NREL/SP-420-7849
DE95004054

Seventeenth Symposium on Biotechnology for Fuels and Chemicals

Program and Abstracts

The Westin Vail
Vail, Colorado, U.S.A.
May 7–11, 1995

Sponsors:

U.S. Department of Energy
Office of Alternative Fuels
Office of Industrial Processes

National Renewable Energy Laboratory

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National Corn Growers Association

Raphael Katzen Associates International, Inc.

South Point Ethanol

Weyerhaeuser

American Chemical Society
Division of Biochemical Technology

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New Energy Corporation of Indiana
Raphael Katzen Associates International, Inc.
South Point Ethanol
St. Lawrence Technologies Inc.
Weyerhaeuser
American Chemical Society
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National Renewable Energy Laboratory
Golden, Colorado

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Oak Ridge, Tennessee

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Standards and Technology
Germantown, Maryland

Jonathan Woodward
Oak Ridge National Laboratory
Oak Ridge, Tennessee

SPECIAL EVENTS AND GUEST PROGRAM

- **Sunday, May 7, 1995**

  - **Evening reception** — for registered attendees and their guests. Light snacks, no charge. *(Cascade Ballroom)*

- **Monday, May 8, 1995**

  - **Morning guest program** — for guests of attendees. A continental breakfast and information on appropriate excursions will be provided. The guest program fee includes participation on Tuesday and Wednesday mornings as well. *(Goldenrod Suite)*

  - **Guest program tour of Redstone and Redstone Castle** — The charismatic town of Redstone is located about one and a half hours from Vail. This sleepy, one-street community was established in the late 1890s by steel magnate John Osgood. Osgood spent a considerable amount of time and money building a hunting castle for himself and his cronies. Enjoy lunch served at the Redstone Inn, a tour of the castle, and time to visit the tiny boutiques and mountain art galleries. Price includes round-trip transportation, lunch at the Redstone Inn, admission to the castle, guide, all taxes, and gratuities. Tour will depart from in front of hotel at 10:00 a.m. and return at 4:00 p.m.

  - **Social gathering and evening banquet at the Westin Vail** — The symposium registration fee includes the social hour and banquet; guests of attendees may purchase tickets. *(Centennial Foyer and Ballroom)*

- **Tuesday, May 9, 1995**

  - **Morning guest program** — see Monday, May 8 for details.

  - **Guest program tour of Silverthorne Factory Outlet shopping** — Take the guided tour of the Silverthorne Factory Outlets. More than 50 stores filled with designer fashion apparel, leather, crystal, china, and jewelry, with prices 20% to 70% below retail. Price includes round-trip transportation, guidebook, store discount book, and all taxes and gratuities. Tour will depart from in front of hotel at 9:00 a.m. and return at 1:00 p.m.

  - **Wine tasting and luncheon buffet** — for registered attendees. Guests of attendees may purchase tickets. *(Cascade Ballroom)*

- **Horseback riding** — Price includes round-trip transportation, two-hour ride, guide, all taxes, and gratuities. Tour will depart from in front of hotel at 1:00 p.m. and return at 4:00 p.m.

- **Hiking trips** — can be arranged at no additional cost.

- **Progressive dinner** — This is a wonderful way to experience some of the local restaurants in Vail. This evening will include dining at four area restaurants. The first stop will be for cocktails and hors d'oeuvres. You will get to renew old acquaintances before venturing on to your second restaurant for an appetizer and salad. At the third restaurant, you will be offered a choice of three entrees for the main course. As the grand finale, the last stop is for dessert and coffee. This will be an evening to remember and an opportunity to sample some of the finest restaurants in Vail. Price includes round-trip transportation, four selected restaurants with four-course dinner, choice of wine, beer, or champagne at cocktail reception, all taxes, and gratuities. Tour will depart from in front of hotel at 6:00 p.m. and return at 9:00 p.m.

- **Wednesday, May 10, 1995**

  - **Morning guest program** — see Monday, May 8 for details.

  - **Guest program day trip to Glenwood Springs** — Glenwood Springs is located about halfway between Aspen and Vail at the junction of the Colorado and Roaring Fork rivers. The town is most renowned for having the world's largest heated outdoor mineral hot springs pool and vapor caves, which have been the center of this resort community since the 1880s. Enjoy lunch at the Hotel Colorado before taking a plunge in the hot springs pool, have a massage at the Vapor Caves, or browse through shops. Price includes round-trip transportation, lunch at the famous Hotel Colorado, pool pass to the hot springs pool, guide, all taxes, and gratuities. Tour will depart from in front of hotel at 10:00 a.m. and return at 4:00 p.m.

  - **Tour of the National Renewable Energy Laboratory** — Tour the National Renewable Energy Laboratory in Golden, Colorado, with an emphasis on the bioprocessing research facilities and the new ethanol pilot plant. Price includes round-trip transportation and box lunch. Tour will depart from in front of hotel at 12:00 p.m. and return at about 6:30 p.m.

  - **Four-wheel jeep tour** — Experience the magnificent Rocky Mountains within the White River National Forest. This four-wheel jeep tour will take you to high mountain meadows and spruce-filled forests. Price includes jeep, guide, refreshments, all taxes, and gratuities. Tour will depart from in front of hotel at 1:00 p.m. and return at 4:00 p.m.

Program

Sunday Evening, May 7, 1995

6:00-10:00 Registration *(Cascade Foyer)*

7:00-10:00 Reception - for registered attendees and guests, light snacks, no charge. *(Cascade Ballroom)*

Monday Morning, May 8, 1995

7:30-8:15 Continental Breakfast - for registered attendees. *(Cascade Ballroom)*

7:30-5:00 p.m Registration *(Cascade Foyer)*

9:00-10:00 Guest Program and Continental Breakfast. *(Goldenrod Suite)*[1]

8:20 Welcome and Introduction to the Symposium, **Charles E. Wyman**, National Renewable Energy Laboratory, Golden, Colorado *(Cascade Auditorium Theatre)*

Session 1. Thermal, Chemical, and Biological Processing. Chair: **Marion Bradford**, A.E. Staley, Decatur, Illinois; Cochair: **Cynthia Riley**, National Renewable Energy Laboratory, Golden, Colorado.

8:30 Introduction and Session Overview *(Cascade Auditorium Theatre)*

8:45 Paper 1. "Initial Operation of a High-Solids, Pilot-Scale Reactor for Dilute-Acid Pretreatment of Lignocellulosic Biomass," **T. Hsu**, M. Himmel, D. Schell, J. Farmer, and C.E. Wyman, National Renewable Energy Laboratory, Golden, Colorado; and M. Berggren, Hazen Research, Inc., Golden, Colorado

9:10 Paper 2. "Steam-Explosion Pretreatment of Sugar Cane Bagasse Without Catalysts: Process Optimization," **F. Teixeira da Silva**, G.J.M. Rocha, A.R. Cotrim, A.R. Goncalves, and A. Ferraz, Centro de Biotecnologia, Lorena, SP, Brazil; and U. Schuchardt, Universidade Estadual de Campinas, Brazil

9:35 Paper 3. "Measurement of the Inhibitory Potential and Detoxification of Biomass Pretreatment Hydrolyzate for Ethanol Production," **C.J. Rivard**, R.E. Engel, T.K. Smith, N.J. Nagle, C. Hatzis, and G.P. Philippidis, National Renewable Energy Laboratory, Golden, Colorado

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[1] Guests may purchase tickets for the continental breakfasts Monday - Wednesday.

9:55 Intermission

10:20 Paper 4. "Conversion of Lignocellulosics Pretreated with Hot Compressed Liquid Water to Ethanol," P. van Walsum, S.G. Allen, M.J. Spencer, and M.J. Antal Jr., University of Hawaii at Manoa, Honolulu, Hawaii; and **L.R. Lynd**, Dartmouth College, Hanover, New Hampshire

10:45 Paper 5. "Recovery of Dilute Ethanol Using a Carbonaceous Adsorbent," R. Hendrickson and **M.R. Ladisch**, Purdue University, West Lafayette, Indiana

11:10 Paper 6. "Downstream Processing of Acetate Fermentation Broths by Nanofiltration," I.S. Han and **M. Cheryan**, University of Illinois, Urbana, Illinois

11:35 Paper 7. "Chemicals Derived From 3-Dehydroshikimic Acid: A Manufacturing Tree Based on D-Glucose," **J.W. Frost,** Michigan State University, East Lansing, Michigan

Noon Session Adjournment

Monday Afternoon, May 8, 1995

Session 2. Applied Biological Research. Chair: **Ting Carlson,** Cargill, Inc., Minneapolis, Minnesota; Cochair: **William Apel**, Idaho National Engineering Laboratory, Idaho Falls, Idaho.

1:30 Introduction and Session Overview *(Cascade Auditorium Theatre)*

1:40 Paper 8. "Fermentation of Lignocellulosic Hydrolyzates—A Review," **B. Hahn-Hägerdal**, Lund University, Lund, Sweden

2:10 Paper 9. "Metabolic Engineering of a Xylose-Fermenting *Zymomonas mobilis* for Efficient Conversion of Lignocellulosic Feedstocks to Ethanol," **S.K. Picataggio**, C. Eddy, K. Deanda, M.A. Franden, M. Finkelstein, and M. Zhang, National Renewable Energy Laboratory, Golden, Colorado

2:35 Paper 10. "Peculiarities in the Regulation of the Ethanol Formation by the Xylose Fermenting Yeast *Pichia stipitis*," **V. Passoth**, M. Zimmermann, and U. Klinner, Institut für Biologie IV (Mikrobiologie), Aachen, F.R.G.

3:00 Intermission

3:20 Paper 11. "Bioconversion of Carbon Dioxide, the Major By-Product of Fermentation, to Ethanol," **T. Conway** and F.R. Tabita, Ohio State University, Columbus, Ohio

3:45 Paper 12. "Metabolic Engineering of *Clostridium acetobutylicum* ATCC 824," **E.M. Green** and G.N. Bennett, Rice University, Houston, Texas

4:10 Paper 13. "Enzymatic Properties of CelS, a Novel Bacterial Exoglucanase," K. Kruus, B. Lytle, and **J.H.D. Wu**, University of Rochester, Rochester, New York

4:35 Paper 14. "Manipulation of Microalgal Lipid Production Using Genetic Engineering," **T.G. Dunahay**, E.E. Jarvis, and P.G. Roessler, National Renewable Energy Laboratory, Golden, Colorado

5:00 Session Adjournment

Monday Evening, May 8, 1995

6:30 Social Gathering *(Centennial Foyer)*[2]

7:30 Banquet *(Centennial Ballroom)*[2]

8:30 After-dinner address: "Future Worlds—Future Minds," **Lowell Catlett**, New Mexico State University, Las Cruces, New Mexico (through Convention Connection)

Tuesday Morning, May 9, 1995

7:30-8:25 Continental Breakfast - for registered attendees. *(Cascade Ballroom)*

7:30-3:00 p.m. Registration *(Cascade Foyer)*

8:00-9:00 Guest Program and Continental Breakfast. *(Goldenrod Suite)*[1]

Session 3. Bioprocessing Research. Chair: **David Ollis**, North Carolina State University, Raleigh, North Carolina; Cochair: **George Philippidis**, National Renewable Energy Laboratory, Golden, Colorado

8:30 Introduction and Session Overview *(Cascade Auditorium Theatre)*

8:45 Paper 15. "Exploiting the Metabolic Oscillations in Continuous Cultures of *Saccharomyces cerevisiae* to Enhance Xylose Fermentation to Ethanol," K.D. Jones and **D.S. Kompala**, University of Colorado, Boulder, Colorado

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[2] For registered attendees, guests may purchase tickets. See Special Events and Guest Program.

9:10 Paper 16. "Biomass 'Toolkit': A New Modeling Approach for Biosystem Evaluation," **E.G. Koukios** and N.J. Kyriazis, National Technical University of Athens, Athens, Greece

9:35 Paper 17. "Production of Cellulase in Solid-State Bioreactor from *Trichoderma reesei* MCG80 on Wheat Straw," **P.S. Chahal** and D.S. Chahal, Université du Québec, Laval, Québec, Canada

10:00 Intermission

10:20 Paper 18. "Thermodynamic Data for Bioprocess Engineering," **R.N. Goldberg** and Y.B. Tewari, National Institute of Standards and Technology, Gaithersburg, Maryland

10:45 Paper 19. "Detailed Material Balance and Ethanol Yield Calculations for the Biomass-to-Ethanol Conversion Process," **C. Hatzis**, G.P. Philippidis, and C. Riley, National Renewable Energy Laboratory, Golden, Colorado

11:10 Paper 20. "Fermentation Process for the Production of Succinic Acid," **N.P. Nghiem**, B.H. Davison, J.E. Thompson, B.E. Suttle, and G.R. Richardson, Oak Ridge National Laboratory, Oak Ridge, Tennessee

11:35 Paper 21. "Rheological, Mass Transfer, Mixing and Biological Kinetics Characterization of Cellulase-Producing *Trichoderma reesei* Suspensions," M. Marten, S. Velkovska, S. Khan, and **D.F. Ollis**, North Carolina State University, Raleigh, North Carolina

Noon Session Adjournment

Noon Wine tasting and luncheon buffet *(Cascade Ballroom)*[2]

Tuesday Evening, May 9, 1995

6:00 Progressive Dinner

7:00-9:00 **Special Topics Discussion Groups**

"Moving Ethanol and ETBE into the Marketplace," Leader, **Frederick L. Potter**, Information Resources, Inc., Arlington, Virginia *(Cascade Auditorium Theatre)*

"Biotechnology for the Forest Products Industry," Leader, **Harry G. Folster**, Weyerhaeuser, Tacoma, Washington *(Centennial Ballroom E & F)*

Wednesday Morning, May 10, 1995

7:30-8:25 Continental Breakfast - for registered attendees. *(Cascade Ballroom)*

7:30-3:00 p.m. Registration *(Cascade Foyer)*

9:00-10:00 Guest Program and Continental Breakfast. *(Goldenrod Suite)*[1]

Session 4. Process Economics and Commercialization. Chair: **Harry G. Folster**, Weyerhaeuser, Tacoma, Washington; Cochair: **Karl Sanford**, Genencor International, Inc., South San Francisco, California.

8:30 Introduction and Session Overview *(Cascade Auditorium Theatre)*

8:45 Paper 22. "Likely Features and Costs of Mature Biomass Ethanol Technology," **L.R. Lynd**, Dartmouth College, Hanover, New Hampshire and Independence Biofuel, Inc.; and R.T. Elander and C.E. Wyman, National Renewable Energy Laboratory, Golden, Colorado

9:10 Paper 23. "Biomass Ethanol Plant Siting Studies: Approaching Commercial Reality," **J. Mielenz**, D. Koepping, and F. Parson, National Renewable Energy Laboratory, Golden, Colorado

9:35 Paper 24. "A Site-Specific Look at Integrating Advanced Biomass-to-Energy Conversion Technologies with an Operating Pulp Mill," M.J. Gradassi, **D.R. Raymond**, and E.R. Zabolotny, Weyerhaeuser Company, Tacoma, Washington

10:00 Intermission

10:20 Paper 25. "Commercial Applications for Industrial Biocatalysis," **K. Sanford** and C. Lehman, Genencor International, Inc., South San Francisco, California

10:45 Paper 26. "The Permitting and Commercialization of Landfill Gas Based Methanol Production Facilities," **P. Wuebben**, R. George, and L. Watkins, South Coast Air Quality Management District, Diamond Bar, California; and A. Bonny, Terameth Industries

11:10 Paper 27. "Hydrogen Production by Photosynthetic Microorganisms," T. Akano, K. Fukatsu, and H. Miyasaka, Kansai Electric Power Co. and Nankoh Laboratories, Hyogo, Japan; Y. Miura, Kansai Electric Power Co., Hyogo, Japan; Y. Ikuta, Mitsubishi Heavy Industries, Ltd., Yokohama, Japan; **H. Matsumoto**, A. Hamasaki, and N. Shioji, Mitsubishi Heavy Industries, Ltd., Takasago, Hyogo, Japan; and T. Mizoguchi, K. Yagi, and I. Maeda, Osaka University, Osaka, Japan

11:35 Paper 28. "Cellulon® Fiber—Bacterial Cellulose from Culture Vial to Industrial Fermentors," D.F. Brinkmann and **H.G. Folster**, Weyerhaeuser Company, Tacoma, Washington

Noon Session Adjournment

Noon Tour of the National Renewable Energy Laboratory, Golden, Colorado *(departs from in front of hotel)*

Wednesday Afternoon, May 10, 1995

2:00-4:00 **Special Topics Discussion Groups**

"Moving Biodiesel into the Marketplace," Leader, **Steven Howell**, National Biodiesel Board, Bucyrus, Kansas *(Cascade Auditorium Theatre)*

"Trends in Commercial Cellulases — Production, Specifications, and Cost," Leader, **Jane L. Kitchar**, Solvay Enzymes, Inc., Elkhart, Indiana *(Centennial Ballroom E & F)*

Wednesday Evening, May 10, 1995

6:30-10:00 Social Gathering *(Centennial Ballroom & Foyer)*[2]

7:00-10:00 **Poster Session**. A tentative list of poster titles is given at the end of this program. Chair: **Antonios Antonopoulos**, Argonne National Laboratory, Argonne, Illinois; Cochair: **Karel Grohmann**, USDA Citrus and Subtropical Products Research Laboratory, Winter Haven, Florida *(Centennial Ballroom & Foyer)*

Thursday Morning, May 11, 1995

7:30-8:25 Continental Breakfast - for registered attendees. *(Cascade Ballroom)*

7:30-Noon Registration *(Cascade Foyer)*

Session 5. Environmental Biotechnology. Chair: **Kerry Sublette**, University of Tulsa, Tulsa, Oklahoma; Cochair: **K. Thomas Klasson**, Oak Ridge National Laboratory, Oak Ridge, Tennessee

8:30 Introduction and Session Overview *(Cascade Auditorium Theatre)*

8:45 Paper 29. "Biodegradation of Thiodiglycol by *Alcalgenes xylosoxidans* (SH91)," **M.Q. Pham**, W.E. Bentley, S.P. Harvey, and W.A. Weigand, University of Maryland, College Park, Maryland

9:10 Paper 30. "Biomethanation of Wood Hydrolyzate in High-Rate Bioreactors," S.K. Chakrabarti and P.K. Bajpai, Thapar Corporate Research and Development Centre, Patiala, India; and **P.K. Raychoudbury**, IIT, New Delhi, India

9:35 Paper 31. "Biodegradation of Mixed Wastes in Continuously Operated Cyclic Reactors," **B.C. Baltzis**, G.A. Lewandowski, K.W. Wang, and D. Tsangaris, New Jersey Institute of Technology, Newark, New Jersey

10:00 Intermission

10:20 Paper 32. "Biodegradation of PCBs in Slurry Reactors," **J.W. Barton**, K.T. Klasson, and M.E. Reeves, Oak Ridge National Laboratory, Oak Ridge, Tennessee

10:45 Paper 33. "Surfactant Concentration Effects on Volatile Contaminant Bioavailability: Partitioning and 'Micellerization'," **J.M. Strong-Gunderson**, B. Summers, S. Carroll, and A.V. Palumbo, Oak Ridge National Laboratory, Oak Ridge, Tennessee

11:10 Paper 34. "Mechanisms of Intrinsic Bioremediation of Gas Condensate Hydrocarbons Under Electron Acceptor Limiting Conditions," **A. Borole**, J.B. Fisher, K. Raterman, N. Kemp, and K.L. Sublette, University of Tulsa, Tulsa, Oklahoma

11:35 Paper 35. "Effect of Micronutrient on Microbial Respiration of a Shallow Coastal Subsurface and Vadosa Zone," **K.D. Chapatwala**, G.R.V. Babu, and E. Armstead, Selma University, Selma, Alabama; and A.V. Palumbo, C. Zhang, and T.J. Phelps, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Noon Session Adjournment and Symposium Adjournment

POSTER PRESENTATIONS
(Invited to Participate)

Thermal, Chemical, and Biological Processing

Poster 36 "Preparation and Characterization of IEA-NIST Standard Biomass Reference Materials," **F.A. Agblevor**, T.A. Milne, H.L. Chum, and D.K. Johnson, National Renewable Energy Laboratory, Golden, Colorado

Poster 37 "Chemical Interactions Between Aqueous and Organic Phases in a Reactive Extraction Process," **R. Bajpai**, E.L. Iannotti, R. Mueller, C. Scheller, and M. Popovic, University of Missouri, Columbia, Missouri

