with ferric sulfate, and accumulating calcium ions are precipitated to calcium carbonate using carbon dioxide. Employing anaerobically digested municipal sewage sludge (AD-MSS) medium as a carbon source, SRB in serum bottles demonstrated an FGD gypsum reduction rate of 8 $mg \cdot L^{-1} \cdot h^{-1} \cdot (10^9 \text{ cells})^{-1}$. A chemostat with continuous addition of both AD-MSS media and gypsum exhibited sulfate reduction rates as high as 1.3 kg FGD gypsum/($m^3 \cdot$day). The increased biocatalyst density afforded by cell immobilization in a columnar reactor allowed a productivity of 152 mg SO_4/(L·h) or 6.6 kg FGD gypsum/($m^3 \cdot$day). Both reactors demonstrated 100% conversion of sulfate, with 75 to 100% recovery of elemental sulfur and a COD utilization as high as 70%. Calcium carbonate was recovered from the reactor effluent upon precipitation using carbon dioxide. The formation of two marketable products—elemental sulfur and calcium carbonate—from FGD gypsum sludge, combined with the use of a low-cost carbon source and further improvements in reactor design, promises to offer an attractive alternative to the landfilling of FGD gypsum.

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Poster 128

RELATING WATER AND SEDIMENT CHEMISTRY TO MICROBIAL CHARACTERIZATION AT A BTEX-CONTAMINATED SITE

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The National Center for Manufacturing Science is investigating bioremediation of petroleum hydrocarbon at a site in Belleville, Michigan. As part of this study, we examined the microbial communities at the site to help elucidate biodegradative processes currently active. We observed high densities of aerobic hydrocarbon degraders (10^{3-4} cells$\cdot g^{-1}$) and denitrifiers (10^6 cells$\cdot g^{-1}$) in the less-contaminated sediments (0.2 mg of BTEX$\cdot kg^{-1}$) as well as low densities (10^2 cells$\cdot g^{-1}$) of iron and sulfate reducers. In contrast, the highly contaminated sediments (10–16 mg of BTEX$\cdot kg^{-1}$) showed low densities of aerobic hydrocarbon degraders, methanogens, and denitrifiers (10^1, 10^2, and 10^5 cells$\cdot g^{-1}$, respectively) and high densities of iron and sulfate reducers (10^4 and 10^3 cells$\cdot g^{-1}$, respectively). Phospholipid fatty acid analyses indicated higher biomass and greater ratios of stress indicators in the highly contaminated area, which was also more reduced (Eh—69 mV) and had lower sulfate (37 mg$\cdot L^{-1}$) than the less-contaminated area (Eh—47 mV, sulfate 68 mg$\cdot L^{-1}$). The microbiological and chemical data suggest that the subsurface environment at the highly contaminated area had progressed into sulfate reduction and methanogenesis. The less-contaminated area appeared to be progressing into primarily iron- and sulfate-reducing microbial communities. The treatment proposed to stimulate bioremediation includes addition of oxygen, but the microbial characterization indicates that other electron acceptors might also be important in implementing treatment to achieve effective and efficient bioremediation.

Poster 129

WASTEWATER TREATMENT WITH AN IMMOBILIZED-CELL REACTOR

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A bench-top bioreactor with immobilized cells was set up to treat municipal wastewater and wastewater from the central kitchen of a major fast-food restaurant in Hong Kong. The biodegradation of the two types of wastewater was carried out by the reactor's microorganisms isolated from the municipal and food wastewaters, respectively. These cells were allowed to be adsorbed to the surface of corrugated plastic plates installed inside the aerated reactor. After the acclimatization period, the bioreactor was fed daily with wastewater, using cycles of steps similar to those of a sequencing batch reactor (i.e., fill, react, settle, and draw).

Results of the biodegradation study of the municipal wastewater indicated that over a period of 24 days, the bioreactor had a daily efficiency of removing 90% of the chemical oxygen demand (COD) in the wastewater containing 1000 COD mg/L with a hydraulic retention time (HRT) of 8 to 12 h. However, for influent with 3000 COD mg/L, the HRT was 20 h.

For fast-food wastewater of 2500 COD mg/L, the bioreactor showed a 96% COD daily removal efficiency over a period of 10 days with a HRT of 7 h.