Poster 38 "Recovery and Recycling of Polymers in Aqueous Two-Phase Extraction," **L. Roy** and R. Bajpai, University of Missouri, Columbia, Missouri

Poster 39 "Biomass Analysis," **J. Brigham,** National Renewable Energy Laboratory, Golden, Colorado

Poster 40 "*In Vitro* Radish Crop-Like Structures as a Possible Source of Natural Anthocyanins," **I.S. Buzovkina,** St. Petersburg State University, St. Petersburg, Russia

Poster 41 "Acid and Enzyme Hydrolysis of Corn Fiber," **P. Tsobanakis**, J. Ulku, and T. Carlson, Cargill Incorporated, Minneapolis, Minnesota

Poster 42 "Enzymatic Hydrolysis of Corn Starch After Extraction of Corn Oil with Ethanol," N.J. Cao, Q. Xu, J.L. Ni, and **L.F. Chen**, Purdue University, West Lafayette, Indiana

Poster 43 "Enzymatic Hydrolysis of AFEX-Treated High-Moisture Corn Fiber," **M. Moniruzzaman** and B. Dale, Texas A&M University, College Station, Texas

Poster 44 "Xylitol Production by Yeasts from Sugar Cane Bagasse: Pretreatments," **J.M. Dominguez**, C.S. Gong, and G.T. Tsao, Purdue University, West Lafayette, Indiana

Poster 45 "The Effect of Storage on Corn Stover as a Feedstock for Biofuels Production," **D.K. Johnson**, F.A. Agblevor, P.A. Ashley, S.D. Deutch, M. Davis, J.A. Fennell, and A. Wiselogel, National Renewable Energy Laboratory, Golden, Colorado

Poster 46 "Ammonia-Recycled Percolation Process for Pretreatment of Herbaceous Biomass," **P.V. Iyer**, S.B. Kim, and Y.Y. Lee, Auburn University, Auburn, Alabama

Poster 47 "Kinetic and Modeling Investigation on Two-Stage Reverse Flow Reactor as Applied to Dilute-Acid Pretreatment of Herbaceous Biomass," **R. Chen** and Y.Y. Lee, Auburn University, Auburn, Alabama; and R. Torget, National Renewable Energy Laboratory, Golden, Colorado

Poster 48 "Fractionation of Herbaceous Biomass by Modified Ammonia-Recycled Percolation Process," **S.B. Kim** (on leave from Geongsang National University, Korea) and Y.Y. Lee, Auburn University, Auburn, Alabama

Poster 49 "Utilization of Acid Hydrolysis Residues as Adhesives for Size Enlargement of Fine Coal Particles," R. Toghiani, G.R. Lightsey, Z. Yusef, **W. Barrier**, and R. Lambert, Mississippi State University, Mississippi State, Mississippi, and Tennessee Valley Authority

Poster 50 "Development of Precipitant Agents for Precipitation of Proteins Based on Hydrophobic Interaction: Precipitation of Bovine Serum Albumin," **E.A. Miranda**, Universidade Estadual de Campinas, Campinas, SP, Brazil; and K.A. Berglund, Michigan State University, East Lansing, Michigan

Poster 51 "Pretreatment of Sugar Cane Bagasse for Enhanced Ruminal Digestion," F.C. Deschamps, Universidade do Vale do Itajaí, Itajaí, SC, Brazil; and **L.P. Ramos** and J.D. Fontana, Federal University of Paraná, Curitiba, Paraná, Brazil

Poster 52 "Foam Fractionation of Barley Malt Amylases in Columns with Reflux," S.L. de Lucena, E.A. Miranda, and **C.C. Santana,** Universidade Estadual de Campinas, Campinas, SP, Brazil

Poster 53 "Biosorption of Precious Metal Ions by Chicken Feather," **K. Suyama** and Y. Fukazawa, Tohoku University, Sendai, Japan

Poster 54 "The Effect of Fermentation (Retting) Time and Harvest Time on Kudzu (*Pueraria lobata*) Fiber Strength," S. Uludag, V. Loha, A. Prokop, and **R.D. Tanner,** Vanderbilt University, Nashville, Tennessee

Poster 55 "Reverse-Flow, Two-Temperature, Dilute-Acid Pretreatment to Enhance Biomass Conversion to Ethanol," **R.W. Torget**, T.K. Hayward, C. Hatzis, and G.P. Philippidis, National Renewable Energy Laboratory, Golden, Colorado

Poster 56 "Palladium-Inactivated Cellobiohydrolase I Does Not Disrupt Cellulose Fibers," **J. Woodward**, L.A. Hamilton, and B.R. Evans, Oak Ridge National Laboratory, Oak Ridge, Tennessee; and J.P. Lassig, Great Lakes Association Student, Albion College, Albion, Michigan

Applied Biological Research

Poster 57 "Synergism and Soluble-Sugar Production in Hybrid Cellulase Systems," **J.O. Baker**, W.S. Adney, R.A. Nieves, S.R. Thomas, and M.E. Himmel, National Renewable Energy Laboratory, Golden, Colorado

Poster 58 "RNA Polymerase Sigma Factors in *Clostridium acetobutylicum* ATCC 824," **J. Wong** and G.N. Bennett, Rice University, Houston, Texas

Poster 59 "Validation of Yeast Viability Stains in an Ethanol Process," P. Meyer and **T. Carlson,** Cargill Incorporated, Minneapolis, Minnesota

Poster 60 "Cloning and Expression of the *Acidothermus cellulolyticus* E1 β-1, 4-Endoglucase Gene in *Streptomyces lividans*," **Y.C. Chou**, T.B. Vinzant, R.A. Nieves, W.S. Adney, R.A. Laymon, M.E. Himmel, and S.R. Thomas, National Renewable Energy Laboratory, Golden, Colorado

Poster 61 "Purification and Characterization of an Acetyl Xylan Esterase from *Aspergillis niger* ATCC 10864," **S.R. Decker** and J.C. Linden, Colorado State University, Fort Collins, Colorado

Poster 62 "Screening for L-Arabinose Fermenting Yeasts," **B.S. Dien**, C.P. Kurtzman, B.C. Saha, and R.J. Bothast, USDA-ARS National Center for Agricultural Utilization Research, Peoria, Illinois

Poster 63 "Production of High Level of Cellulase-Free Xylanse by *Aspergillis niger* Using the Extracts of Steam Exploded Corn Stover," **W. Dong**, Z. Xin, Q. Yinbo, and G. Peiji, Shandong University, Jinan, P.R. China

Poster 64 "Lignocellulases and Ethanol Production From Cellulose by Two Soil Fungi," **L.R. Durrant,** State University of Campinas, Campinas, SP, Brazil

Poster 65 "Cellulases and Xylanases Produced by *T. longibrachiatum*: Properties and Application to the Hydrolysis of Cellulosic Material," V. Reginatto and **L.R. Durrant,** State University of Campinas, Campinas, SP, Brazil

Poster 66 "Microbial Conversion of Synthesis Gas Components to Useful Fuels and Chemicals," **B.B. Elmore**, Louisiana Technical University, Ruston, Louisiana

Poster 67 "Development of a Metalloxylanase and Its Application Biobleaching," **B.R. Evans** and J. Woodward, Oak Ridge National Laboratory, Oak Ridge, Tennessee; L.M. Stephan, Oak Ridge Research Institute, Oak Ridge, Tennessee; R. Margalit, Jet Propulsion Laboratory, Pasadena, California; and A.J. Ragauskas, Institute of Paper Science and Technology, Atlanta, Georgia

Poster 68 "Use of Sugar Cane Juice and Other Disaccharide Sources in the Growth of Yeast and Bacterial Carotenoid Producers," **J.D. Fontana**, M.F. Guimarães, C.A. Fontana, and M. Baron, Federal University of Paraná, Curitiba, Paraná, Brazil; and N.T. Martins, PESCOBRAS Piscicultura do Brazil Ltda., Brazil

Poster 69 "Studies on the E2 Endoglucanase from *Thermomonospora fusca*," **J.A. Franco** and J.C. Linden, Colorado State University, Fort Collins, Colorado

Poster 70 "Saccharification of Native Sugar Cane Bagasse Pith by the Cross-Synergistic Action of Cellulases from *Penicillium sp.* CH-M-001 and *A. terreus* CH-M-013," **O. Garcia-Kirchner** and C. Huitrón, National University of Mexico, Ciudad Universitaria, México, D.F., México

Poster 71 "Ferulate Cross-Linking Limits Degradation of Lignified Grass Walls by Fungal Hydrolases," **J.H. Grabber**, R.D. Hatfield, and J. Ralph, U.S. Dairy Forage Research Center, Madison, Wisconsin

Poster 72 "Fermentation of Orange Peel Hydrolyzates by Ethanologenic *Escherichia coli:* Effects of Nutritional Supplements," **K. Grohmann** and R.G. Cameron, U.S. Citrus and Subtropical Products Laboratory, Winter Haven, Florida; and B.S. Buslig, Florida Department of Citrus, Winter Haven, Florida

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Abstracts for Oral Presentations

Paper 1

INITIAL OPERATION OF A HIGH-SOLIDS, PILOT-SCALE REACTOR FOR DILUTE-ACID PRETREATMENT OF LIGNOCELLULOSIC BIOMASS

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Dilute sulfuric acid pretreatment of lignocellulosic biomass for conversion of the carbohydrate fraction into ethanol and other fuels and chemicals has been intensively investigated over the past 15 years, and despite some potential limitations, it is one of the most effective and promising pretreatment techniques for use in the near term. Almost all of the studies reported thus far have used bench-scale equipment with reactor sizes ranging from a few milliliters to a few liters, and the reactor solids loading has been limited largely to 10% or less. However, an economic analysis of a conceptual biomass-to-ethanol process showed that the solids loading should be significantly higher than 10% for the process to be economically viable. To demonstrate the feasibility of the technique, extend the research database to cover higher solids loadings, provide scale-up data, and produce large quantities of pretreated solids for fermentation research, a 100-L shaft mixer/reactor, made of Carpenter 20Cb3, was designed and fabricated. Following the recent installation, several preliminary runs with a hardwood and a herbaceous biomass have been conducted. This paper describes the high solids pretreatment reactor and its initial operation and compares the results obtained from this reactor with conventional bench-scale reactors.

Paper 2

STEAM-EXPLOSION PRETREATMENT OF SUGAR CANE BAGASSE WITHOUT CATALYSTS: PROCESS OPTIMIZATION

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Sugar cane bagasse was pretreated by the steam-explosion process in a reactor with a capacity of 0.65 L. The process optimization was carried out at temperatures from 165°C to 2109°C and residence times from 5 to 60 min., using 10 g of bagasse (dry weight). The results showed that at 190°C, 15 min., about 36% of the sugar cane bagasse was solubilized and 68% of this percentage was determined as identified compounds. Under these conditions 86% of glucan was recovered as cellulose in the pretreated bagasse, 3.2% was hydrolyzed to glucose, 0.12% was decomposed into hydroxymethylfurfural and 11% into unknown compounds. 73% of xylan was hydrolyzed to arabinose (2.5%), arabinose linked to xylan (1.0%), xylose acetyl groups were hydrolyzed to acetic acid (39%), and acetyl groups linked to xylans (36%); 12% remained in the pretreated bagasse and 13% was lost to unidentified compounds. The quantity of lignin hydrolyzed to soluble products was 20%. Pretreatments under milder conditions showed to be inefficient, whenever high recovery of the hydrolyzed hemicellulose was desired. More drastic conditions promoted condensation reactions among lignin, hydroxymethylfurfural, and furfural. The mass balance was performed for all experiments studied and the results were compared with those obtained in a pilot plant, under optimum conditions. The main reactions involved in this process will be discussed.

Paper 3

MEASUREMENT OF THE INHIBITORY POTENTIAL AND DETOXIFICATION OF BIOMASS PRETREATMENT HYDROLYZATE FOR ETHANOL PRODUCTION

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The Microtox® assay represents a rapid, accurate, and reproducible method for determining the potential toxicity of various samples. This assay was used to evaluate the relative toxicity of a variety of batch dilute and alkaline biomass pretreatment hydrolyzate samples. Toxicity is caused by carbohydrate by-products such as furfural, hydroxymethylfurfural, and acetic acid, generated during pretreatment. The data indicate that the pretreatment samples examined ranged from 2 to 102 toxicity units (TU). Correlations of TU and sample absorbance at several wavelengths were evaluated for all series of samples. Sample TU values best agreed with absorbance at 230 nm, but the unsatisfactory goodness of fit suggests that absorbance should not be used as an absolute measure of sample toxicity.

Microtox data for pretreatment hydrolyzate samples were correlated with the inhibition experienced by the ethanologenic yeast, *Saccharomyces cerevisiae* strain D_5A, during the simultaneous saccharification and fermentation (SSF) process of pretreated biomass. In general, fermentation inhibition was demonstrated for hydrolyzate samples with values exceeding 45 TU. Preliminary studies that focused on reducing hydrolyzate sample toxicity (detoxification) indicate that adding perlite, zeolite, and ion exchange resins had little effect. However, the use of charcoal or a universal flocculent resulted in significant reductions in sample toxicity, holding promise for the efficient bioconversion of pretreated biomass to ethanol. Moreover, the developed toxicity measurement assay can serve as a fast means of monitoring the quality of the pretreatment process. In this way, reliable process control can be exercised on the operation of biomass conversion at the pilot and commercial scale.

Paper 4

CONVERSION OF LIGNOCELLULOSICS PRETREATED WITH HOT COMPRESSED LIQUID WATER TO ETHANOL

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Lignocellulosics pretreated using only hot compressed liquid water were fermented to ethanol at high conversion by saccharification and simultaneous fermentation (SSF). Sugar cane bagasse, aspen chips (25 mm longest dimension), and mixed hardwood flour (-60 +70 mesh) have been evaluated. Reducing the particle size of the lignocellulosics before pretreatment was not required to make the fibers reactive. Batch SSF achieved 90% conversion to ethanol in 2–5 days at enzyme loadings of 15–30 IU/g. In most cases, 90% of the final conversion was achieved within 75 h of inoculation. Comminution of the pretreated bagasse did not affect the conversion to ethanol. In addition, the hydrolyzate produced from the liquid water, pretreatment showed no inhibition of batch growth of *S. cerevisae*. The substrates were pretreated in a custom-built reactor (250 mL) at a variety of solids concentrations. The effect of solids concentration on the conversion to ethanol will also be discussed.

Paper 5

RECOVERY OF DILUTE ETHANOL USING A CARBONACEOUS ADSORBENT

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Modern ethanol distillation processes are designed to ensure removal of all ethanol from the column bottoms; i.e., to levels less than 100 ppm ethanol, and utilize substantial stripping steam to achieve this result. An alternate approach is proposed as a means to reduce energy requirements in the stripping section, and thereby reduce cost. Adsorbents tested for use in such an application showed that carbonaceous supports, in particular Ambersorb XEN 572, completely remove ethanol for a 1% starting alcohol concentration at both 70° and 80°C. A liquid-phase separation apparatus consisting of a 25-mm × 600-mm jacketed column with a 590-mm bed packed with Ambersorb was set up and further experiments were carried out with CO_2 scrubber water obtained from an ethanol plant, which contained 1% alcohol. The feed was passed downflow at 1 bed volume/hr and alcohol-free water was obtained as effluent. Regeneration was readily achieved at 90°C using hot air, vacuum, or superheated steam to strip the ethanol from the column.

The square-wave character of the breakthrough profile and the absence of a temperature increase during sorption indicates an equilibrium mechanism, where alcohol is physically trapped into the pores of this material. These observations combined with the experimentally determined operating conditions have enabled specification of fixed bed adsorption design for sorptive concentration of dilute ethanol. Characteristics of the sorbent, mechanisms of the separation, and mass and energy balances relevant to alcohol plant design will be presented.

Paper 6

DOWNSTREAM PROCESSING OF ACETATE FERMENTATION BROTHS BY NANOFILTRATION

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Acetate can be separated from fermentation broths and partially purified by nanofiltration (NF). Performance of the NF membranes was a function of pressure, pH, concentration of acetate, temperature, and the presence of other media components. Acetate rejection and the degree of dissociation of acetate were closely related. Of all the membranes screened, the best for separation and purification of acetate was Nitto-Denko's NTR-729, with an average acetate rejection of 40%, glucose rejection of 99%, and flux of 60 $Lm^{-2}h^{-1}$ at 250 psig, 50°C, and pH 5.6. Other good membranes were Desal's DS5 and Fluid System's PZ. Based on a preliminary economic analysis, the best downstream strategy is to clarify the fermentation broth by microfiltration (MF), recycle the cells for improving fermenter productivity, nanofilter the cell-free broth, and then evaporate the permeate. The added cost of NF-purification is about $32/ton acetate compared to the cost of potassium acetate ($950/ton) or calcium-magnesium acetate ($650/ton).

Paper 7

CHEMICALS DERIVED FROM 3-DEHYDROSHIKIMIC ACID: A MANUFACTURING TREE BASED ON D-GLUCOSE

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One of the intermediates, 3-dehydroshikimic acid (DHS), in the common pathway of aromatic amino acid biosynthesis is proving to be a highly versatile starting point for synthesis of aromatic chemicals. DHS is synthesized in high yield from D-glucose using a genetically modified strain of *Escherichia coli*. Abiotic oxidation of DHS leads to gallic acid which can, in turn, be enzymatically decarboxyolated to pyrogallol. Dehydration of DHS catalyzed by DHS dehydratase (AroZ) produces protocatechuic acid (PCA) that can be converted into catechol by PCA decarboxylase (AroY). Oxidative ring cleavage of the catechol catalyzed by catechol 1,2-dioxygenase affords *cis*, *cis*-muconic acid that is catalytically hydrogenated to adipic acid. The enzymatic and mechanistic details of each of these conversions will be outlined. Particular emphasis will be placed on the mechanism of abiotic oxidation of DHS to gallic acid and characterization of the *aroY* and *aroZ* genes and the encoded enzymes.

Paper 8

FERMENTATION OF LIGNOCELLULOSE HYDROLYZATES—A REVIEW

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Lignocellulosic raw materials such as forest products and agricultural residues constitute a major renewable energy source for the fermentative production of a renewable liquid fuel such as ethanol, also assuring a sustainable development of the transportation sector. Lignocellulosic raw materials are composed of hemicellulose, cellulose, and lignin. The hemicellulosic and cellulosic fractions are made up of the monosaccharides glucose, mannose, galactose, xylose, and arabinose, the composition being dependent on the nature of the raw material. In soft woods and in agricultural residues, the pentose sugars may account for as much as 40% of the raw material. The raw materials are pretreated and/or hydrolyzed to make the sugars accessible to fermenting microorganisms. In these processes, inherent inhibitors such as acetic acid from acetyl groups in the hemicellulose fraction are released. In addition, inhibitors, such as furfurals and phenolics compounds, are formed due to the chemical reactions in pretreatment and/or hydrolysis. The sugars can be fermented to ethanol with bacteria, yeast, and filamentous fungi. For optimal process economy, all sugars should be fermented to ethanol, preferentially simultaneously. Microorganisms fermenting all sugars in lignocellulosic hydrolyzates to ethanol are either slow or highly susceptible to the inhibitors, whereas efficient fermenting microorganisms such as the yeast *Saccharomyces cerevisiae* only ferment certain sugars. Lignocellulose hydrolyzates have been detoxified to enhance their fermentability, and both bacteria and yeasts have been metabolically engineered to enhance their sugar utilization for ethanol production.

Paper 9

METABOLIC ENGINEERING OF A XYLOSE-FERMENTING *Zymomonas mobilis* FOR EFFICIENT CONVERSION OF LIGNOCELLULOSIC FEEDSTOCKS TO ETHANOL

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An economical biomass-to-ethanol process depends on the rapid and efficient conversion of both its cellulose and hemicellulose components. While many microorganisms can ferment the glucose component in cellulose to ethanol, conversion of pentose sugars in the hemicellulose fraction, particularly xylose, has been hindered by the lack of a suitable biocatalyst. *Zymomonas mobilis* demonstrates many potential advantages for ethanol production, including high ethanol yield and tolerance, high specific productivity, and the ability to ferment sugars at low pH. Unfortunately, *Z. mobilis* is unable to ferment the xylose found in lignocellulosic feedstocks because it lacks the essential xylose assimilation and pentose metabolism pathways. To broaden its range of fermentable sugars, we have metabolically engineered *Z. mobilis* to grow on and ferment xylose to ethanol by introducing the required metabolic pathways. The engineered strain now rapidly and efficiently ferments both glucose and xylose, and provides a unique biocatalyst for the economical conversion of lignocellulosic biomass to ethanol. The metabolic engineering and fermentation performance of this unique biocatalyst will be presented.

Paper 10

PECULIARITIES IN THE REGULATION OF THE ETHANOL FORMATION BY THE XYLOSE FERMENTING YEAST *Pichia stipitis*

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The capacities of fermentation and respiration of the xylose fermenting yeast *Pichia stipitis* were found to be independent of the concentration of glucose or xylose in the medium. The capacity of respiration was not decreased either under semiaerobic conditions. Moreover, the activity of the pyruvate dehydrogenase (PDH) was not changed in reaction to changes in the tension of oxygen. Additionally, the activity of this enzyme was not changed in reaction to different carbon sources. However, pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase were induced by a decrease of the oxygen tension. Contrary to *Saccharomyces cerevisiae*, no induction of the PDC occurred as a reaction to the addition of glucose to ethanol grown cells. By measurements of the intracellular concentrations of glycolytic metabolites in the induction experiments, it was determined that the main signal for the induction of the PDC is not derived from the glycolysis. Therefore, it can be concluded that the regulation of the PDC in *P. stipitis* is achieved by another mechanism than in *S. cerevisiae*. We further investigated some kinetic properties of the PDH and PDC. The PDH was found to be similar in its K_m to that of *S. cerevisiae*. The PDC was found to be similar in its K_m to that of *S. cerevisiae* but it was found to have a higher Hill-constant and it was not inhibited by phosphate.

Paper 11

BIOCONVERSION OF CARBON DIOXIDE, THE MAJOR BY-PRODUCT OF FERMENTATION, TO ETHANOL

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The one billion gallons of fuel ethanol produced in this country each year are primarily derived from corn by using yeast fermentation, a biological process that is inherently inefficient. Fully one-third of the corn starch used for ethanol production is lost through carbon dioxide gas (CO_2), which is the major waste product. The goal of the proposed research is to overcome this inefficiency by developing a microbial based system that is capable of capturing the waste CO_2 for additional ethanol production. This technology would increase the yield of ethanol by one-third, regardless of the biomass source, and make ethanol production more attractive economically.

This research is based on a novel concept: We are using recombinant DNA techniques in our laboratories to genetically engineer a CO_2 fixing bacterium with the ability to make ethanol. Initially, we have chosen to use well-characterized strains of *Rhodobacter sphaeroides* and *R. capsulatus* as recombinant hosts for the cloned ethanol production pathway (comprised of pyruvate decarboxylase and alcohol dehydrogenase) from *Zymomonas mobilis*. We have succeeded in developing two plasmid-based gene transfer systems that work with *Rhodobacter sp.* and many related organisms. Expression of the plasmid-borne *pdc* and *adh* genes has been achieved by fusion to the highly active *Rhodospirillum rubrum cbbM* promoter that normally drives transcription of a key Calvin cycle gene. Both PDC and ADH activities can be detected in recombinant strains of *Rhodobacter* containing the described plasmid. These recombinant strains of *Rhodobacter* are able to produce modest amounts of ethanol when growing on CO_2 as the sole carbon source with light serving as the primary energy source. This result confirms that the recombinant pathway of CO_2 fixation via the Calvin cycle and subsequent conversion to ethanol is functional. Modification of these recombinant bacteria for improved ethanol production from CO_2 is in progress.

Paper 12

METABOLIC ENGINEERING OF *Clostridium acetobutylicum* ATCC 824

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Clostridium acetobutylicum is a Gram-positive, spore-forming bacterium capable of producing acids (acetate and butyrate) and solvents (ethanol, acetone, and butanol). Much research has focused on the regulation of the acetone-butanol fermentation with the aim of developing a more efficient and competitive industrial process to produce solvents. We plan to redirect cellular metabolism in *C. acetobutylicum* by integrating fragments from acidogenic and solventogenic genes into the chromosome. Integration may inactivate, replace, or amplify metabolic genes and improve both the yield and selectivity of desirable products.

Integrational non-replicative plasmids (containing fragments from butanol dehydrogenase, butyrate kinase, and phosphotransbutyrylase genes) have been introduced into the chromosome. Transformation was accomplished by electroporation with relatively high concentrations of methylated plasmid DNA. Southern hybridization experiments revealed that integration occurred by single crossover homologous recombination. The integrants were relatively stable after several generations, and the frequency of integration was dependent upon the length of the homologous DNA fragment. Culture analysis by gas chromatography revealed that the integrants produced different quantities of solvents than wild-type clostridial cells.

Paper 13

ENZYMATIC PROPERTIES OF Ce1S, A NOVEL BACTERIAL EXOGLUCANASE

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CelS is the most abundant and a key catalytic subunit of the *Clostridium thermocellum* cellulosome, a complicated multi-component cellulase aggregate with a total molecular weight of millions. Its enzymatic properties were studied using the recombinant CelS expressed in *Escherichia coli*. The purified rCelS displayed typical exoglucanase characteristics. The hydrolysis products from the crystalline cellulose were cellobiose and cellotriose at a ratio of 5:1. However, cellotetraose was also transiently produced from the amorphous cellulose. The rCelS activity on the amorphous cellulose was optimal at 70°C and at pH5–6. Its thermostability was increased by Ca^{++}. Sulfhydryl reagents only had a mild adverse effect on the rCelS activity. Cellotetraose was the smallest oligosaccharide substrate for rCelS and the hydrolysis rate increased with the substrate chain length. The rCelS activity on cellopentaose was strongly inhibited by cellobiose. Many of these properties were consistent with those of the cellulosome, indicating the key role of CelS in the function of cellulosome. The abundance of CelS in cellulosome is consistent with the generally accepted concept that an exoglucanase is essential for the degradation of crystalline cellulose. It may also contribute significantly to the difference between the fungal and the clostridial cellulase systems.

Paper 14

MANIPULATION OF MICROALGAL LIPID PRODUCTION USING GENETIC ENGINEERING

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Many microalgal species accumulate significant quantities of triacyglycerol storage lipids under conditions of nutrient starvation. These lipids can be isolated and chemically converted into fatty acid methyl esters, which can be used as a substitute for petroleum-derived diesel fuel ("biodiesel"). A goal of our research is to use genetic engineering to optimize the quantity and quality of algal storage lipids for cost-effective production of algal-derived biodiesel. Previous to this report, genetic transformation techniques have not been available for any industrially important microalga. We report here on the successful genetic transformation of two species of oleaginous microalgae. Chimeric transformation vectors were developed in which algal regulatory sequences were used to drive high levels of expression of a foreign gene in the algal cells. The foreign DNA was stably integrated into the host cell genome, and production of the foreign protein was confirmed by Western analysis. Progress is being made toward using this transformation system to affect lipid production in microalgae with potential for biodiesel production.

Paper 15

EXPLOITING THE METABOLIC OSCILLATIONS IN CONTINUOUS CULTURES OF *Saccharomyces cerevisiae* TO ENHANCE XYLOSE FERMENTATION TO ETHANOL

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Saccharomyces cerevisiae exhibits spontaneous oscillations in the concentrations of cell mass, glucose, ethanol, dissolved oxygen, pH, NAD(P)H concentrations over a wide range of continuous culture operating parameters (dilution rate, oxygen supply rate, glucose feed concentration). We have shown that these sustained oscillations are the manifestations of the dynamic competition between the three metabolic pathways (glucose fermentation, glucose oxidation, and ethanol oxidation), but not causally related to the observed cell cycle synchrony. We have developed a metabolic competition model for *S. cerevisiae* that incorporates the complex dynamics of the intracellular enzyme repression and inhibition with a cybernetic perspective as the optimal response to the current environmental conditions. This model predicts accurately the batch aerobic growth dynamics on glucose, including the two exponential growth phases (fermentation of glucose and oxidation of ethanol), with an intermediate diauxic lag phase. We have recently shown that the same dynamic mechanisms of enzyme repression and inhibition create the necessary feedback loop to cause the spontaneous oscillations between the available modes of metabolism in continuous cultures. This metabolic competition model also predicts the experimentally observed spontaneous generation and cessation of these oscillations, when the culture operating parameters are changed into and out of their critical ranges. With this thorough understanding of yeast metabolic oscillations through the simple cybernetic model, they may now be exploited in a number of metabolic engineering applications, such as the enhanced xylose fermentation by recombinant *S. cerevisiae* containing the two *Pichia* genes for conversion of xylose to xylulose.

Paper 16

BIOMASS "TOOLKIT": A NEW MODELING APPROACH FOR BIOSYSTEM EVALUATION

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The complexity of biomass systems acts as an additional constraint in designing feasible and sustainable bioprocesses and biosystems for the large-scale production of fuels and chemicals from biological feedstocks. The object of this paper is to present and discuss a new modeling approach, and the corresponding PC-aided tools ("Toolkit"), making possible the quantitative description of such complex processes and production systems by performing mass and energy balances, as well as other related calculations (of inputs, pollutants, CO_2 effects, other side effects, etc.). This work is based on recent developments in the emerging field of biosystems dynamic modeling; biosystems dynamics are described with the help of a minimum number of parameters, having clear physical and/or technical significance; default values of all those parameters are provided to assist the non-expert user. Two types of biosystems are considered: those aiming at the conversion of typical energy crops; e.g., sweet sorghum, to fuel bioethanol; and integrated utilization of agricultural residues as biofuels. "Toolkit" is a user-friendly, interactive computer program, written in Borland C++ language, and operating under Windows PC environment.

Paper 17

PRODUCTION OF CELLULASE IN SOLID-STATE BIOREACTOR FROM *Trichoderma reesei* MCG80 ON WHEAT STRAW

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Cellulosic material is one of the most abundant solid wastes generated in the developing countries. The fungus *Trichoderma reesei* produces cellulase enzymes that could convert this cellulosic waste to useful products: sugars, pharmaceuticals, single-cell protein, biofuel (ethanol), etc. The cellulase systems of most of the *T. reesei* mutants are deficient in β-Glucosidase. Additional β-Glucosidase from other sources is required for complete hydrolysis of cellulose. In this paper we tried different environmental conditions to improve the yield of Filter Paper (FP) activity with a high ratio of β-Glucosidase (β-G) to FP cellulase by using *T. reesei* MCG80, because accumulation of high cellobiose concentration suppresses further cellulose hydrolysis. A mild pretreatment of wheat straw with 2.5% NaOH (w/w of wheat straw) was sufficient (among the various other concentrations tried: up to 15% w/w) prior to inoculation to expose cellulose to the fungus. Among different nitrogen sources, urea was eliminated from the medium; thus, the medium contained only ammonium sulphate and yeast extract as nitrogen sources. Liquid state cultures gave a maximum of 2 IU FP activity/mL and 2.6 IU β-G activity/mL. However, FP activity of about 4 IU/mL and β-G activity between 4.8 and 6.8 IU/mL was obtained in 24 days of growth in solid-state fermentation on 4 g wheat straw when the cellulase system was extracted with total volume of 100 mL water. This corresponded to 250 IU FP activity/g cellulose and 300–425 IU β-G activity/g cellulose. The hydrolytic potential was more than 75% in 72 h of incubation of delignified wheat straw at 50°C when enzyme in 20 IU FP/g was supplied.

Paper 18

THERMODYNAMIC DATA FOR BIOPROCESS ENGINEERING

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The combined use of equilibrium and calorimetric measurements, coupled with thermodynamic modeling calculations, enables the characterization of the thermodynamics of enzyme-catalyzed reactions. The information obtained allows for the prediction of the position of equilibrium of the variety of biochemical reactions over wide ranges of temperature, pH, and ionic strength. This in turn can be used both to predict the feasibility of possible reactions and to optimize product yields. Some of the classes of reactions which have been studied include isomerization, hydrolysis, and phosphorylation of sugars, and ammonia and water elimination reactions. This methodology has been used to study many of the key industrial reactions, including the conversion of glucose to fructose, catalyzed by glucose isomerase, and the conversion of trans-cinnamic acid and ammonia to L-phenylalanine, catalyzed by L-phenylalanine ammonia-lyase. We have recently studied the hydrolysis of N-acetyl-L-phenylalanine ethyl ester in water and in several organic solvents. The experiments have been coupled with a substantial effort aimed at a critical compilation of all available thermodynamic data for enzyme-catalyzed reactions. Current efforts are being geared toward the study of reactions used for the efficient utilization and conversion of biomass into chemicals and fuels.

Paper 19

DETAILED MATERIAL BALANCE AND ETHANOL YIELD CALCULATIONS FOR THE BIOMASS-TO-ETHANOL CONVERSION PROCESS

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Application of detailed material balance calculations to the evaluation and optimization of biomass conversion processes is of fundamental importance. Due to the lack of a generally accepted framework for material balance calculations, comparison of results from different research groups has been difficult. Inconsistencies are mainly due to difficulties with compositional analysis of lignocellulosic substrates. Evaluation of the data under a detailed material balance framework can reveal internal inconsistencies caused by analysis errors. Material balance templates have been developed to accurately follow the distribution of carbon in lignocellulosic substrates through the pretreatment and SSF processes. The balance computations provide information on the overall carbon recovery, on the recovery of individual sugars after substrate pretreatment, and also on the fractional loss of sugars to degradation products. Since material balances are performed on the solids and liquid streams, information on the solubilized fraction of each biomass component is also available.

Alternative pretreatments are usually evaluated on the basis of ethanol yields, which are typically based on the sugar content of the pretreated solids. A more appropriate basis for such calculations is the sugar content of the untreated substrate since, depending on the pretreatment conditions, different portions of the sugars are solubilized or degraded during the pretreatment. Based on material balance considerations, a set of equations was developed to allow the correct computations of overall ethanol yields for biomass conversion processes involving pretreatment and SSF.

Paper 20

FERMENTATION PROCESS FOR THE PRODUCTION OF SUCCINIC ACID

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A fermentation process is being developed for the production of succinic acid. In rich media with glucose as the main carbon source, a strain of *Anaerobiospirillum succiniciproducens (An. s.)* produced 44 g/L succinic acid in a batch fermentor maintained at 39°C and pH 6.0. In order to achieve high yield of succinic acid, it was necessary to supplement CO_2 produced by the organism with external sources. This was accomplished by using 1.5 M sodium carbonate for pH control and bubbling the gas through the fermentation broth. The main by-products of the fermentation included acetic and lactic acids. This initial process is based on the Michigan Biotechnology Institute patents. Current research is performed to (1) develop fermentation media more suitable for large-scale production, (2) optimize process parameters to improve yield and productivity, and (3) compare other succinic acid-producing organisms to *An. s.* These results will be discussed.

Paper 21

RHEOLOGICAL, MASS TRANSFER, MIXING, AND BIOLOGICAL KINETICS CHARACTERIZATION OF CELLULASE-PRODUCING *Trichoderma reesei* SUSPENSIONS

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Viscous fungal fermentations for cellulase production from cellulosic feedstocks are challenging to characterize because of the simultaneous influence of biological kinetics coupled with several aspects of physical kinetics, including mixing, non-Newtonian fluid behavior, and gas-liquid mass transfer of the oxygen required for aerobic metabolism. We describe studies in which we develop deeper understanding of this complex bioconversion through two achievements: (1) use of steady and dynamic shear measurements on fungal suspension samples which allow us to characterize the instantaneous suspension properties as a Casson fluid and which also indicate operating regimes in which we have reasonable mixing (liquid-like suspension) or poor mixing (gel-like suspension) in the bioreactor, and (2) development of a biological kinetic model in which we for the first time provide simultaneous inclusion of four major Trichodermal characteristics: (a) structured biomass (primary and secondary mycelia), (b) synthesis of cellulases by secondary mycelia only, (c) Monod model dependence of substrate conversion on cellulose concentration, and (d) a fractional conversion dependence of the cellulose reactivity with cellulase. Air-only feeds lead to oxygen mass transfer limitations even at only 5% cellulose; oxygen supplemented feeds avoid this circumstance.

Paper 22

LIKELY FEATURES AND COSTS OF MATURE BIOMASS ETHANOL TECHNOLOGY

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What would be the features and cost of technology for producing ethanol from cellulosic biomass at a level of technical maturity comparable to that of an oil refinery? Although challenging to approach, and currently impossible to answer precisely, this question is of vital importance in considering the possibility and desirability of a major biomass ethanol industry. Scenarios will be defined involving very advanced conversion technology, and the results analyzed in terms of issues such as cost, performance parameters, energy balance, and greenhouse gas emissions.

Paper 23

BIOMASS ETHANOL PLANT SITING STUDIES: APPROACHING COMMERCIAL REALITY

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Technology for enzymatic conversion of lignocellulosic biomass to fuel ethanol has reached the pilot plant scale with the recent commission of the NREL process development unit. In the near future, construction will begin on pre-commercial scale engineering demonstration units (EDUs) to demonstrate this technology at selected biomass-rich locations around the country. These EDUs will likely be built and operated at or near proposed commercial plant sites. Proper site selection can be critical to the long-term profitability of the new commercial biomass ethanol plants. With this in mind, NREL has initiated a program to assist industrial partners in developing a sound business plan that includes a detailed siting study. Included in the siting study is an examination of biomass availability (production and/or delivery systems) and the projected cost, delineation of site requirements and the identification of qualifying sites, examination of environmental impacts and community issues, as well as a detailed financial *pro forma* financial evaluation and projection. The NREL ethanol project currently is supporting eight such business plans as a means of assisting industry to identify economic viable commercial opportunities for biomass ethanol.

Paper 24

A SITE-SPECIFIC LOOK AT INTEGRATING ADVANCED BIOMASS-TO-ENERGY CONVERSION TECHNOLOGIES WITH AN OPERATING PULP MILL

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Weyerhaeuser—together with Amoco Corporation, Carolina Power and Light, and Stone and Webster Engineering Corporation—carried out a feasibility study sponsored by the National Renewable Energy Laboratory and the Electric Power Research Institute to assess the economic merits of expanding the use of biomass at its New Bern, North Carolina, facility to produce electric power and liquid fuels. To reduce oil consumption and energy costs at its facilities, Weyerhaeuser has determined that a combined-cycle power system of about 60 MW for internal use and sale has the potential for significantly increased efficiencies and economy. Biomass gasification is expected to be a means for improving utilization of wood and process wastes while producing gas turbine fuel and reducing environmental impact. The team also assessed the technical merits and business potential of integrating a nominal 1,000 ton/day biomass processing facility to produce ethanol at the New Bern plant. The primary objectives were to evaluate biomass as a source of electric power and liquid fuels within the context of a specific operating pulp mill and to assess the relative value of biomass-derived electric power and liquid fuel. An additional objective was to define the most cost-effective process for commercializing advanced biomass to energy technologies. The paper will describe the conditions that make these technologies potentially attractive, outline the approach taken to develop comparative economics and economic sensitivities, present results of the analysis, and suggest options for proceeding to commercialization of these advanced conversion technologies.

Paper 25

COMMERCIAL APPLICATIONS FOR INDUSTRIAL BIOCATALYSIS

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Significant progress has been made in commercializing a number of products for the enzyme and fine chemical markets over the past five years. Progress has depended upon the integration of science, engineering, and marketing for production of products which deliver benefits to the customer at an acceptable price and a strategy using technology platforms. A technology platform has the potential of yielding a number of products, thereby leveraging resources and investment in addition to providing a strategic approach to technology-driven business opportunities. Examples of this approach include use of protein engineering, expression and secretion technology of proteins and metabolites from microbial strains, and enzymology to connect opportunities in the enzyme business and the chemical industry. In addition, the rapid pace of technology and market needs have necessitated the use of commercial and technology partnerships to successfully develop products. Examples of this strategy will be used to highlight its application to biotechnology and industrial markets.

Paper 26

THE PERMITTING AND COMMERCIALIZATION OF LANDFILL GAS BASED METHANOL PRODUCTION FACILITIES

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Current landfills emit copious amounts of methane which are typically flared on-site. Such flaring results in significant emissions of NO_x as well as potentially serious levels of toxics, greenhouse gases, and other flare gas contaminants. The District has therefore worked closely with Terameth Industries to demonstrate a first-in-the-world plant which diverts this gaseous waste stream, through a syn-gas synthesis process for 50 metric tons per day methanol production at the BKK landfill in West Covina.