This bioreactor system could be further modified for effective application in treating wastewater from small food factories or restaurants and for home use in large populated cities to remedy the pollution of underground and coastal waters and to offset the surcharge fees for effluent of high COD.

Poster 130

MODIFIED SBR TECHNOLOGY FOR WASTEWATER PRODUCED BY FOOD INDUSTRIES

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Studies of modified sequencing batch reactor (SBR) systems have been conducted to investigate their potential applicability to the treatment of the high-strength wastewater produced by food industries. These modified SBR systems have used immobilized bacterial cells. In this study, modified SBR systems using immobilized cells and using suspended cells were operated in parallel, and the wastewater treatment efficiencies were compared. Significant removal rates for COD (75–95%), BOD_5 (50–75%), and suspended solids (70–80%) were achieved. Studies of both modified SBRs using immobilized cells and those using suspended cells were outlined and details given of the experimental results. The advantages of each system and its potential applicability to the food industry were compared. The experiment demonstrated that the modified SBRs are stable reactor systems that can produce high-quality effluent. The immobilized-cell SBR could be most suitable for on-site treatment of food industry effluent.

Poster 131

THE BENEFITS OF INTEGRATED BIOLOGICAL PROCESSES IN THE PRETREATMENT OF AN INDUSTRIAL WASTEWATER

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The increasing demand for improved effluent quality from wastewater treatment plants coupled with the concept of user charges has proved to be an effective stimulus for the application of various waste pretreatment processes in industry. As an example, the wastewater streams of the meat packing and processing industry are characterized by relatively high wastewater volumes generated per unit animal slaughtered. These waste streams are commonly high in BOD, suspended solids, and total Kjeldahl nitrogen (TKN).

An analysis of the wastewater treatment charges applied by a municipality to an industrial food processing waste indicates a potential for savings to the industry by the application of a combination of anaerobic, anoxic, and aerobic treatment processes. An existing anaerobic treatment process, operated for almost 10 years, has generated savings in the form of methane generation and reduced BOD discharges to the municipality, but the continuing wastewater treatment costs to the company from its discharge of BOD, TKN, and suspended solids to the municipal wastewater plant suggest a potential for further savings through the application of anoxic and aerobic stages in the company's waste treatment process.

Poster 132

REMOVAL OF COLOR FROM PAPER MILL BLEACH PLANT EFFLUENT BY SEQUENTIAL ENZYMATIC AND MICROBIAL TREATMENT

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A characteristic of paper mill effluent is its dark brown color, which is associated with the lignin and tannin compounds. The color must be removed before the effluent can be discharged, normally to nearby natural streams. Several biological methods for paper mill effluent color removal have been investigated. One of these methods involved the use of horseradish peroxidase plus catalytic amounts of hydrogen peroxide. In this treatment, the disappearance of color was very fast initially, but gradually decreased and could not be restored to the initial rate, even with the addition of enzyme and hydrogen peroxide. When the peroxidase/hydrogen peroxide method was used, the ultimate color removal from a bleach plant effluent having initial color of 3000 color units was 75%. In a new process, the peroxidase/hydrogen peroxide–treated bleach plant effluent was inoculated with a *Baccilus subtilis* strain that was isolated from a soil sample and was selected for its capability of growing on a commercial tannin as the sole carbon source. After 24 h, peroxidase and hydrogen peroxide were added to the microbially treated wastewater. Upon this addition, the color was removed at an initial rate comparable with that observed initially in the first enzymatic treatment. The ultimate removal of color was 92%. The results of this work and its potential commercial application will be discussed.

Poster 133

THE KINETICS OF THE BIOLOGICAL REDUCTION OF CHROMATE

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The development of techniques for bioremediation of soil and groundwater contaminated with toxic chromate ions is discussed. A consortia of subsurface bacteria, obtained from a site contaminated with chromate, were cultured and grown *in vitro*. The reactor consisted of a 1-L polycarbonate vessel fitted with gas and liquid sampling ports and instrumentation to measure pressure, temperature, pH, and oxidation/reduction potential. All laboratory apparatuses were autoclavable and metal free. The consortia demonstrated the ability to reduce hexavalent chromate to the less-toxic trivalent chromic form. Kinetic expressions were developed to describe microbial growth and chromate reduction. These expressions account for effects of microbial growth, nutrient consumption, and contaminant reduction. Analytical methods for chromium speciation are discussed.