Scale-up of this technology is projected to be very commercially competitive with world-wide methanol production economics. For example, contractual commitments for the long-term purchased product have been obtained. Permitting issues unique to landfills have also been addressed as part of this project. This paper will (1) present the commercial and economic implications of the plant design, (2) identify key permitting issues which were addressed to facilitate construction and operation of this facility, (3) identify potential future markets for this conversion technology, and (4) identify the end-use markets expected for fuel methanol by the year 2000 and beyond.

Paper 27

HYDROGEN PRODUCTION BY PHOTOSYNTHETIC MICROORGANISMS

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Hydrogen is a clean energy alternative to the fossil fuels, the main source of greenhouse gas emissions. We developed a stable and efficient system for the conversion of solar energy into hydrogen using photosynthetic microorganisms. Our system consists of the following three stages: (1) photosynthetic starch accumulation in green microalgae (400 L × 2), (2) dark anaerobic fermentation of the algal biomass to produce hydrogen and organic compounds (150 L × 2), and (3) further conversion of the organic compounds to produce hydrogen using photosynthetic bacteria. (three types of reactor, Parallel plate, raceway, and tubular).

We constructed a test plant of this process at Nankoh power plant of Kansai Electric Power Company in Osaka, Japan. We carried out a series of tests using CO_2 obtained from a chemical absorption pilot plant using actual power plant flue gas. The photobiological hydrogen production process used a combination of a marine alga, *Chlamydomonas* Sp. MGA161 and marine photosynthetic bacterium, *Rhodopseudomonas* Sp. W-1S. The dark anaerobic fermentation of algal biomass was also investigated. A sustained and stable starch accumulation, degradation in the algal cell, and hydrogen production from algal fermentation and photosynthetic bacteria were demonstrated during several experiments of about one month duration each.

Paper 28

CELLULON® FIBER—BACTERIAL CELLULOSE FROM CULTURE VIAL TO INDUSTRIAL FERMENTORS

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A number of bacteria produce small quantities of cellulose in static culture. Weyerhaeuser, in a collaborative effort with Cetus Corporation, developed an agitated fermentation process for producing bacterial cellulose. This process has been scaled up and demonstrated at large commercial scale. The product, Cellulon® Fiber, is a novel form of cellulose with unique properties. It is now being used in several commercial products. This presentation will discuss the Cellulon® Fiber project from its initiation through commercialization, with emphasis on the process development and scale-up.

Paper 29

BIODEGRADATION OF THIODIGLYCOL BY *Alcaligenes xylosoxidans* (SH91)

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Thiodiglycol is the primary hydrolysis product of sulfur mustard (2, 2^1-dichlorodiethyl sulfide), commonly referred to as "mustard gas." A bioreactor is used to mineralize the hydrolysis products resulting from the treatment of sulfur mustard with aqueous sodium hydroxide. Primary concerns for this process are cost, reproducibility, and safety. The cost is directly related to reactor volume, and so the growth of *Alcaligenes xylosoxidans* (SH91) on thiodiglycol was modeled by an Andrews type inhibition equation for use in a simulation study of bioreactor type and operating mode. The following reactor configurations were explored: batch, CSTR, CSTR with cell recycle, two CSTRs in series, and repeated fed-batch. The model constants were determined from shake flask and fermentor experiments, and were found to be μ_{max}=0.23 hr^{-1}, K_s=50 mM, K_i=82 mM, and $Y_{x/s}$=0.3 OD/mM. From these constants, the model predicted superior performance of repeated fed-batch reactors over the others, since this type of reactor maintains the highest average cell growth rate. Further analysis of the fed-batch reactor showed that the volume was relatively insensitive to the optimal draw down volume and the feed and reactor effluent concentrations of interest. In practice, this greatly simplifies the operation of the fed-batch fermentor. Moreover, experimental results also confirmed the model predictions and showed that the volume required for a repeated fed-batch reactor was much smaller than the other reactor configurations.

Paper 30

BIOMETHANATION OF WOOD HYDROLYZATE IN HIGH-RATE BIOREACTORS

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Wood hydrolyzate liquor generated during the autohydrolytic pretreatment of wood in the rayon pulp-making process is a highly polluting wastewater; COD 80,000–100,000 mg/L and BOD_5 60,000–75,000 mg/L. Anaerobic treatment of this wastewater in high-rate bioreactor seems to be the most promising proposition for generation of bioenergy and pollution abatement. Maximum organic loading rates achieved in upflow biofilm reactor (AF) with cross-flow filter media and sludge blanket reactor (UASB) are 16 and 11.5 kg COD/m^3/d respectively at 37±2°C. Methane content in the biogas was almost same (64%–67%) in both the reactors. Higher COD turnover of 85% has been observed in AF reactor as compared to 80% in UASB reactor. Sludge washout and excessive foaming were experienced in UASB reactor above organic loading rate of 12.0 kg COD/m^3/d, whereas performance of AF reactor was quite steady. Accumulation of volatile fatty acids beyond organic loading rate of 17.0 kg COD/m^3/d was, however, observed in AF reactor, the revival of which to normal state after shock loading was better than that of UASB.

Paper 31

BIODEGRADATION OF MIXED WASTES IN CONTINUOUSLY OPERATED CYCLIC REACTORS

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The problem of simultaneous biological treatment of liquid wastes containing industrial and urban components has been examined at a fundamental level with a simple model system in order to address issues of optimal process design. Glucose and phenol were chosen as representatives of urban and industrial wastes, respectively. The kinetics of biodegradation of the two compounds by a pure culture of *P. putida* were investigated in batch experiments. It was found that although the two substrates can be simultaneously degraded, they are involved in cross-inhibitory interactions. Based on this information, a mathematical model was derived to describe biodegradation in continuously operated cyclic reactors. These reactors have the features of sequencing batch reactors (SBRs), which are employed in a number of biotreatment applications. The model was subjected to detailed mathematical/numerical studies in order to investigate the effect of various parameters such as rate of filling, fraction of cycle time allocated to the filling phase, ratio of minimum to maximum volume of reactor contents, and feed concentrations of the two substrates. Experiments were then performed with a continuously operated cyclic reactor under various values of the operating parameters. In this presentation, results from the theoretical and experimental studies will be shown and compared. Despite the relative simplicity of the model system examined in this study, the results suggest a detailed methodology which can be applied in optimizing actual biotreatment processes.

Paper 32

BIODEGRADATION OF PCBs IN SLURRY REACTORS[1]

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Workable, inexpensive remediation methods for on-site treatment of soils and sediments contaminated with polychlorinated biphenyls (PCBs) remain an elusive goal for many industries and government agencies. Our current work focuses on bench-scale studies in support of an innovative field bioreactor design that is planned for use in a demonstration of biological remediation of soils at an electrical power substation. The design incorporates a desorption mechanism to remove PCBs from soil, followed by adsorption onto an artificial slurry medium (diatomaceous earth) and subsequent degradation in a sequential, anaerobic-aerobic slurry bioreactor system. Batch reactors containing artificially contaminated diatomaceous earth, sewage sludge, and/or soil slurries were tested under aerobic, anaerobic, and sequential aerobic/anaerobic conditions for PCB degradative capacities. Anaerobic inoculum was obtained from Hudson River sediments, while strains of aerobic, PCB-degrading bacteria were obtained commercially. Effects of agitation, addition of supplementary carbon sources, presence of residual desorption agents, and initial contamination levels were also examined. Data presented will provide documentation of two important steps toward the realization of an overall bioremediation scheme for PCBs: the deployment of anaerobic dechlorinating organisms from foreign environments for transformation of PCBs from soils and a workable bioreactor design incorporating both anaerobic and aerobic degradation pathways.

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

Paper 33

SURFACTANT CONCENTRATION EFFECTS ON VOLATILE CONTAMINANT BIOAVAILABILITY: PARTITIONING AND "MICELLERIZATION"

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Several biosurfactant-producing microbes were stimulated to produce enhanced levels of cell-associated exopolymers. These exopolymers decreased the surface tension of water from 76 dynes/cm to <50 dynes/cm, dependent upon the stimulant (n = 8 compounds tested). In addition to being contaminant-specific, biosurfactant (exopolymer) production and persistence was dependent on the microbial growth phase.

The biosurfactant (crude extract) was added to aqueous suspensions containing volatile contaminants and equilibrated for 24 hr. The effect of biosurfactant on partitioning into the headspace versus liquid versus micelle was measured. The results showed increased bioavailability for degradation with increasing surfactant concentration **until** the critical micelle concentration was reached. This increased bioavailability was measured with two bioreporters and contaminant degradation confirmed with gas chromatography analyses.

The enhanced bioavailability was compared to Tween's ability to enhance bioavailability. As the Tween concentration increased above the CMC, the degradation rates decreased. This interference in bioavailability was likely caused by contaminant partitioning into the micelle, thus becoming unavailable to the microbes.

The proposed use of surfactants for enhanced site remediation requires a clear understanding of surfactant/contaminant interactions.

Paper 34

MECHANISMS OF INTRINSIC BIOREMEDIATION OF GAS CONDENSATE HYDROCARBONS UNDER ELECTRON ACCEPTOR LIMITING CONDITIONS

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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 Production Co. 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 electrons acceptors and slurry experiments which are hydrocarbon limiting. The microcosm studies are described in this paper.

In the microcosm studies 25 g of soil obtained from the field site was saturated with 6 mL of an aqueous phase containing nutrients and electron acceptors in 50-mL serum bottles. The soil was a mixture of pristine soil and soil obtained from a contaminated region. The latter served as an inoculum. In a second series of experiments, Ottawa sand was used in place of the pristine soil. Two types of electron acceptor conditions were used, anoxic and hypoxic. The anoxic condition corresponded to zero oxygen, while the hypoxic condition corresponded to microcosms which initially contained a limiting amount of air. In the anoxic series either nitrate, Fe(III), or sulfate and carbon dioxide were added as electron acceptors. In the hypoxic series each of the four electron acceptors was supplied in addition to air. Weathered condensate was added as a separate phase to about 10,000 mg/kg TPH in the soil (dry weight). The incubation temperature was 10°C. Microcosms were sacrificed at six different times over a total of 402 days. Contents were analyzed for electron acceptors, hydrocarbons, and possible products of hydrocarbon degradation.

The results of this study have verified that hydrocarbon 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. Results also suggest that limited amounts of oxygen may lead to partial oxidation of hydrocarbons which are then more amenable to degradation through anoxic mechanisms.

Paper 35

EFFECT OF MICRONUTRIENT ON MICROBIAL RESPIRATION OF A SHALLOW COASTAL SUBSURFACE AND VADOSA ZONE

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As part of our study on microbial heterogeneity in the subsurface, we have studied the microbial respiration of soil (sediment) samples obtained from a coastal site near Oyster, Virginia. The sediments at the site are unconsolidated, fine to coarse beach sand and gravel. Columbus Instruments Micro-Oxymax Respirometer was used to measure rates of CO_2 production and O_2 consumption/production. The soil samples showing maximum and minimum production of CO_2 were selected to study the effect of micronutrient, yeast extract (0.5 and 1.0 ug per gram of soil) on the CO_2 production of the selected soil samples. The rate of CO_2 production was significantly increased by several folds when the yeast extract was added to the soil samples. Studies dealing with the effect of specific nutrients such as carbon, nitrogen, and phosphorus on the respiration of these soil samples are in progress.

Abstracts for Poster Presentations

Poster 36

PREPARATION AND CHARACTERIZATION OF IEA-NIST STANDARD BIOMASS REFERENCE MATERIALS

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Research and development of bioenergy as a renewable energy source has made significant progress during the past decade. As bioenergy matures into a commercial energy source, the need for biomass standards cannot be overemphasized. The bioenergy community recognized this need more than a decade ago and identified four biomass feedstocks to use as standards for biomass analysis and conversion processes. The International Energy Agency (IEA), Bioenergy Agreement, the Voluntary Standards Activity Group in collaboration with the National Institute of Standards and Technology (NIST) prepared four biomass materials as standard reference materials: eastern cottonwood (*Populus deltoides* clone #ST-66), monterey pine (*Pinus radiata*), sugar cane bagasse (*Saccharum spp*), and wheat straw (*Triticum aestivum*, Thunderbird variety).

The four biomass materials were carefully prepared in 10-g packages, and part of the lot was used for the analytical round robin on whole biomass feedstocks in 1991. Twenty laboratories worldwide participated in the round robin summative analysis of the whole biomass feedstocks, but only five commercial laboratories in the USA participated in the ultimate, proximate, higher heating value, and ash analyses of these samples.

We report the results of the summative, ultimate, proximate, ash, and heating values of the four standard whole biomass feedstocks. The reference standard biomass materials are available at NIST for purchase by the public.

Poster 37

CHEMICAL INTERACTIONS BETWEEN AQUEOUS AND ORGANIC PHASES IN A REACTIVE EXTRACTION PROCESS

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In a membrane reactive extraction process for recovery of lactic acid from fermentation broths, significant fouling of the membrane has been observed. In an effort to understand the factors responsible for the membrane fouling, we have investigated the chemical interactions between the aqueous and organic phases. The solubility of the reactive agent trioctylphospheneoxide (TOPO) in kerosene, and partitioning of lactic acid between aqueous and kerosene/topo solutions were carefully measured. Lactic acid/topo complex was investigated with the help of [^{32}P]NMR and FT-IR spectroscopy. These results will be presented and their impact on fouling of membranes will be discussed.

Poster 38

RECOVERY AND RECYCLING OF POLYMERS IN AQUEOUS TWO-PHASE EXTRACTION

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Aqueous two-phase extractions have been explored for the past two decades for downstream processing of bioproduct streams. This method of separations and recovery is very attractive for labile compounds and offers the unique advantages of specificities of extractive processing. However, the high costs of the biopolymers used for formation of aqueous two-phase systems have been a major roadblock in widespread use of this technology. This paper will deal with the impact of recovery and recycling of the biopolymers on the economics of an integrated aqueous two-phase extraction process. It will include purification of a desired protein from a mixture containing other proteins and nucleic acids, back extraction of the protein(s) and nucleic acids from their respective phases, and reuse of the recovered biopolymers on protein separation. A simple mathematical model of the aqueous two-phase process and design of a continuous countercurrent extractor for the recovery of an intracellular target protein will be presented.

Poster 39

BIOMASS ANALYSIS

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Analysis of biomass is a very important part of research into alternative fuels. In many cases the data which drives the research is a result of analysis of biomass or process intermediates. This area of analytical chemistry has challenges in many areas. Compositional analysis of biomass cannot be accomplished without first degrading the subject material which certainly has an impact upon the analysis. There are inherent complexities in the HPLC which result in interesting analytical chemistry. The substituents of biomass are unstable. They can degrade and polymerize in unpredictable ways.

Only with great care toward technique and specialized instruments can useful analytical data be generated.

Poster 40

IN VITRO RADISH CROP-LIKE STRUCTURES AS A POSSIBLE SOURCE OF NATURAL ANTHOCYANINS

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Previously we have shown that excised apexes of radish seedlings are able to produce crop-like structure (CRS) when cultured *in vitro* in presence of exogenically supplied phytohormone. Here we report very intensive pigmentation of such CRS with anthocyanins. This pigmentation takes place in surface as well as internal CRS tissues and is stably expressed during CRS sub-cultivation at least for 12 passages (25 days each, growth index about 10–12 passages). Under sub-cultivation, successive selection for further increased CRS pigmentation with anthocyanins is possible.

The pigmentation occurs only in CRS derived from radish forms possessing anthocyanin synthesis *in vivo*. Since CRS-producing ability of radish apexes is under polygenic control, the choice of appropriate initial radish line is a crucial step in successful obtaining of the anthocyanin-pigmented CRS. We believe that such pigmented CRS may be used as a suitable source of natural food dyes. For this purpose, hybrid radish forms simultaneously possessing ability to produce *in vitro* pigmented CRS and hormone-independent growth (Buzovkina et al. 1993) should be of special interest.

Poster 41

ACID AND ENZYME HYDROLYSIS OF CORN FIBER

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Corn fiber, a by-product from the corn wet milling process, is rich in carbohydrates and can be used as a potential sugar source for ethanol production. The easily hydrolyzable hemicellulose and residual starch portions of corn fiber can be released by a dilute-acid process. To maximize the yield of monomeric sugars and minimize further sugar degradation, a kinetic model of this reaction was established. The model was used to optimize the process and reactor design. The unhydrolyzed fiber containing mostly cellulose, lignin protein, and fat has a similar nutritive value as the starting corn fiber and can be used as a new feed ingredient. The yield of monomeric sugars from corn fiber hydrolyzed with commercial cellulases was unsatisfactory. A comparison of the hydrolysis by acid or enzymes will be discussed. Further work is necessary to optimize the efficiency of cellulases on corn fiber hemicellulose.

Poster 42

ENZYMATIC HYDROLYSIS OF CORN STARCH AFTER EXTRACTION OF CORN OIL WITH ETHANOL

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An alternative, a solvent corn milling process was tested in a pilot scale to recover corn oil and zein from the cracked corn kernels. Corn oil was extracted by 95% ethanol, and zein was extracted by 65% ethanol operated at different temperatures with a liquid:solid ratio of 0.5. At 65°C, over 90% of the corn oil was extracted. When the cracked corn was dried to about 1%, each kilogram of the cracked corn was able to absorb water from 500 mL of 95% ethanol to yield 99.6% (w/w) of ethanol in the oil miscella.

After corn oil extraction, starch in the residue could be hydrolyzed to glucose by α-amylase and glucoamylase without separation of fiber and residual protein. The glucose yield in hydrolysis is a function of temperature, enzyme concentration, and solid:liquid ratio. With a two-step heating hydrolysis, corn starch was converted to glucose with a 98% yield and syrup contained 24% glucose (w/v) after filtration and washing of the hydrolyzed mass.

Poster 43

ENZYMATIC HYDROLYSIS OF AFEX-TREATED HIGH-MOISTURE CORN FIBER

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Corn fiber is a crop processing residue containing significant amounts of cellulose, hemicellulose, and starch which is already gathered in facilities where fuel ethanol is currently manufactured. Preliminary research has shown that corn fiber (30% moisture) responds well to ammonia fiber explosion (AFEX) pretreatment. However, an important AFEX pretreatment variable which has not been adequately explored for corn fiber is sample moisture. In the present investigation we determined the best AFEX operating conditions for pretreatment of corn fiber at high moisture content (about 150% moisture dwb). The optimized AFEX treatment conditions are defined in terms of the moisture content, ammonia:biomass ratio, temperature, residence time, as well as the response of the pretreated biomass to enzymatic hydrolysis. Fermentable sugar yields by enzymatic hydrolysis of AFEX-pretreated high moisture samples are comparable to those with low moisture content. Enzymatic hydrolysis was performed with several different enzyme preparations, Cytolase 300, and various SPEZYME preparations. SPEZYME preparations showed improved efficiency compared to the Cytolase 300 and effectively hydrolyzed all the carbohydrate components (cellulose, hemicellulose, and starch) of AFEX-treated corn fiber. An advantageous "fed-batch" hydrolysis process is demonstrated using SPEZYME preparations at low enzyme loadings.

Poster 44

XYLITOL PRODUCTION BY YEASTS FROM SUGAR CANE BAGASSE: PRETREATMENTS

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Xylitol is a naturally occurring, five-carbon polyalcohol with high sweetening power. Besides it's completely safe for the teeth, it has anticaries properties and it's tolerated by diabetics. Xylitol is formed as a metabolic intermediary product of D-xylose fermentation by yeasts. Different yeasts were compared for the xylitol production from pure xylose under microaerobic conditions. A yeast mutant 11-2, that led to the best xylitol production: 95.4 g/L (Qp = 0.993 g/L) was chosen to perform the subsequent fermentations.

The sugar cane bagasse can be hydrolyzed with dilute acid, to get a mixture of fermentable sugars with xylose as the major component. When the hydrolyzate was only neutralizated, the xylitol production was 2.55 g/L (Qp = 0.053 g/Lh). This poor value is due to substances in the fermentation media generated in the hydrolysis, that make the fermentation processes difficult. When the media was diluted, xylitol production increased to 4.32 g/L (Qp = 0.066 g/Lh) because the effect of these substances is less important. However, when the hydrolyzate was treated with activated charcoal or cation exchange resins (packed into a column 1.5 x 25 cm) and neutralized, the xylitol was produced readily, reaching 10.54 g/L (Qp = 0.205 g/Lh) and 10.10 g/L (Qp = 0.195 g/Lh), respectively. In order to increase the initial xylose concentration, the hydrolyzate treated with activated charcoal was concentrated by vacuum evaporation in rotavapor. When this hydrolyzate was only concentrated and neutralized, the xylitol productivity and final xylitol concentration were 0.136 g/Lh and 9.7 g/L, respectively, while when after concentrating it was treated with cation exchange resins and neutralized, the xylitol productivity and final xylitol concentration increased to 0.297 g/Lh and 21.4 g/L, respectively.

Poster 45

THE EFFECT OF STORAGE ON CORN STOVER AS A FEEDSTOCK FOR BIOFUELS PRODUCTION

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For large-scale production of biofuels it will be necessary to store feedstocks for extended periods that may last up to one year. A study was conducted examining the effect of storage on several woody and herbaceous feedstocks. Changes in composition were measured, and the impact of storage on converting these feedstocks into ethanol and pyrolysis oil was assessed. Corn stover was one of the feedstocks most affected. The corn stover bales experienced periods of heavy rainfall (up to 8 cm in one day) during storage. The moisture appeared to initiate microbial activity, observed as rapid increases in internal bale temperatures (up to 40°C). Loss of extractives, protein, and minor hemicellulose components, and increased lignin and ash contents, were the compositional changes observed. Pretreatment residue yields increased with feedstock storage; however, ethanol yields decreased. This is partly because of the higher lignin contents of the stored feedstocks. Pyrolysis oil yields and properties were also affected by storage. Oil yields were higher from stored corn stover, which possibly reflects the higher lignin contents. The oils' carbon contents decreased with the length of storage, resulting in lower oil heating values. The largest change in oil properties was the very large increase in alkali contents, indicating that the increases in ash contents were probably caused by accumulating dirt.

Poster 46

AMMONIA-RECYCLED PERCOLATION PROCESS FOR PRETREATMENT OF HERBACEOUS BIOMASS

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An ammonia-based pretreatment method termed as ammonia recycled percolation (ARP) was investigated for corn cobs/stover mixture (CCSM). The process involves treatment with aqueous ammonia through a percolation reactor (packed bed flow-through type) operated in a recirculation mode. The effects of process parameters of temperature, reaction time, flow rate, and ammonia concentration were studied. The experimental data on CCSM indicate that the ARP is a highly effective pretreatment method. The rate of enzymatic hydrolysis of the ARP samples was substantially higher than that of filter paper or α-cellulose. The 72-hour digestibility of ARP samples was slightly higher than that of filter paper and significantly higher than that of α-cellulose. The extent of delignification in the ARP process was in the range of 65–85%. The ARP process solubilized significant amounts of xylan fraction into the pretreatment effluent, yet left most of the glucan fraction intact. The decomposition products of glucose and xylose (HMF and furfural) were undetectable in any of the ARP effluents. The ARP effluents were evaluated for fermentability/toxicity by *P. stipitis*. The ARP solid samples were also put through the final test of simultaneous saccharification and fermentation (SSF) based on cellulase and *Saccharomyces cerevisiae*. The results on these microbial tests are reported.

Poster 47

KINETIC AND MODELING INVESTIGATION ON TWO-STAGE REVERSE FLOW REACTOR AS APPLIED TO DILUTE-ACID PRETREATMENT OF HERBACEOUS BIOMASS

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The kinetics of dilute-acid pretreatment/hydrolysis of corn cobs/stover mixture hemicellulose was investigated. The kinetic data have confirmed that hemicellulose in this herbaceous feedstock is of biphasic. The kinetic model recognizes the presence of soluble xylose-oligomers, xylose monomer as well as the decomposition of xylose. The experimental data were used to determine the associated kinetic parameters including activation energies, acid exponents covering the conditions of 120° - 150°C, and sulfuric acid concentration of 0.44%–1.90%. The biphasic nature of the kinetics brings about an interesting concept regarding the reactor design and operation strategy. We have proven that an unconventional reactor, a two-state percolation reactor operated in reverse flow mode is advantageous for biphasic substrates. We have extended this work to see the applicability of this reactor for herbaceous biomass feedstock incorporating the aforementioned kinetic data. The modeling results indicate that under proper adjustment of operating parameters, the performance of this novel pretreatment reactor is indeed far superior to that of batch reactor or single-stage percolation reactor, especially attaining high product yield (xylose).

Poster 48

FRACTIONATION OF HERBACEOUS BIOMASS BY MODIFIED AMMONIA-RECYCLED PERCOLATION PROCESS

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Ammonia-recycled percolation (ARP) process was proven to be a highly effective pretreatment method in terms of the enzymatic digestibility and delignification. However, a significant portion of the hemicellulose was solubilized during the process. From a process viewpoint, this is an undesirable attribute of the ARP because an additional processing is required for the recovery of the residual hemicellulose. To alleviate this problem, the ARP process was modified to add hydrogen peroxide into ammonia stream. This modified process, as was the case with the unmodified version, is environmentally safe. It was found to give not only high level of enzymatic digestibility, but also a high degree of fractionation of biomass to the three major components. The extent of delignification was 94%–99% for corn cobs/stover mixture and switchgrass under a near optimum treatment condition of 170°C and 0.28 g loading of H_2O_2/g dry biomass. The concentrations of ammonia and H_2O_2 in the process stream were at 10 wt.%, and 5 wt.% respectively. At the same reaction condition, hemicellulose recovery of above 80% was achievable. The sugar decomposition during the process was negligible. The 72-hour enzyme digestibilities of the herbaceous samples generated from the modified ARP process were essentially the same as that of filter paper.

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[1] On leave from Geongsang National University, Korea.

Poster 49

UTILIZATION OF ACID HYDROLYSIS RESIDUES AS ADHESIVES FOR SIZE ENLARGEMENT OF FINE COAL PARTICLES

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Coal dust is produced during the desulfurization of coal and often accounts for up to 30% of the processed material. Previous research has shown that these fine coal particles, unusable because of their small size, are desirably low in sulfur but have poor combustion properties due to a low volatile matter content. The small size of the particles also makes them difficult to transport due to dusting.

One proposed solution to the problem of poor combustion and small particle size is to pelletize the dust with residues derived from the acid hydrolysis of biomass. Acid hydrolysis of biomass converts much of the cellulose and hemicellulose content present to simple six- and five-carbon sugars for subsequent conversion to fuels or chemicals. The solid residue remaining has a high lignin content. This residue has good adhesive characteristics, a relatively high heat content, and is inexpensive. The addition of the residue as adhesive for fine coal particles also serves to increase the percentage of volatile matter in the resulting coal/adhesive mixture, improving its combustibility.

Research at Mississippi State has shown that combining coal dust with the residues from the acid hydrolysis of wood, agricultural products, and municipal solid wastes results in a pelletized coal/residue particle that is nondusting, high in volatile matter and heat content, and low in sulfur. Combining the coal dust and acid hydrolysis residue results in a value-added product, low-sulfur solid fuel pellets.

Poster 50

DEVELOPMENT OF PRECIPITANT AGENTS FOR PRECIPITATION OF PROTEINS BASED ON HYDROPHOBIC INTERACTION: PRECIPITATION OF BOVINE SERUM ALBUMIN

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The high cost of the downstream processing requires the development of techniques and materials that provide at the same time efficiency in the separation and recovery of bioproducts as well as simplicity of operation at low cost. In order to improve one of the more used operations in protein recovery (precipitation), we developed new precipitant agents based on food grade polymer of moderate cost. These precipitation agents were developed to perform protein precipitation based on hydrophobic interaction. They consisted of ligands, saturated linear chains of fatty acids, attached by esterification to a carrier molecule, methylcellulose. Precipitation of bovine serum albumin was achieved at 50% saturation of ammonium sulfate. The butyric acid derivative showed a higher efficiency in precipitating this protein than other derivatives tested. The data provide evidence that the interaction between the protein and the derivatives is hydrophobic as expected.

Poster 51

PRETREATMENT OF SUGAR CANE BAGASSE FOR ENHANCED RUMINAL DIGESTION

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Crop residues such as sugar cane bagasse have been largely used as a source of nutrients for cattle feeding. However, the close association which exists among the three major plant cell wall components, cellulose, hemicellulose, and lignin, limits the efficiency by which ruminants can degrade these materials. Previously we have shown that pretreatment with 3% (w/w) orthophosphoric acid, under relatively mild conditions, increased the nutritional value of sugar cane bagasse from 35% to 70% in relation to its dry weight. This was considered ideal because it allows good rates of passage of the diet through the digestive tract, therefore increasing animal production. We have now extended our study to other pretreated materials such as alkali-washed and steam-exploded sugar cane bagasse. We have determined the ruminal lag phase for each of the pretreated residues and monitored their products of ruminal digestion. This and other data were shown to fit very nicely to nonlinear models which describe the kinetics of ruminal digestion. Several analytical methods, such as Fourier-transformed infrared spectroscopy and x-ray crystallography, were used to determine how the structure and chemical composition of the feedstock had been affected by the process of ruminal digestion.

Poster 52

FOAM FRACTIONATION OF BARLEY MALT AMYLASES IN COLUMNS WITH REFLUX

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Foam fractionation in columns is a technique that has long been used to recover and purify solutions of proteins, including enzymes. This method is specially advantageous in treating dilute solutions where other separation methods run into economical limitations. An improvement in the efficacy of separation can be obtained adding external reflux to the column. The use of this more complex operational mode is indicated on those cases where structural damage of the protein is small when submitted to repeated foaming and foam breaking. This work analyzes operational variables like superficial gas velocity, solution concentration, and external reflux ratio on the study of the foam phase enrichment of amylases solutions from barley malt. The experimental setup includes a 3.2-cm diameter and 105-cm high column operated by bubbling nitrogen in pH-controlled solutions. The measurement of top and bottom protein concentration and amylases activity enables the determination of solute enrichment and purification factor for several reflux ratios. Activity in the top fraction was increased up to seven times using gas superficial velocities of 0.30 cm/s and reflux ratio of 1.1. This separation approach demonstrates foam fractionation as a reliable method to concentrate amylases from barley malt dilute wastewaters.

Poster 53

BIOSORPTION OF PRECIOUS METAL IONS BY CHICKEN FEATHER

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Chicken feather (C-feather) is an intricate network of stable and water-insoluble fibers with high surface area and is an abundant bio-resource. We found C-feather protein accumulated various precious metal ions (gold and platinum metals) selectively from their dilute aqueous solutions in high yield and in short contact time, depending on pH and characteristics of the individual precious metal ions. Under certain conditions, the accumulation level of precious ions, Au(III), Pt(II), Pd(II), and Rd(II) approaches about 17%, 13%, 7%, and 2% of dry wt of C-feather, respectively. Gold(I) potassium cyanide was also accumulate in 5.5% at pH 2.0. No significant changes on uptake rate and capacity of precious metal ions in the presence of 100 folds (mol) of coexisting ions (Na+, Fe3+, Cu2+, and Ni2+) were observed. One of the poisonous metal ions Hg(II) also occluded in 5% yield of that of dry wt under the acidic condition. The experiment suggested C-feather is promising to use for the purpose of removal/recovery of precious metals and of water pollution control.

Poster 54

THE EFFECT OF FERMENTATION (RETTING) TIME AND HARVEST TIME ON KUDZU (*Pueraria lobata*) FIBER STRENGTH

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Kudzu (*Pueraria lobata*) is an unwanted plant growing wild in the southern United States with no obvious economic value. On the other hand, its starch and fiber components have been used for centuries in the Orient. Kudzu fibers, which have been used traditionally for producing clothing, paper, baskets, and fishing line are investigated for possible application to the western market. In this study, vines, sorted according to their diameter, were placed in water for various time periods at room temperature. Naturally occurring microorganisms were used to remove the outer sheath from the fibers in the water-vine medium by fermentation. Fibers were then separated from the outer sheath and the underlying inner core using a laboratory spatula. The tensile strength of the fiber was measured by a static weight test taking the fiber to the breaking point. The kudzu fiber tensile strength varied as a function of the fermentation time, the vine diameter, and the time of year when the vines were harvested.

Poster 55

REVERSE-FLOW, TWO-TEMPERATURE, DILUTE-ACID PRETREATMENT TO ENHANCE BIOMASS CONVERSION TO ETHANOL

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Previously, a sensitivity analysis performed at NREL using a wood-to-ethanol economic model showed that yield-related process improvements can significantly lower the estimated production cost of biochemically converting the carbohydrate portion of hardwoods to fuel ethanol. In order to maximize the conversion yields from both C_5 and C_6 carbohydrates, we have recently described a two-temperature, dilute-acid pretreatment process for hardwood sawdust, which not only prehydrolyzes hemicellulose and preserves 92% of the theoretical yield of xylose equivalents in the liquor, but also greatly increases cellulose digestibility and hence the rate of ethanol biosynthesis from the carbohydrates in the solid residue using the SSF process.

The current study was undertaken to further optimize the two-temperature prehydrolysis scheme for hardwood sawdust as to yields of total sugar produced, sugar concentration in the prehydrolyzate liquor, residence times and temperatures, acid concentrations, and the rate and extent of ethanol production in the SSF process. Two percolation reactors (packed-bed up-flow-through-type) were used in a reverse-flow mode to demonstrate the advantages of counter-current flow. Process design considerations will be discussed in light of producing a separate solid and liquor stream, neutralizing the acid at elevated temperatures, and ethanol production rates and solids loading in the SSF process. Results from the optimization studies will be used in process analysis simulations to evaluate the technical potential of the proposed technology.

Poster 56

PALLADIUM-INACTIVATED CELLOBIOHYDROLASE I DOES NOT DISRUPT CELLULOSE FIBERS[1]

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Palladium complexes have been shown to irreversibly inhibit several *Trichoderma reesei* cellulase activities. The inhibition of cellobiohydrolase I (CBH I) is virtually instantaneous and dependent on the stoichiometry of Pd:CBH I. Although the amino acids histidine, cysteine, and cystine can prevent the inhibition, the mechanism whereby palladium completely inhibits CBH I appears to involve the disulfide bonds in the enzyme. CBH I modified by the attachment of pentaammine ruthenium(III) to histidine 206 or 228 exhibits greater sensitivity to palladium inhibition compared to native CBH I. Ruthenium-modified CBH I partially inhibited by palladium was much less thermally stable compared to the uninhibited enzyme, suggesting a degradation of disulfide bonds.

Although palladium inhibited the catalytic activity of CBH I, adsorption to crystalline cellulose was unaffected. This prompted an examination of the effect of palladium-inactivated CBH I on the surface of crystalline cotton linters by scanning electron microscopy. The results obtained suggested that binding of inactive CBH I to cellulose fibers did not result in their disruption. The latter was shown to be dependent on catalytic activity.

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[2] Oak Ridge National Laboratory Postdoctoral Research Associate through the Oak Ridge Institute for Science and Education, managed by the Oak Ridge Associated Universities for the U.S. Department of Energy.

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

Poster 57

SYNERGISM AND SOLUBLE-SUGAR PRODUCTION IN HYBRID CELLULASE SYSTEMS

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Each of a series of six highly purified bacterial and fungal endoglucanases has been evaluated for synergism with each of three highly purified exoglucanases (two fungal and one bacterial) in the solubilization of crystalline cellulose. Endoglucanases studied were *Acidothermus cellulolyticus* E1, *Thermomonospora fusca* E_5, *Trichoderma reesei* EG I, *Microbispora bispora* Endo-A, *Thermotoga maritima* Endo-B, and a xylanase/cellulase from *M. bispora*. Exoglucanases included CBH I and CBH II from *T. reesei* and E_3 from *T. fusca*. Artificial binary combinations of cellulases performed well, with a phylogenetic interdomain combination providing both the highest synergism numbers and the highest soluble-sugar release. Of the eighteen binary combinations studied, four of the six most synergistic pairs, and four of the top six producers of soluble sugar, were also bacterial/fungal hybrids. Comparisons of the patterns of synergism and sugar-release for the different endoglucanases in combination with the three exoglucanases suggested a basis for classification of the endoglucanases into four, and perhaps as few as three, activity classes.

Poster 58

RNA POLYMERASE SIGMA FACTORS IN *Clostridium acetobutylicum* ATCC 824

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Clostridium acetobutylicum is a sporulating, Gram-positive bacterium that produces acids (acetate and butyrate) and solvents (acetone, ethanol, butanol). Although correlation between solvent production and sporulation has been known for a long time, regulation of either process has not been established. Using a PCR probe based on the amino acid sequence of the sporulation factor sigma E of *Bacillus subtilis*, the sigma E analog from *C. acetobutylicum* was cloned in *Escherichia coli*. The gene was found in a gene cluster: upstream was a gene encoding the putative processing enzyme of sigma E, while downstream was a gene encoding sigma G, another sporulation factor. This gene arrangement was conserved in two *Bacillus* species. The sequence suggested possible variations in gene regulation between the species and could explain the morphological differences in sporulation development.

Two methods were examined to introduce these genes back into *C. acetobutylicum*. Transformation (in vivo methylation followed by electroporation) was unsuccessful, suggesting that overproducing the minor sigma factors may affect growth. Integration by recombination was then attempted in three ways: gene duplication (single crossover), gene inactivation (single crossover), and gene replacement (double crossover). The selected strains were then analyzed for their ability to sporulate and produce solvents.

Poster 59

VALIDATION OF YEAST VIABILITY STAINS IN AN ETHANOL PROCESS

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In a continuous ethanol process with a large number of recycled yeast cells, the sensitivity of a viability stain is crucial. Two fluorescent dyes (FungoLight and EukoLight from Molecular Probes, Inc.) based on esterase and redox reactions inside yeast cells were evaluated against methylene blue and plate count for yeast viability determination in an ethanol fermentation process. A growth curve and a heat kill curve of *Saccharomyces cerevisiae* in a batch culture were used as test cases. Methylene blue gave inconsistent results among different staining conditions. The esterase activity stain (EukoLight) was easy to use and differentiate live from dead yeasts, but lost its efficacy in fermentation broth containing high ethanol concentrations. FungoLight stain was the most promising in all situations studied. The correlation between viable cell counts as determined by various methods and ethanol productivity will be discussed.

Poster 60

CLONING AND EXPRESSION OF THE *Acidothermus cellulolyticus* E1 β-1, 4-ENDOGLUCANASE GENE IN *Streptomyces lividans*

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In 1985, under permit from the National Park Service, NREL researchers isolated a thermophilic eubacterium, *Acidothermus cellulolyticus*, from an acidic thermal spring in Yellowstone National Park. This organism produces a highly thermostable cellulase complex which includes a ß-1,4-endoglucanase called E1 (T_{opt}=81°C, pH_{opt}=6.0). A 3.7-kb genomic DNA fragment containing the entire E1 gene was subcloned into plJ702, producing E1-plJ702, which was introduced into *Streptomyces lividans* TK24 via protoplast transformation. Recombinant E1 protein (rE1) is efficiently secreted in active form by the recombinant host strain. rE1 has a molecular mass of 72 kDa which is indistinguishable from the native form of E1 and exhibits other characteristics which are very similar to native E1 (e.g., thermostability, immunoreactivity, and substrate range).

The net yield of rE1 protein from the native E1 gene in *S. lividans* batch culture is modest after a 3-step chromatographic purification. Initial attempts to increase the productivity of rE1 in *S. lividans* involved substitution of the native E1 promoter with the *S. lividans*-derived thiostrepton-inducible promoter, *ptipA*, according to three different strategies. Expression levels of rE1 can be significantly altered by changing the promoter context. We are currently investigating additional possibilities for engineering the E1 gene using other strong promoters in *Streptomyces*.

Poster 61

PURIFICATION AND CHARACTERIZATION OF AN ACETYL XYLAN ESTERASE FROM *Aspergillus niger* ATCC 10864

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A major resistance factor of woody biomass to enzymatic degradation is the high degree of acetylation of the xylan component. Heavy acetylation impedes the binding of xylanolytic enzymes to the xylan, inhibiting hydrolysis. Acetyl Xylan Esterase (ACE) overcomes this problem by removing a percentage of the ester-linked acetates from the xylose backbone, allowing other xylanolytic enzymes better access to the xylan chain. An acetyl xylan esterase was isolated and purified from *Aspergillus niger* ATCC 10864 grown under conditions optimal for expression of xylanolytic enzymes. The AXE was purified from the culture filtrate using ammonium sulfate precipitation and low pressure hydrophobic interaction chromatography. Assays for enzymatic activity were carried out using chemically acetylated oat spelt xylan as a substrate. The K_m, V_{max}, temperature, and pH optima were also determined using acetylated oat spelt xylan. The molecular weight and pI were determined by 2D SDS-PAGE. The extent of deacetylation by the purified enzyme was determined by comparison to chemically deacetylated oat spelt xylan. The actions of other xylanases were also measured against acetylated oat spelt xylan concurrent with and subsequent to the action of AXE.