Poster 134

REDUCTION OF Cr(VI) TO Cr(III) IN A PACKED-BED BIOREACTOR

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Hexavalent chromium, Cr(VI), is a common and toxic pollutant in soils and waters. Reduction of the mobile Cr(VI) to the less mobile and less toxic trivalent chromium, Cr(III), is achieved with conventional chemical reduction technologies. Alternatively, Cr(VI) can be biochemically reduced to Cr(III) by anaerobic microbial consortia which appear to use Cr(VI) as a terminal electron acceptor. A bioprocess for Cr(VI) reduction has been demonstrated using a packed-bed bioreactor containing ceramic packing and then compared with a similar bioreactor containing DuPont Bio-Sep™ beads. An increase of volumetric productivity [from 4 mg $Cr(VI)\cdot L^{-1}\cdot h^{-1}$ to 260 mg $Cr(VI)\cdot L^{-1}\cdot h^{-1}$], probably due to an increase in biomass density, was obtained using Bio-Sep™ beads. The beads contained internal macropores which were shown by scanning electron microscopy to house highly dense concentrations of bacteria. Comparisons with conventional Cr(VI) treatment technologies indicate that a bioprocess has several economic and operational advantages.

Poster 135

SEQUENCING BATCH BIOFILM REACTOR FOR TREATING HIGH-STRENGTH TRADE EFFLUENT

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A novel sequencing batch filter (SBF) was designed by incorporating immobilized biofilms into a conventional sequencing batch reactor (SBR) to improve treatment efficiency, increase tolerance to shock loadings, and reduce sludge wasting.

The 20-L vessel was packed with fire-expanded clay pellets. A dense biofilm was established on the packaging medium after a seeding and acclimatization procedure. The system was used to treat a restaurant kitchen wastewater, which had an average COD level of 5500 mg/L, more than 50 wt % of which was oil and grease. The organic strength of the wastewater characteristically fluctuated, resulting in significant organic shock loadings. The SBF was studied under different hydraulic retention times (HRTs), and the packing medium was extracted for observation of the biofilms.

The SBF system, operated at batch loading rates not exceeding 1.2 mg COD·(mg VSS)$^{-1}$·day^{-1} with a reaction–to–contact time ratio of 0.8, could achieve at least 97% COD removal. The performance surpassed the loading rates of 0.2–0.4 mg COD·(mg VSS)$^{-1}$·day^{-1} commonly associated with the conventional SBR. The immobilized biofilms also made the SBF system more tolerant to organic shock loadings due to changes in HRT. The COD levels in the treated effluent remained unaffected by shock loadings throughout the entire 260-day study. The VSS in the treated effluent and the MLVSS remained low, indicating that the immobilized biofilm had low specific-growth yields. This reduced the sludge-wasting requirements in the SBF system.

Poster 136

SELECTION OF HYDROCARBON-DEGRADING BACTERIA BASED ON DRYING-RESISTANCE CRITERIA

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Different bacterial products have been developed for bioremediation of contaminated soils. These products are commercially available primarily as desiccated starter culture. However, the efficiency of such starters is, in most cases, not well examined [A. D. Venosa et al., *J. Ind. Microbiol.* **10**, 13–23 (1992)] for various reasons. Most of the experiments carried out in the field of bioremediation are focused on degradation studies *in vitro*, metabolic analyses, etc. The question of implementation of a bacterial product (i.e., the form in which it is applied and the behavior of this product in different environments) has attracted little attention. For example, despite their intrinsic importance, the effects of the desiccation process on microorganisms seem to continually escape the critical attention of researchers.

The work presented here consists of applying a new selection strategy to hydrocarbon-degrading bacteria based on tolerance to desiccation. Two techniques have been applied in pursuing this goal.

With the first procedure, a sample of diesel-polluted soil was dried by fluidization. Microorganisms resistant to this drying process were then selected on solid medium with diesel as the sole carbon source. Sixteen different bacteria were isolated. Their growth in liquid medium in the presence of diesel or a mixture of three polycyclic aromatic compounds was tested. Two of them were able to grow well on diesel liquid medium.

With the second technique, a collection of 12 bacteria belonging to different genera (*Acinetobacter*, *Alacaligenes*, *Arthrobacter*, *Pseudomonas*, *Rhodococcus*, and *Sphingomonas*) and possessing a high level of hydrocarbon degradation potentialities was submitted to a standardized freeze-drying procedure. A classification of bacteria was established on the basis of drying tolerance. Their viability after spray-drying, freeze-drying, and fluidization is under investigation.