Poster 62

SCREENING FOR L-ARABINOSE FERMENTING YEASTS

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Utilization of pentose sugars (xylose and arabinose) is essential for the economic conversion of lignocellulosic biomass to ethanol. Xylose fermenting yeasts were discovered in 1980 but to date, no yeasts are able to ferment L-arabinose to ethanol in significant quantities. Discovery of a yeast that efficiently ferments L-arabinose to ethanol would enhance the yield from such potential feedstocks as sugar beet residues, corn hulls, rice bran, and some grasses. We have screened over 100 different yeasts from the ARS Culture Collection for the ability to ferment L-arabinose under microaerophilic conditions. While no yeasts screened produce ethanol in appreciable quantities, yeast isolates have been identified that are promising candidates for mutational enhancement of L-arabinose fermentation. It has been hypothesized that the inability of yeasts to ferment L-arabinose arises from a redox imbalance in the fermentation pathway. Further experiments have been conducted to test this hypothesis.

Poster 63

PRODUCTION OF HIGH LEVEL OF CELLULASE-FREE XYLANASE BY *Aspergillus niger* USING THE EXTRACTS OF STEAM EXPLODED CORN STOVER

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Corn stover, xylose, and extracts of steam exploded corn stover were respectively selected as the substrates for the production of xylanase by 10 strains which belong to bacteria, yeasts, filamentous fungi, and basidiomyces.

When corn stalk was as the substrate, xylanase and cellulase were simultaneously produced by 6 strains tested, and in the xylose culture, the production of both xylanase and cellulase had been all strongly inhibited. When extracts of exploded corn stover (main components were oligo-xylosides DP 3-6) was applied as the only carbon source, high level of cellulase-free xylanase was obtained by *A. niger* and *A. niger* L-22.

The components of *A. niger* An-76 xylanolytic enzymes, a ß-xylosidase, and three endo-ß-xylanases, were purified by gel filtration and ion exchange chromatography. This cellulase-free xylanase was successfully used in decomposition of xylan in the biobleaching process.

Poster 64

LIGNOCELLULASES AND ETHANOL PRODUCTION FROM CELLULOSE BY TWO SOIL FUNGI

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The microbial production of chemicals by lignocellulose fermentation has generated considerable research interest. Since the commercially available celluloses are unlikely to be used as substrates for industrial fermentations, fuel production from biomass in the form of agricultural and forest residues is attractive as an alternative renewable energy resource.

The present work examines the production of ethanol via direct fermentation of pure celluloses and lignocellulosic wastes by two soil fungi isolated under anaerobic conditions. One of the isolates was identified as *Trichocladium canadense* and the other as a basidiomycete species. The strains were cultured on a defined medium containing ball-milled filter paper slurry as the carbon source under anaerobic, microaerophilic, and aerobic conditions. After complete degradation of the cellulose source, lignocellulases and fermentation products were determined. For both strains, high levels of xylanase activity followed by CMCase activity were detected in the culture supernatants. ß-glucosidase was present at lower levels than the CMCase. Avicelase was also detected but at much lower levels than the other activities. Highest activities for *Trichocladium canadense* and the basidiomycete strain were obtained when cultures were incubated under microaerophilic conditions and air, respectively. Ethanol was the major nongaseous fermentation product. Acetic acid was also produced but at much lower concentrations than ethanol. Highest conversion of available cellulose to ethanol was obtained with strain Q 10 (90%–96%), under non-aerated conditions. Strain H2 showed 81% under aerated conditions. Ethanol production decreased when microcrystalline cellulose and lignocellulosic substrates were used.

Poster 65

CELLULASES AND XYLANASES PRODUCED BY *T. longibrachiatum:* PROPERTIES AND APPLICATION TO THE HYDROLYSIS OF CELLULOSIC MATERIAL

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A mesophilic fungus, identified as *Trichoderma longibrachiatum* strain Blu 6.5, was isolated from degraded plant material collected in Blumenau - SC, Brazil. The production and characterization of the enzymes responsible for cellulose degradation and their use in the hydrolysis of cellulosic material were investigated.

The time course for the production of cellulolytic enzymes by Blu 6.5 in liquid medium, using Solka-Floc BW 40 as a carbon source was monitored for 13 days. The highest filter paper hydrolyzing, carboxymethylcellulase, avicelase, and β-glucosidase specific activities were obtained on the sixth day of growth. Cellulolytic activity reached its maximum in the pH range 4.6–5.0 and maintained 80% of its original activity after 24 hours incubation at a pH between 4.4 and 5.0. The optimum temperature for activity was 55°C. Following incubations of 1 hour or 24 hours at this temperature, the enzyme retained 82% or 50% of its activity, respectively.

After hydrolysis for one hour, 60% and 49% of the glucose and total reducing sugar, respectively, produced by carboxlmethylcellulose were obtained in the avicel hydrolyzate. The production of reducing sugars at a concentration of 10% of newspaper or bagasse to enzyme extract was determined. The newspaper was hydrolyzed with more efficiency than the bagasse, producing more than 6.0 g/L of reducing sugars. The peak production for bagasse was about 1.6 g/L. Cellulases and xylanase production by strain Blu 6.5 and a mutant strain (M 38) in various carbon sources was also studied.

Poster 66

MICROBIAL CONVERSION OF SYNTHESIS GAS COMPONENTS TO USEFUL FUELS AND CHEMICALS

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The combustion of carbonaceous materials (e.g., oil, coal, natural gas, and lignocellulosics) to produce energy results in the release of tremendous quantities of carbon dioxide—one of the predominant greenhouse gases. However, these materials may be gasified under controlled conditions to produce **synthesis gas**. This gas may, in turn, be upgraded to valuable fuels and chemical feedstocks.

One approach to upgrading synthesis gas employs microorganisms with a demonstrated ability to grow autotrophically on synthesis gas components. Utilization of microbial cultures in a syngas conversion strategy offers a strong potential for essentially complete conversion of these carbon-based materials to higher-value chemicals and fuels. An attractive feature of such a process is the ability to convert a variety of complex carbon materials to the molecularly simple components of synthesis gas—thus improving the process concerns for the microbial conversion step (e.g., no solids handling, enhanced mass transfer, and reaction kinetics).

This work reports on preliminary studies for syngas conversion by a variety of cultures isolated from raw inocula. Inoculum sources include oil-field wastes and hydrocarbon contaminated soils, municipal sludge, paper industry effluent, and animal wastes.

Poster 67

DEVELOPMENT OF A METALLOXYLANASE AND ITS APPLICATION BIOBLEACHING[1]

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Two xylanases, xynA of *Bacillus pumilus* and xyn II of *Trichoderma reesei*, were purified from culture filtrates obtained from NovoEnzymes, Danbury, Connecticut. Purified xylanases were modified by attachment of pentaammineruthenium groups to histidine residues on the xylanases. All of the modified xylanases had veratryl alcohol oxidase activity. Hydrolytic activity of *T. reesei* xyn II on soluble xylans was unchanged by modification with pentaammineruthenium. Modification of *B. pumilus* xyn A was found to greatly reduce xylan hydrolysis unless the active site of the xylanase was protected with xylose during the modification. Incubation with histidine, but not with glycine, restored xylanase activity to pentaammineruthenium-modified xynA.

Bleaching pretreatment with modified xynA was found to substantially improve the brightness and the removal of lignin in hardwood kraft pulp samples. Bleaching pretreatment of kraft pulps using xylanases modified with pentaammineruthenium was shown to reduce the requirement for chlorine dioxide to a greater degree than pretreatment with native xylanases.

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

3 Operating Division of the California Institute of Technology under contract NAS7-918 with the National Aeronautics and Space Administration.

Poster 68

USE OF SUGAR CANE JUICE AND OTHER DISACCHARIDE SOURCES IN THE GROWTH OF YEAST AND BACTERIAL CAROTENOID PRODUCERS

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Fish farming is an increasing enterprise in Brazil. The basidiomicetous yeast *Phaffia rhodozyma* is becoming an attractive source for the production of the pink diketo-dihydroxy-carotenoid astaxanthin for fish feeding and meat staining. As compared to synthetic pigment, the yeast whole cellular material may contribute, besides the pigment itself, with appreciable amounts of protein, carbohydrate, lipids, and micronutrients when used as a fodder complement for fish farming. So, there is a need of less expensive C sources for *Phaffia* growth. Sucrose from crude sugar cane juice may fulfill both scopes: good cell growth and cellular pigment co-production. Other suitable disaccharide sources could be ∝-amylolyzed raw starches from Brazilian crops and cellobiose-enriched cellulolyzed cane bagasse. *Phaffia* cell wall architecture is another feature of paramount importance, since yeast cell digestion is required for an efficient astaxanthin delivery.

A different group of pigments was obtained from AMRL, an inulinolytic bacterium isolate able to grow in starch and sugar cane juice. Its pigment population displayed some similarity to carrot and tomatoes carotenoids in the classical assay with antimony trichloride.

Data on *Phaffia* and AMRL cultivation, carotenoids production, yeast cell wall composition/polysaccharide release, and microbial pigments extractability will be presented.

Funding: CNPq, CAPES, and UFPR (Brazil).

Poster 69

STUDIES ON THE E2 ENDOGLUCANASE FROM *Thermomonospora fusca*

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Technologies to decrease the cost and usage of cellulase could have a major impact on the viability of ethanol from biomass conversion. The degradation of woody biomass by thermophilic enzyme systems is advantageous in terms of reaction kinetics and catalyst stability. The E2 endoglucanase from *Thermomonospora fusca* has been studied as part of a program to establish alternate means of cellulase production. The enzyme was purified using hydroxyapatite chromatography and gel filtration. Electrophoresis was conducted as SDS-PAGE and as zymograms for detecting cellulase activity on native gels. Highly sensitive assays were developed using filter paper, carboxymethylcellulose and 4-methylumbelliferyl-ß-$_D$-cellobiopyranoside to test potential expression of the enzyme in alternative expression systems.

Poster 70

SACCHARIFICATION OF NATIVE SUGAR CANE BAGASSE PITH BY THE CROSS-SYNERGISTIC ACTION OF CELLULASES FROM *Penicillium* sp. CH-M-001 and *A. terreus* CH-M-013

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Industrial and agricultural lignocellulosic wastes are potential sources for the production of energy, food, chemicals, and other useful products by enzymatic and microbial methods. Enzymatic hydrolysis by microbial cellulases has advantages over the chemical processes, especially the avoidance of environmental pollution, but the efficient saccharification of cellulose requires the cooperative actions of different enzyme activities, namely exoglucanase, endoglucanase, beta-glucosidase, and the synergism mainly between the two first has an important role in the course of hydrolysis.

By this reason and taking account the abundance of sugar cane bagasse to be annually available and is regarded as underutilized in Mexico (approximately 13 million ton/year, about 35% in this total was bagasse pith), our aim is to examine the saccharifying capacity of the cellulases of three strains isolated in our laboratory acting alone or together. Cellulases from *Aureobasidium sp.* CH-M-018 or *Penicillium sp.* CH-M-001, mixed with the *Aspergillus terreus* CH-M-013 cellulase in different volumetric ratios, were tested under the proper conditions for the yield of soluble reducing sugars on non-pretreated sugar cane bagasse pith. These three cellulolytic fungi were selected mainly because of their different relations in enzyme activities obtained on the same material.

A large enhancement of saccharification rate of native bagasse pith due to cross-synergism was observed in all experiments with a maximum increase of 42% in the rate of reducing sugar production when culture fluids of *Penicillium sp.* and *A. terreus* with the same protein content, were mixed in 4:6 volumetric proportion than that obtained with the most active filtrate alone and gives 12% higher rates and extent of saccharification than would obtained from the sum of hydrolytic activities of individual cellulases.

Poster 71

FERULATE CROSS-LINKING LIMITS DEGRADATION OF LIGNIFIED GRASS WALLS BY FUNGAL HYDROLASES

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Ferulate cross-linking of arabinoxylans to lignin may limit degradation of grass walls by fungal hydrolases. Wall-bound peroxidase and *in situ* generated H_2O_2 was used to form dehydrogenation polymers of coniferyl alcohol within nonlignified walls isolated from maize (*Zea mays* L.) suspension cultures. Cell walls with 17 mg g^{-1} and 4.5 mg g^{-1} of ferulates were lignified to Klason lignin levels up to 150 mg g^{-1}. Ferulate concentrations in cell walls were reduced by growing cultures with 2-aminoindan-2-phosphonic acid or by selectively methylating wall ferulates with diazomethane prior to lignification. Structural carbohydrates in nonlignified walls were extensively degraded (>90%) after a 72 h incubation with hydrolases from *Trichoderma reesei* and *Aspergillus niger*. In cell walls with high ferulate concentrations, each unit of lignin was reduced by 70%, structural carbohydrate degradation was increased by about 20%. Ferulate cross-linking reduced the release of all neutral and acidic sugars from cell walls, particularly that of xylose. Our results indicate that modification of grasses to achieve low ferulate concentrations will improve the enzymatic degradation of structural polysaccharides for bioconversion into ethanol.

Poster 72

FERMENTATION OF ORANGE PEEL HYDROLYZATES BY ETHANOLOGENIC *Escherichia coli:* EFFECTS OF NUTRITIONAL SUPPLEMENTS

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Orange peel, an abundant by-product of the citrus processing industry, can be easily converted to a mixture of monomeric sugars by hydrolysis with mixed pectinase and cellulase enzymes. Glucose, galacturonic acid and fructose are the main sugars in orange peel hydrolyzates, but arabinose, galactose, and traces of xylose are also present. These sugars can be fermented to ethanol or ethanol and acetic acid by a recombinant bacterium *Escherichia coli* KO11 in the presence of 2.5 g/l each of yeast extract and tryptone. Since these complex nutritional supplements are relatively expensive and supplemented peel hydrolyzate was fermented at a low rate, we have undertaken a more extensive nutritional study of these fermentations. The results indicate that peel hydrolyzates contain sufficient levels of mineral nutrients and vitamins to support growth and fermentation by *E. coli* KO11, but provide inadequate supply of amino acids. This nutritional deficiency can be eliminated by supplementation with protein hydrolyzates, corn steep liquor, or by proteolytic digestion of endogenous proteins. *E. coli* KO11 does not have an auxotrophic requirement for any individual amino acid, but its growth and fermentation are stimulated by supplementation with a complete mixture of these acids.

Poster 73

RECOMBINANT XYLOSE-FERMENTING *Saccharomyces* CAPABLE OF EFFECTIVE FERMENTATION OF SUGARS FROM LIGNOCELLULOSIC HYDROLYZATES

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Ethanol has been identified as a desirable alternative liquid fuel for transportation. This is partly because renewable lignocellulosic materials, which are inexpensive and abundantly available, can be readily converted to ethanol. However, there are at least two prerequisites that must be fulfilled in order for lignocellulosic materials to be used as the feedstocks for the production of fuel ethanol. The first is to have one or more microorganisms that can effectively ferment the sugars present in the cellulosic hydrolyzates. The other is that the microorganism used for fermentation must not be inhibited by other substances present in the hydrolyzates. In addition to glucose, xylose is the second major fermentable sugar present in most lignocellulosic materials. Microorganisms chosen as the host to convert cellulosic materials to fuel ethanol must be able to effectively ferment both glucose and xylose to ethanol. It is known that *Saccharomyces* yeasts traditionally used in industry to produce ethanol can only ferment glucose, but not xylose, to ethanol. Recently, we have developed recombinant *Saccharomyces* that can ferment both glucose and xylose effectively. Nevertheless, our recombinant yeasts were characterized by using purified sugars for fermentation. In this presentation, we will demonstrate that our recombinant xylose-fermenting *Saccharomyces* can also very effectively ferment both glucose and xylose present in the crude acid hydrolyzates of lignocellulosic materials.

Poster 74

INTRODUCTION AND EXPRESSION OF FOREIGN GENES IN *Candida shehatae* AND *Candida magnoliae*

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The yeasts *Candida shehatae* (CBS 22984) and *Candida magnoliae* (YB4266) were mutagenized with ethylmethane sulfonate and uracil auxotrophs were selected by growth on 5' fluoroorotic acid. Some *C. shehatae* and *C. magnoliae ura* mutants gave rise to Ura+ colonies when plasmids bearing an *URA3* gene and an autonomously replicating sequence (ARS) from *P. stipitis* were introduced by electroporation. The *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I (*ADH1*) or the *Pichia stipitis* gene for xylose reductase (*XYL1*) were introduced into mutant (*ura3*) strains of *C. shehatae* and *C. magnoliae* using the transformation system designed for *P. stipitis* (Yang et al. AEM 60:4245 1994). Ura+ colonies arising after electro-transformation bore the correct transformed DNA as an extrachromosomal plasmid. The transformed strains maintained the plasmids under fermentative conditions over a period of days. The introduced heterologous genes were expressed as shown by increased enzyme activity. In shake flask fermentations, sugar metabolism in transformed strains is changed relative to untransformed control strains.

Poster 75

INCREASED XYLOSE REDUCTASE ACTIVITY IN *Pichia stipitis* BY OVEREXPRESSION OF *XYL1*

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Autonomous and integrative yeast expression vectors were developed in order to genetically engineer strains for improved xylose fermentation. The known sequence of the *Pichia stipitis* xylose reductase (*XYL1*) promoter and coding region was cloned into a plasmid that *P. stipitis* maintains in high copy number. The expression vector and a control vector without the *XYL1* insert was used to transform *P. stipitis*. Promoter control was tested in transformants grown on glucose and on xylose. When grown on xylose under relatively aerobic conditions, the strain transformed with the expression vector had up to 1.8 fold higher xylose reductase (XOR) activities than that of the control strain. Oxygen limitation led to higher activity in both test and control transformants. When grown on glucose under aerobic or oxygen-limited conditions, the activity of the test strain was up to ten times higher than that of the control. Ethanol production was not improved with the introduction of the expression vector, and under most conditions the ethanol yield was lowered. The physiological and metabolic response of the yeast to elevated XOR levels indicates that this enzyme does not limit ethanol production under the conditions employed. The promoter can be used to express other heterologous proteins important for xylose fermentation.

Poster 76

SOLID-STATE FERMENTATION WITH *Trichoderma reesei* FOR COMPLEX GLUCOSIDASES PRODUCTION

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The complex glucosidases are extensively used in processing of plant side-products of agriculture. Thus it is important that the processing need the complex glucosidases rather than only an enzyme. *Trichoderma reesei* Rut-C30 was selected for complex glucosidases production from a series of the higher cellulase production strains with solid-state fermentation. The optimum conditions for enzyme production were maize straw powder 7 g, wheat bran 3 g, 2% $(NH_4)_2SO_4$ solution 30 ml, pH5-6 at 30°C for 5 days. Under those conditions, its enzyme activities for filter paper, CMC, -glucosidase, xylanase, amylase, and pectinase reached 21.35, 123.49, 41.78, 869.07, 81.02, and 46.36 IU/g dry wt of medium.

Poster 77

THE RELATIONSHIP BETWEEN GROWTH ENHANCEMENT AND *pet* EXPRESSION IN *Escherichia coli*

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Wild-type *E. coli* is heterofermentative with lactic acid being the predominant end-product of anaerobic glucose metabolism. Transformation of *E. coli* 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 from lactate to ethanol as the major end-product.[1,2] Transformants are recognized by an increased colony morphology on solid media. This observation coupled to that of enhanced growth of an ethanologenic recombinant culture in aerobic shake flask cultivations (in the absence of pH control) prompted the conclusion that *pet* had a positive influence on growth yield.[3] Subsequent anaerobic fermentations conducted under glucose-limiting conditions, at constant pH, confirmed that the growth yield of *pet* expressing recombinants was improved over that of the wild-type culture.[4] Furthermore, from the results of a separate investigation, it was concluded that growth enhancement could not be attributable to effects on external pH or ΔpH, but rather to the effect of the switch in pathways from mixed acid to ethanol fermentation.[5]

In this study, the growth yield of wild-type (*E. coli* B ATCC 11303) and *pet* expressing recombinants were compared in pH-controlled batch fermentations using LB medium with growth limiting concentrations of both hexose and pentose sugar substrates. Two recombinant types were used—a strain in which *pet* expression was via a multicopy plasmid (pLOI297) and a chromosomally integrated construct (KO11). The results of this study are at variance with the conclusions of others.

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1 Ingram, L.O. et al. (1987) Appl. Environ. Microbiol., 53, 2420–2445.

2 Ingram, L.O. et al. (1991) U.S. Patent 5,000,000.

3 Ingram, L.O. et al. (1988) Appl. Environ. Microbiol., 54, 397–404.

4 Diaz Ricci, J.C. and Bailey, J.E., (1992), Biotechnol. Bioeng., 39, 59–65.

5 Diaz Ricci, J.C. et al., (1991) Biotechnol. Prog., 7, 305–310.

Poster 78

FACTORS CONTRIBUTING TO THE LOSS OF ETHANOLOGENICITY OF RECOMBINANT *E. coli* B (pLOI297) and KO11

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To be compatible with modern continuous bioconversion systems, it is imperative that the process organism exhibit an extremely high degree of stability, which in the case of ethanol production, can be functionally assessed in terms of the high selectivity of ethanol as end-product of the fermentation of biomass-derived sugars. Ingram and his research associates (University of Florida) have cloned the genes for pyruvate decarboxylase and alcohol dehydrogenase from the bacterial ethanologen, *Zymomonas mobilis* and expressed them in *Escherichia coli* B (ATCC 11303). The original constructs depended on expression from multicopy plasmid vectors; however, more recently chromosomally integrated recombinants have been engineered. From a variety of different constructs two recombinant cultures seem to have emerged as candidate process biocatalysts for ethanol production from lignocellulosic feedstocks—these being *E. coli* B bearing the *pet* plasmid pLOI297 and the chromosomally integrated variant KO11. Both of these constructs have been shown to exhibit high ethanol yields from hexose and pentose sugars in pH-controlled batch fermentations. With both types of recombinant, antibiotic resistance has been linked to high expression of the *Zymomonas* genes. Although improved stability has been claimed for the chromosomally integrated construct KO11, the literature is silent with respect to the fermentation performance of KO11 in the absence of chloramphenicol.

In this study we examined the compatibility of these recombinants to continue flow systems. Stability was assessed in terms of the requirement for the constant selective pressure due to the presence of antibiotics. Other environmental factors which contribute to the loss of ethanol selectivity are also described.

Poster 79

STUDIES ON NUTRIENT REQUIREMENTS AND COST-EFFECTIVE SUPPLEMENTS FOR ETHANOL PRODUCTION BY RECOMBINANT *E. coli*

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Whereas *Escherichia coli* B (ATCC 11303) utilizes all biomass-derived sugars, lactic acid is the major end-product of anaerobic metabolism. A recombinant that expresses *Zymomonas mobilis* genes for pyruvate decarboxylase and alcohol dehydrogenase II on a multicopy plasmid designated pLOI297 (Ingram et al., U.S. Patent 5,000,000) has been shown to convert both hexose and pentose sugars to ethanol at near maximum theoretical efficiency. However, the majority of this research has been conducted using a complex and nutrient-rich medium (Luria broth) composed of laboratory-grade chemicals. The use of expensive nutrient supplements has serious negative impact on the economics of ethanol production. The extent to which these complex nutrients are essential for high performance fermentation is not known. The objective of this study was to establish the basic nutritional requirements of recombinant *E. coli* using defined media with LB as the reference for comparative fermentation performance in terms of both ethanol yield and productivity in pH-controlled batch fermentations with xylose as substrate. The use of less costly industrial-grade complex nutritional supplements (yeast extracts, protein hydrolyzates, corn steep liquor) was also investigated.

Poster 80

CLONING AND EXPRESSION OF FULL-LENGTH *Trichoderma reesei* CELLOBIOHYDROLASE I (CBH I) cDNAs IN *E. coli*

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CBH I, a ß-1,4-exoglucanase (EC 3.2.1.91), comprises about 60% of the cellulase protein produced by *Trichoderma reesei* and has been shown to boost the catalytic activity of many cellulase systems, both fungal and bacterial. CBH I is able to synergistically degrade crystalline cellulose in combination with a variety of fungal and bacterial ß-1,4-endoglucanases (EC 3.2.1.4). Although isolation of the CBH I gene was reported nearly a decade ago, it has not been readily available for heterologous expression studies.

A cDNA library was constructed from mRNA isolated from lactose-induced, cellulose-grown *T. reesei* mycelia. Two distinct full-length CBH I cDNA clones were isolated from this library using digoxigenin-labeled PCR probes via plaque hybridization. These clones contain the entire CBH I coding sequence and differ slightly in the 5' and 3' noncoding regions. These coding sequences will be employed in the development of heterologous expression systems for the preparation of rCBH I for biochemical studies.

Poster 81

DISCOVERY OF A NEW PHOTOSYNTHETIC WATER-SPLITTING REACTION FOR PRODUCTION OF HYDROGEN AND OXYGEN[1]

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Sustained simultaneous photoevolution of molecular hydrogen and oxygen has been observed in mutant B4 of *Chlamydomonas reinhardtii*. B4 lacks Photosystem I, containing Photosystem II only. Data are presented which indicate that Photosystem II is capable of spanning the potential difference between water oxidation/oxygen evolution and proton reduction/hydrogen evolution. In addition, B4 is capable of photoassimilation of atmospheric carbon dioxide. Direct kinetic time-profile experiments on CO_2 uptake with mutant B4 and control experiments with wild-type *Chlamydomonas* 137c indicated that whereas the wild-type alga, containing both light reactions, had stable photosynthetic activity in air and anaerobiosis, photosynthesis in mutant B4 was stable under anaerobiosis only. Results are discussed in terms of thermodynamic limits of photosynthesis, especially known energetic properties of primary donor and acceptor of Photosystem II, accessibility of ferredoxin to accumulated reduced species on the reducing side of Photosystem II in mutant B4, and origin of the photosynthetic function in early and present-day atmospheres.

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

Poster 82

PROPOSED FLAVIN K_{eq}(O/R) CONSTANT

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New biphasic rate equations: 1-quantitate total $FLDH_{2Av}$ formed by this unique NADPH: Flavodoxin Oxidoreductase, 2-further predict the comproportionation reaction, and 3-describe a quadratic equation for each reductase reaction. The rate equations for the 5 mM FLD_{Av} oxidation and reduction rates appeared as polynomial coefficients of the Least Squares analysis of the 5 mM FLD_{Av} trial fit the equations: $f(t) = 0.01358+0.6482*t+0.0007293*t^2$ and $f(c) = 0.02093+1.5127*c-0.0026126*c^2$. The standard deviation of this fit is 0.38 and the correlation is 0.99. Formation of $FLDH_{2Av}$ satisfies the quadratic equation $c = 0.007899+0.65049*t+0.0001311*t^2$. Also, formation of $FLDH^*_{Av}$, a zero order reaction, originates from these equations: $f(t) = (0.3776 + (0.1194*t))$ and $f(c) = 4.1700 + (0.01189*c))$.

Application of these equations quickly stimulates fresh speculation concerning the considerable excess formation of $FLDH_{2Av}$. Primarily controlled by O/R potential, leading to large excesses of flavodoxin hydroquinone under very reduced, low oxygen potential and very low O/Rs. Conversely, relatively high oxidation states impose very large excesses of flavodoxin quinone under low reduction potential and high O/Rs. The presence of this tri-molecular flavin equilibrium is very beneficial to environmentally competitive microorganisms.

Poster 83

SCIENTIFIC AND PRACTICAL APPLICATIONS OF STEROL'S MUTANTS OF MICROALGAE

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Great variety of sterols in plants compared with those in mammals and fungi enables to obtain numerous specific substrata. Using mutants (it is preferable scheme) and inhibitors of sterol biosynthesis results in accumulation of intermediates instead of major sterols too. Thus plant mutants with altered sterol content may provide commercial source of precursors for further chemical and microbiological transformations to steroid hormones and others. There are some difficulties with selection and fertility of mutants in higher plants, so investigations with microalgae may be useful.

More than 500 spontaneous and induced by set of mutagens clones of *Chlamydomonas* were isolated on selective media. Ten strains with stable phenotype (after reseeding) were tested biochemically and many differences in sterol content have been detected. To study mechanisms of regulation and control of sterol's metabolism for construction of sterol's super-producers the genetic analysis is carrying out now.

On the other hand, some microalgae are biotechnological objects and production of sterols as main or co-products may be an effective way for the complex utilization of algal biomass (by analogy with ergosterol and its intermediates in yeast). So, 5 clones of *Chlorella* with altered sterol composition which were isolated in the same manner are testing now in the laboratory, conditions for optimization of the cultivation regimes.

Poster 84

NOVEL "BROWN" STRAINS OF CHLORELLA— PORPHYRIN-PRODUCERS

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Chlorella is one from a few species of microalgae which is cultivating on a large scale now. Biomass of Chlorella can yield various pigments (and its derivatives) for further processing down to the commercial products. Colonies of pigment mutants with altered color may be isolated easily by visual observation.

So, algal strains accumulating precursor of chlorophyll—pure protoporphyrin IX (contrary to other microorganisms, which produce mixture of porphyrins) form brown colonies usually. This ability is suitable marker for primary selection and now numerous light- and dark- "brown" strains are collected in our lab. They were induced mainly by UV-irradiation, nitrosoguanidine, and streptomycin action.

There are three types among them which are interesting in the great extent from point of view of biotechnological applications. First of them secrets pigment products into cultural media in some cases. Second is able to grow phototropically, i.e., this strain differs from such kind mutants in that it has resistance to light. Specific feature of mutant of third type is chaining of pigmentation from green color to grayish-brown color during replacement from the light to the dark cultivation conditions. (1,2 obtained by Stolbova, 3 by Nakonechny.)

These mutants are useful tools for studying of general problem of photosynthetic apparatus biogenesis. Current screening procedures direct to selection more productive and technological strains of Chlorella with desirable properties.

Poster 85

EVALUATION OF COMMERCIAL CELLULASE PREPARATIONS FOR USE IN ETHANOLOGENIC FERMENTATIONS OF CELLULOSIC BIOMASS

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Lignocellulosic biomass, such as short rotation woody crops, herbaceous plants, agricultural residues, and wastepaper represent a cost-effective source of carbohydrates for ethanol fermentation. Enzymes capable of degradation of the recalcitrant cellulose are known as cellulases. Commercial cellulase enzymes have been used in the food, detergent, and textile industries and are also effective for the processing of biomass feedstocks.

A survey was undertaken to identify major manufacturers/ distributors of cellulase and to evaluate representative commercial products for enzyme activities, protein content, and chemical composition. Samples were subjected to activity measurements on filter paper, carboxymethylcellulose, xylan, and cellobiose. To ascertain the microbial origin of the cellulase preparations, Western blots utilizing monoclonal antibodies specific for the major cellulases of *Trichoderma reesei* were developed. Additionally, polyvalent antiserum raised in rabbits immunized with purified *Aspergillus niger* ß-D-glucosidase was used to screen enzyme preparations to detect supplementation with the enzyme. The results of enzyme assays, immunorecognition tests, and physicochemical analysis of these preparations will be reported.

Poster 86

GENERATING OF THE EXOPOLYSACCHARIDES WITH SETUP CHARACTERISTICS BY *Acinetobacter sp.*

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A strain of *Acinetobacter sp.* bacteria, the producer of high-viscous exopolysaccharide (EPS) had been isolated. The EPS appeared to comprise glucose, mannose, galactose, and rhamnose residues etherified by the fatty acids.

The acidic groups are presented by glucuronic and pyruvic acids residues. EPS was determined to be heterogenous and to consist of one neutral and two acidic polysaccharides, one of which is acylated. The method of separation of acidic polysaccharides basing on the emulsifying ability of acylated exopolysaccharides has been elaborated.

The acidic exopolysaccharides are identical in monosaccharides, pyruvic, and glucuronic acids ratio. Due to the fact that EPS comprises fatty acids, its solutions have some significant characteristics: e.g., emulsifying ability, viscosity increasing in the presence of cations as well as in the low shearing rates area at low pH values.

During the producer cultivation the nonacylated and acylated polysaccharides ratio changes in the content of the synthesized EPS. It results in changes of the EPS solutions characteristics.

The conditions of producer cultivation which made it possible to obtain EPS with steady, setup characteristics have been established. It makes EPS attractive for oil extracting, chemical, and food industries.

Poster 87

COAL-INDUCED ENHANCEMENT OF ETHANOL AND BIOMASS PRODUCTION

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The effect of coal on ethanol production (from dextrose) by *Saccharomyces cerevisiae* was explored. Inclusion of solid or soluble forms of lignite coal in basal growth medium enhanced ethanol production to the same overall level that was attained when yeast extract was added to the medium. Coal and yeast extract caused similar maximum ethanol production yields. Coal-induced enhancement required a longer time to reach maximum ethanol level in comparison to yeast extract-induced enhancement. Both coal and yeast extract also enhanced biomass production levels in direct correlation to ethanol production levels. However, ethanol/biomass ratios were twice as high for coal-induced enhancement in comparison to yeast extract-induced enhancement. In addition, humic acid (a chemical similar to coal) enhanced ethanol and biomass production. Coal was found to supply nitrogen, phosphorus, magnesium, and zinc for cell growth. Coal also appeared to facilitate the uptake of cobalt, and it reversed the toxic effects of high concentrations of aluminum and manganese. This suggests that part of the mechanism for coal-induced enhancement of metabolism is due to a chelator effect; this was supported by the observation that ethylenediamine-tetraacetic acid mimicked the enhancing effect of coal. Coal also enhanced ethanol and biomass production by *Zygosaccharomyces rouxii*. In conclusion, coal appears to enhance ethanol and biomass production by supplying macronutrients, micronutrients, and by detoxifying growth medium.

Poster 88

DEVELOPMENT AND VALIDATION OF A NOVEL MATRIX FOR IMMOBILIZATION OF CELLS AND CELL FRACTIONS[1]

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A novel matrix for immobilization of cells or cell fractions used in biotechnological applications has been developed. This polyurethane-based material possesses a number of characteristics that make it a desirable candidate for use in a wide variety of processes. These characteristics include high mechanical strength, good biomass retention when properly formulated, ability to be formed into small-diameter spherical beads, excellent mass-transfer potential, and resistance to failure when subjected to harsh chemical solutions or repeated usage. Details of the formulation of this material and the unique laboratory-scale system was deployed as a matrix for immobilization of a microbial species being used as a biosorbent for uranium in aqueous solution. In this application, biomass loadings of up to 33% were achieved without any noticeable leakage of biomass over time. Kinetic data from both batch and flow-through column studies using this unique biosorbent product will be presented. Performance under a variety of chemical and physical conditions will be discussed.

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Poster 89

EVALUATION OF ACETYL CELLULOSE ESTERASE (ACE) ACTIVITY IMPORTANT IN THE ANAEROBIC BIOCONVERSION OF COMMERCIAL CELLULOSE ACETATE POLYMERS

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In a previous study, we determined that anaerobic biodegradation of cellulose was inhibited by acetylation at levels exceeding 1.5 degrees of substitution (DS). The proposed mechanism for the biodegradation of cellulose acetate polymers by cellulase enzymes requires the cooperative action of an acetyl esterase to reduce polymer acetylation prior to the hydrolysis of the cellulose. An anaerobic culture capable of biodegrading commercially available cellulose acetate (DS = 2.4) was developed. This culture was maintained on commercial-grade cellulose acetate (Eastman Chemical Co. #04655) using a 3.5-L continuously stirred tank reactor (CSTR) system. The CSTR was operated at 37°C with an organic loading rate of 1.2 g of volatile solids per liter per day. The retention time was maintained at 14 days, and digester effluent was collected for analysis. Active ACE was detected in both the supernatant and the solids fractions of digester sludge. Protocols that employ dilute detergents were used to extract active acetyl esterases from the digester effluent solids. Both sodium dodecyl sulfate (SDS) and Triton X-155 were effective in extracting solids-bound esterase activity. More than 60% of the total acetyl esterase activity was associated with the solids fraction. Using a variety of substrates, digester-resident acetyl esterase activity was evaluated. The optimal acetyl esterase activity was identified with respect to temperature and pH.

Poster 90

BIOCONVERSION OF RICE STRAW HYDROLYZATE FOR XYLITOL PRODUCTION

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Lignocellulosic residues as rice straw represent an inexpensive and abundant source of biomass that can be used as a substrate in bioprocesses. The use of this biomass is a challenge since it is a cause of serious environmental problems. In recent years there has been a constant increase in studies of the utilization of residues in the production of useful chemicals.

Xylitol, a valuable product with anticariogenic and clinical properties is one of the substances that can be produced by fermentation processes using this biomass as substrate. Currently, xylitol is produced by chemical means using nickel as a catalyst. However, this process is expensive since it requires several purification steps for xylose before the chemical reaction proceeds. Microbial production of xylitol from agroindustrial residues is a more economical and simple approach since it does not require pure xylose. Furthermore, this bioprocess occurs at lower temperatures and pressures compared to the ones for the chemical method. In our investigation, we selected the yeast *Candida guilliermondii* FTI 20037 and determined some fermentative conditions that affected xylitol production from rice straw as substrate. The batch fermentations were conduzed in acid hydrolyzate treated with NaOH pellets. The experiments were performed in Erlenmeyer flasks under 200 rpm at 30°C for 72 hours in semi-aerobic conditions. Xylose and xylitol were determined by HPLC and cell concentration indirectly by correlation of the dry weight of the cells and absorbance at 600 nm.

The maximum xylitol concentration was 38.8 g/L at pH 6.0 using urea as a nitrogen source. However, the higher fermentation rates were obtained at pH 5.3 in a medium containing ammonium sulfate in the same C/N ratio. Statistical analysis shows that the xylitol production was significantly affected by pH and the nitrogen source as well as by the interactions between both of these factors. According to the results, the bioconversion of rice straw hydrolyzate has potential application in large-scale fermentation due to the high yield and simple pretreatment method employed.

Poster 91

EFFECT OF CULTURE CONDITIONS ON XYLITOL PRODUCTION BY *Candida guilliermondii* FTI 20037

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Xylitol, a 5-carbon sugar with anticariogenic properties and clinical applications, can be obtained from hemicellulosic biomass. Currently, xylitol production is carried out by chemical conversion; however, this process is very expensive due to several purification steps performed before the catalytic hydrogenation of D-xylose. A biotechnological approach has significant advantages, since no purified D-xylose is required and no toxic products are produced. Nevertheless, the use of microorganisms implies the evaluation of physiological parameters which can lead to higher xylitol conversion and productivity.

According to the results, the age of inoculum seemed to influence only the productivity and not the final xylitol concentration. The highest xylitol productivity (0.66 $g.L^{-1}.h^{-1}$) was reached using a 24h-culture. The yeast extract concentration significantly affected the xylitol formation and the best results were obtained using 12.5 $g.L^{-1}$ of this nutrient. The specific growth rates (μ) in the different media ranged from 0.04 h^{-1} for xylose to 0.11 h^{-1} for glucose with intermediate values obtained for mixtures of these sugars. Cultivation of the inoculum using different carbon sources did not significantly influence the latter fermentation. Finally a batch fermentation in a 1-L fermenter using sugar cane bagasse hydrolyzate was conducted. The yeast was able to ferment this medium, producing 18.40 $g.L^{-1}$ of xylitol from 29.50 $g.L^{-1}$ of xylose, at a production rate of 0.38 $g.L^{-1}.h^{-1}$.

These results increase our overall understanding of optimization of biological xylitol production and demonstrated the great potential of the *Candida guillermondii* FTI 20037 yeast in this bioprocess.

Poster 92

ISOZYME POLYMORPHISM OF ENDOGLUCANASE ENZYME OF *Streptomyces*

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Isozymes are multiple molecular forms of enzymes which have been found to be important in the study of differential gene action of an organism. The electrophoretic analysis of endoglucanase enzyme of various *Streptomyces* species revealed the existence of multiple city of its isozymes. Ten zones of the enzyme designated as EG I, EG II, EG III, EG IV, EG V, EG VI, EG VII, EG VIII, EG IX, EG X were detected in natural population of *Streptomyces*. EG IX was found to be the most common zone which was detected in 48 out of 103 isolates. All the zones were found to be polymorphic with 2–8 electromorphs in different zones. A similarity in the banding patterns of the isolates having similar morphological features was observed. Depending upon the banding pattern and electrophoretic mobility of isozymes, these isolates of *Streptomyces* could be grouped into 38 categories which manifested the potential of isozyme technique as a taxonomic tool for the differentiation of various *Streptomyces* species.