Poster 137

REDUCTION OF URANIUM BY MICROBIAL CELLS AND CELL FRACTIONS

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Certain sulfate-reducing bacteria have been shown to effect a reduction in the valence state of uranium by means of a "uranium reductase" enzyme. This microbially catalyzed process converts the highly soluble oxidized form of uranium, U(VI), to the highly insoluble reduced species, U(IV). The subsequent binding of an anion results in the precipitation of a uranium mineral. Recent research suggests that this biogenic precipitation of uranium is a major factor in global uranium deposition. In the anaerobic sulfate-reducing bacterial genus *Desulfobvibrio*, the electron-transport protein cytochrome c_3 has been shown to be responsible for uranium reduction. Our research explores the extraction and immobilization of cytochrome c_3 uranium reductase for development of an *in vitro* uranium precipitation system. Preliminary research has shown that the facultative aerobe *Escherichia coli* may also have uranium reductase ability, a phenomenon never before demonstrated.

Poster 138

EFFICIENT BIOTREATMENT OF HAZARDOUS WASTES

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There are two main processes for the treatment of organic (hazardous) wastes: (1) aerobic and (2) anaerobic. At times a combination of these treatments is used to treat organic wastes. However, not every organic waste is biodegradable under anaerobic fermentation conditions. Anaerobic fermentation affords biogas (and sometimes manure also) from the organic wastes to meet part of the process energy needs. A technique such as Aerobic-Anaerobic-Cometabolic Biotreatment (AACB) may help in degrading organic wastes to generate biogas. It is suggested that studies on the AACB technique may be extended to make the waste treatment processes efficient.

Poster 139

DECHLORINATION OF γ-HEXACHLOROCYCLOHEXANE (LINDANE) BY CYANOBACTERIUM *Anabaena* sp. REQUIRES FUNCTIONAL NITRATE/NITRITE REDUCTASE OPERON

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Biodegradative capacities of different groups of microorganisms are enhanced in the presence of nitrate. Nevertheless, no strong experimental evidence supporting the mechanism of this stimulation has been offered. Recently, we showed that cyanobacteria *Anabaena* sp. strain PCC7120 and *Nostoc ellipsosporum* can dechlorinate γ-hexachlorocyclohexane (HCH, or lindane) and that this dechlorination is dramatically enhanced in the presence of nitrate, similar to the enhancement of biodegrading capacities of anaerobic denitrifiers.

We showed that the rate of degradation of HCH by *Anabaena* sp. was enhanced proportionally to nitrate concentration in the medium. Substitution of nitrite for nitrate also caused enhancement in lindane degradation. In the absence of nitrate or nitrite, *Anabaena* sp. was unable to degrade lindane. Furthermore, 100 μ*M* ammonium inhibited HCH degradation by cyanobacteria. Therefore, patterns of induction and inhibition of HCH degradation coincided with those known for nitrate/nitrite reductase operon. *Anabaena* sp. with a transposon-induced mutation in the upstream portion of this operon was unable to degrade lindane. We propose the role of nitrate/nitrite reductase operon in dechlorination by *Anabaena* sp.

Poster 140

MICROBIAL DEGRADATION OF A NOVEL POLYMER REDUCES THE VOLUME OF HAZARDOUS WASTE MATERIAL

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Materials that come into contact with hazardous or radioactive wastes become contaminated themselves, and costly disposal practices must be followed. Most of the volume of these waste materials is not hazardous and could be disposed of as conventional waste if it is separated from the hazardous material. We have used a novel biodegradable plastic to fabricate articles of clothing for the routine handling of hazardous and radioactive materials. The biodegradable plastic, a polybutyrate-polysuccinate polymer, is easily broken down by microorganisms, and a substantial fraction of the mass is converted to carbon dioxide. The resulting volume reduction of the waste material translates into substantial savings in landfill space and disposal costs. We have partially characterized a microbial community that actively degrades the biopolymer and describe the conditions of incubation that enhance its biodegradation. Oxygen is continuously supplied through a rotary incubation unit, while nitrogen and phosphate are added at a ratio to carbon that is calculated from the biopolymer added. Properties of the biopolymer are provided, and applications of this technology to waste reduction are described.

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