Poster 93

PROPERTIES OF PURIFIED ENDOGLUCANASES OF *Chaetomium erraticum*

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The endoglucanase, a component of cellulase enzyme complex of *Chaetomium erraticum* revealed three forms viz. Endo I, Mw 25,000; Endo II, Mw 33,000; and Endo III, Mw 50,000, after purification using gel chromatography and preparative electrophoresis. All of these could withstand a pH range of 5.0–9.0 without loss in activity. Even after 30 min. of incubation at 50°C, the enzyme was fully active. Endo II and Endo III were more active at 60°C. Ag^{++} inactivated all the three components where Endo III was less prone to HG^{++} and Fe^{++}. A significant promontory effect was exhibited by all the three forms in the presence of Cu^{++}, Ca^{++}, Co^{++}, Na^{+}, Zn^{++}, Mg^{++} and EDTA. The K_m values were 6.0, 4.8, and 5.05, while V_{max} was 18.0, 10.0 and 18.0 for Endo I, Endo II and Endo III, respectively. The carbohydrate content was 10, 14.8, and 24.0, which indicated the glycoprotein nature of these enzyme forms.

Poster 94

A RECOMBINANT STRAIN WITH CELLULASES OVER-PRODUCTION PREPARED BY PROTOPLAST FUSION OF *Aspergillus niger* AND *Trichoderma reesei*

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In an attempt to improve the saccharification of cellulose, intergeneric protoplast fusion of taxonomically different *A. niger* and *Trichoderma reesei* was carried out. The fusion produced two types of hybrids that were identified on the basis of DNA content and nuclear diameter compared to parental strains. The first type was unstable diploid that showed segregation on MM, while the other type has been segregated on either MM or CM with or without benlate. The majority of segregants showed elevated activity values in more than one enzyme of different cellulases than their corresponding parental strains. The increase in CMCase activity varied between 7.37 and 23.4 times that of the parental strains. Whereas the increase in the ß-glucosidase activity was 1.15 and 1.72 times the activity values of either *A. niger* or *T. reesei*. On the other hand, a tremendous increase in the avicelase activity (cellobiohydrolase) of certain recombinant strains was detected.

Poster 95

ETHANOL FROM LACTOSE BY MODIFIED *Saccharomyces cerevisiae*

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In order to construct a yeast strain having ethanol tolerance with good lactose fermentation ability, the protoplast fusion using an osmotolerant strain of *S. cerevisiae* ATCC 26603 and a lactose fermenting *Kluyveromyces fragilis* ATCC 8608 was carried out. Mutants of the two strains were selected with respect to resistance toward systemic fungicides including imazalil and pimaracine as markers. The fusion frequency using polyethylene glycol and calcium ions solution was 3×10^{-6} leading to a number of fusant diploids that have been selected on the basis of their nuclear diameter, DNA content, and their ability to grow on MM in the presence of imazalil and primaracine. Haploid recombinants revealed best results with respect to their ethanol tolerance, ß-galactosidase activity, and lactose fermentation. The performance of lactose fermentation and ethanol tolerance by these fusants was better than that of *K. fragilis*.

Poster 96

AN OSMOTOLERANT, HEAT-RESISTANT *Saccharomyces cerevisiae* FOR ENHANCEMENT OF ETHANOL PRODUCTION

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A dual purpose strain of *S. cerevisiae* was prepared from an osmotolerant strain ATCC 26603 and a heat resistant strain ATCC 4126. Diploid strains obtained were identified on the basis of their vigorous growth on MM containing imazalil and cyclohexamide at 10 and 5 ug ml^{-1}, respectively. DNA content and nuclear diameter of the diploids were found double the values obtained for parental haploid strains. Upon segregation of the diploids on CM in the presence of benlate, recombinant haploids were identified on the basis of their growth on 50% sucrose at 40°C and the percentage of ethanol produced in the fermentation broths.

Poster 97

PRODUCTION OF CELLULASES AND XYLANASES BY *Trichoderma viride* AND BIOLOGICAL PROCESSING OF LIGNOCELLULOSE AND RECYCLED PAPER FIBERS

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The cellulases and the xylanases are the main enzymes used for recycled fiber modification. *Trichoderma spp.* are well known as producers of cellulolytic and hemicellulolytic enzymes. The production of cellulases and xylanases was studied with *T. viride L-333* (Institute of Microbiology and Biotechnology, University of Latvia). Technological parameters of the cultivation were studied to elucidate the most attractive way of cultivation method and bioreactor design (submerged and solid state). Profiling of pH, carbon source variations, Tween-80, etc. were used to increase mycelial growth and extracellular enzyme production. Significant amounts of cellulase (3–4 FPA U/ml) and xylanase (36–48 IU/ml) were obtained on cellulose based medium at pH = 4.8–5.5 when the submerged fermentation was performed in the fed-batch regime. The endoglucanase peak activities were attained by soluble inducers (sophrose). Production of xylanase was increased by pH-control between 5.5 and 6.0 using birch xylan or wheat bran as inducers. Studies were performed to characterize recycled fiber modification during processing with *T. viride* fermentation both with different amounts of cellulases and xylanases. Hydrolysis of carbohydrates was tested by SEC chromatography using UV and RI detectors. All enzyme treated recycled fibers showed a decrease in fines and an increase in freeness, brightness, WRV-values, and strength properties. The most significant improvement was observed at increased xylanase and comparatively low cellulase activities.

Poster 98

HEXOKINASE PRODUCTION FROM *S. cerevisiae*: CULTURE CONDITIONS

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The effect of pH, temperature (T) and dissolved oxygen (O_2) on hexokinase (HK) and invertase (INV) formation by yeast were studied. Tests were carried out batchwise in a 5-L NBS fermentor as follows: Culture Medium = 2.55 L (3.0 g/L) yeast extract, 5.0 g/L peptone, 2.0 g/L glucose, 15.0 g/L sucrose, 2.4 g/L $Na_2HPO_4.12H_2O$, 0.075 g/L $MgSO_4.7H_2O$ and 5.1 g/L $(NH_4)_2SO_4$); Inoculum (0.70 g dry cell/L) = 0.45 L; Impeller Speed = 500 rpm; Antifoam: silicone; pH: 4.0, 4.5, or 5.0; O_2:0.2, 2.0, 4.0, or 6.07 mg/L and T: 30°C, 35°C, or 40°C. At each hour, 10 mL aliquots of culture medium were taken for determination of cell and sugars concentrations and the activities of HK [in cell debris-free extract attained after cell disruption in presence of glass beads (0.55 mm) in a vortex] and of INV (in intact yeast cells). The best culture conditions for HK and INV formation were: T = 35°C, pH = 4.0, and O_2 = 4.0 mg/L. Furthermore, the HK activity, although remained unchanged against T variation, increased about 30% as pH was varied from 5.0 to 4.0. The data obtained allowed us to assume that the sugar concentration in the medium was not a determining factor for enzymes production. Instead, the intracellular metabolic state could actually regulate the HK and INV production.

Poster 99

Sesbania rhizobiae — A MISNOMER?

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Sesbania rostrata is a popular green manure crop nodulating not only root but also stem. The host specificity of both stem and root nodulating *Rhizobia* of *S. rostrata* was investigated. Cross-inoculation studies proved that *Rhizobia* isolated from the root nodule induced stem nodules and similarly stem nodule *Rhizobia* isolated from the root nodule induced stem nodules and similarly stem nodule *Rhizobia* induced root nodules. To find out whether these *Rhizobia* are specific strains that could nodulate only *S. rostrata* or any other plant belonging to Fabaceae as is the case with cowpea strain, attempts were made to nodulate green gram (*Vigna radiata*) and black gram (*Vigna mungo*) with the Sesbania rhizobial late green gram (*Vigna radiata*) and black gram (*Vigna mungo*) with the Sesbania rhizobial strains. In contrast to the belief, these *Rhizobia* were found to infect and nodulate *V. radiata* and *V. mungo*. As another attempt, the root nodule *Rhizobia* of groundnut were prepared for inoculating the seedlings of *S. rostrata*. Incredibly, the groundnut *Rhizobia* nodulated *S. rostrata* both on the root and stem. These experiments clearly proved that the *Rhizobia* of *S. rostrata* are not genus specific and probably common to any legum-rhizobial symbiotic nexus. It is also of interest to note that stem nodulation does not commonly occur in *S. rostrata* but only under water-logged conditions. To surmise, the *Rhizobia* of *S. rostrata* are not of a special type and erecting a separate generic or even species status seems to be ambiguous and unjustified. Probably, the *Rhizobia* of *S. rostrata* belong to the wide-spectral cowpea strain.

Poster 100

FACTORS AFFECTING POLYHYDROXYBUTYRATE BIOSYNTHESIS IN HALOPHILIC PHOTOSYNTHETIC BACTERIUM *Rhodopseudomonas sp.* STRAIN W1S

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Halophilic photosynthetic bacterium, *Rhodopseudomonas sp.* strain W1S accumulates polyhydroxybutyrate (PHB) as intracellular granules. Cells were incubated under various conditions to study the factors affecting PHB accumulation. We found that the addition of NaCl is essential and vitamins exert inhibitory effect on the PHB accumulation. No PHB accumulation was observed in the absence of NaCl, while the amount of PHB reached 54% of the total cell dry weight in the presence of 5% NaCl. The amount of PHB was decreased to around 20% of the total cell dry weight by the addition of nicotinic acid, biotin, thiamine, and *p*-aminobenzoic acid. These vitamins might stimulate the production of pigment, such as carotinoid, because cells incubated in the presence of vitamins had very deep pink color. Next, we studied the relationship between PHB accumulation and hydrogen evolution. The amounts of PHB accumulated and hydrogen evolved were compared in the presence or absence of carbon monoxide, which is an inhibitor of nitrogenase. Carbon monoxide completely inhibited hydrogen evolution and stimulated PHB accumulation. Under anaerobic conditions there might be a competition between PHB accumulation and hydrogen evolution for reducing equivalent. Depending on the purpose, we could control the metabolism of the strain for the production of sources of clean energy and biodegradable polymer.

Poster 101

ACQUISITION OF ABILITY TO GROW UNDER AUTOTROPHIC CONDITIONS IN HETEROTROPHIC BACTERIA BY INTRODUCTION OF DNA FRAGMENTS FROM HYDROGEN-OXIDIZING BACTERIA

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We have been trying to produce useful materials, such as biodegradable polymer and antibiotics, using carbon dioxide as a carbon source. Hydrogen-oxidizing bacteria have been known to grow autotrophically with hydrogen as an energy source and carbon dioxide as a sole carbon source. Gene clusters, which are responsible for the ability of autotrophic growth in hydrogen-oxidizing bacterium, *Alcaligenes hydrogenophilus*, have already been cloned using broad host range plasmid R68.45. In this study we tried to transfer the recombinant plasmid by conjugation to heterotrophic bacteria, *Pseudomonas acidophila, P. cepacia,* and *P. mesoacidophila*, which are known to secrete antibiotics into the media. Three strains could successfully acquire the ability to grow with gaseous substrates, such as hydrogen, carbon dioxide, and oxygen. Activities of hydrogenase and ribulosebisphosphate carboxylase, which are key enzymes of hydrogen oxidation and Calvin cycle, respectively, were detected in those strains. The recombinant plasmid in three strains were stably retained even after the heterotrophic cultivation with L-broth in the absence of any selection pressure. Moreover, *P. acidophila* secreted the antibiotic inhibiting the growth of *Bacillus subtilis* into the medium under autotrophic conditions. Thus, transferring the ability of autotrophic growth could be a very promising method for the utilization of carbon dioxide.

Poster 102

COMPARISON OF XYLOSE-FERMENTING STRAINS OF *Zymomonas mobilis* FOR ETHANOL PRODUCTION FROM LIGNOCELLULOSIC FEEDSTOCKS

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Efficient conversion of xylose, which is the second most abundant sugar after glucose in lignocellulosic feedstocks, is critical for an economical biomass-to-ethanol process. *Zymomonas mobilis* is recognized for its ability to produce ethanol at high yield and productivity from glucose. However, wild-type strains only ferment glucose, fructose, and sucrose. Recently, we have metabolically engineered *Z. mobilis* to ferment xylose by introducing several key enzymes in xylose assimilation and pentose phosphate pathways. Previous studies have shown significant fermentation performance differences between *Z. mobilis* strains on lignocellulosic hydrolyzates. In this study, we focus on a comparison of xylose-fermenting strains using selected *Z. mobilis* hosts which exhibited superior growth and fermentation performance on glucose-supplemented hydrolyzates. Two different plasmid constructs carrying the key enzymes in assimilation and pentose phosphate pathways were transformed to the selected *Z. mobilis* strains. All the transformed *Z. mobilis* strains were to grow on and ferment xylose to ethanol. A comparison of performance characteristics, such as ethanol yield and productivity, for these xylose-fermenting strains on xylose as well as on a mixture of glucose and xylose at different temperatures was conducted to select the best strains for further process development.

Poster 103

COMPARATIVE PERFORMANCE OF IMMOBILIZED INULINASES I AND II

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Fructose is a well established good in the whole sweetener market due to its native occurrence, easy production from starch, and completely known metabolism, adding its condition of non-insulinogenic monosaccharide. Fructans such as inulin, being composed by about 97% of fructosyl units, are simpler than starch to be processed. Only one step enzymolysis, depending on the inulinase type, may lead to complete depolymerization of inulin to fructose, DFA III (defructose anhydride), or inulooligosaccharides.

YLW (from its yellow pigmentation) is a versatile inulinolytic bacterium previously isolated from rotting dahlia tubers. This glycolipid-rich microbe may be considered a superior DFA producer if compared to the reference strain, *Arthrobacter ureacaciens*, and then, a suitable source for type II inulinase. The excreted enzyme displays some degree of thermostability and hence the interest for immobilization purposes. Incidentally, inulin presentas a limited solubility which can be improved by warming.

Type I fungal inulase, an enzyme which depolymerizes inulin to free fructose, was used as a second model when studying the comparative performance on immobilization.

Current prospects are: a) the immobilization procedures for YLW extra-cellular DFA "synthase" and commercial frutofuranosidase, and their uses in fluidized bed columns; b) intracellular YLW "synthase" and its counterpart DFA hydrolase; and c) chemical nature of the YLW bacterium glycolipid.

Funding: CPNq, CAPES, and UFPR (Brazil).

Poster 104

BIOREFINERY SYSTEM ECONOMICS

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Over the past 15 years, the Tennessee Valley Authority has developed technology for fuel and chemicals production from biomass using acid hydrolysis process for hemicellulosic and cellulose conversion to sugars. Recently, studies were conducted by TVA to evaluate the feasibility of integrating fuel and chemical processes with thermal conversion/power generation processes. Economic evaluations indicate that these integrated systems have enhanced benefits over the individual processes. The results of these evaluations will be discussed in this paper.

Poster 105

MICROBIAL SCREEN FOR ETHANOL PRODUCTION ON CORN FIBER HYDROLYZATE

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Thirty pentose-utilizing yeast strains in the genus of *Pichia stipitis, Pachysolen tannophilus, Candida shehatae, Candida tenius*, and *Pichia segobiensis* from public culture collections were screened for ethanol production on acid converted corn fiber hydrolyzate. None of the strains utilized arabinose in corn fiber hydrolyzates containing 6–8% total sugars of glucose, xylose, and arabinose. The sugar utilization profile and ethanol yield in both simulated hydrolyzate composed of pure sugar mixes and in corn fiber hydrolyzate of each stain was determined. One *Pichia stipitis* and one *Pachysolen tannophilus* strain were chosen for further studies. *P. stipitis* showed better ethanol yield and xylose utilization than *P. tannophilus*. But *P. stipitis* was more sensitive to inhibitors in hydrolyzate and corn steep liquor, required a better aeration control, and showed an inhibition on xylose utilization by arabinose as compared to *P. tannophilus*. Low overall yield and productivity and the requirement of careful aeration control made these strains difficult to use in a commercial process.

Poster 106

CELLULASE COMPLEX PRODUCTION BY SELECTIVE MUTANTS OF *Trichoderma reesei* IN SOLID-STATE FERMENTATION AND ITS HYDROLYTIC POTENTIAL

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The term cellulase complex comprises cellulases and β-glucosidase. Cellulases are assayed as filter paper units (FPU) due to the synergistic action of endo- and exo-glucanases on Whatman #1 filter paper. The cellulase complex containing the ratio of β-glucosidase activity to filter paper activity close to one is considered suitable for hydrolysis of cellulose. In our present study, selective mutants of *Trichoderma reesei*; QMY-1, MCG 80, Rutger C-30 and QM9414 individually and in combination with *Aspergillus phoenicis*, were cultivated in solid state fermentation. MCG 80 and QMY-1 were able to produce cellulase complex with high FPU/g cellulose as well as high ratios of β-glucosidase units to FPU. *A. phoenicis* alone and in combination with the mutants of *T. reesei* was able to produce cellulase complex with high ratios of β-glucosidase units to FPU but had low FPU/mL. *A. phoenicis* dominated the growth of all the mutants of *T. reesei* when grown in co-cultures. High hydrolytic potential was observed with cellulase complex having both high FPU/mL and high ratios of β-glucosidase units to FPU. The low hydrolytic potential of cellulase complex that had low ratios of β-glucosidase units to FPU was attributed to the accumulation of cellbiose in the hydrolyzate, which inhibited further hydrolysis of cellulose.

Poster 107

PRODUCTION OF BIODEGRADABLE COPOLYESTERS OF 3-HYDROXYBUTYRATE AND 3-HYDROXYVALERATE BY *Alcaligenes eutrophus*

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Biodegradable copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate (3HB-3HV) were produced by *Alcaligenes eutrophus* in a two-staged (growth stage and polyester-accumulation stage) process. The choice of carbon sources in the culture medium for the polyester-accumulation stage was used as the variable to control the polymeric compositions, and hence the physical properties, of the copolyesters. When valeric acid was used as the sole carbon source, the copolyester contained 90 mol % of 3HV. A range of copolyesters within 0–90 mol % of 3HV could be produced by using a medium containing butyric and valeric acids, and controlling the butyric-valeric concentration ratio. A mathematical model was constructed to predict the polymeric composition of the copolyesters produced from different fatty acids. The model was based on the assumption that fatty acids in the medium were directly incorporated into the copolyesters without decomposition of the molecular carbon skeletons.

Poster 108

DEVELOPMENT OF AN EPI-FLUORESCENCE ASSAY FOR MONITORING YEAST VIABILITY AND PRETREATMENT HYDROLYZATE TOXICITY IN THE PRESENCE OF LIGNOCELLULOSIC SOLIDS

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Monitoring biocatalyst activity is of fundamental importance in the biomass-to-ethanol conversion process. Although the enzymatic activity can be measured with relatively straightforward assays, determination of the activity of the yeast cells is confounded by the presence of solids in a typical simultaneous saccharification and fermentation (SSF) environment. An assay was developed for determining cell number and percent viability of yeast cells in the presence of SSF solids. The assay utilizes a fungal-specific dye which fluoresces green within yeast cells. The dye is converted to a different form, which fluoresces red, by metabolically active cells. Therefore, by comparing the two forms of the stain, a viability ratio from the yeast culture can be determined.

We have demonstrated success in measuring the viability of *Saccharomyces cerevisiae* D_5A in the presence of paper, sawdust, and corn stover using an automated fluorescence microplate reader. The fluorescence staining procedure is sensitive enough to distinguish live from dead yeast despite background staining of the solid substrate. In addition, we could assess the toxicity of hydrolyzates produced from pretreating cellulosic substrates for fermentation using the same technique. The measured loss in viability of *S. cerevisiae* when incubated in various hydrolyzates became in additional criterion in evaluating effective pretreatments of cellulosic material. The developed approach shows promise as a fast means for monitoring pilot-scale biomass conversion processes.

Poster 109

EFFECTS OF IMMOBILIZATION ON CELLULASE PRODUCTION BY A MODIFIED STRAIN OF *Pseudomonas* BACTERIUM

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The bacterium *Pseudomonas fluorescens var. cellulosa* has been subjected to a mutagenesis program to select for high cellulase activity. The cellulase is constitutively excreted from the cells both during growth and resting states. A promising technique for the continuous production of cellulase from this bacterial source is immobilization of the bacterium in hydrogel beads placed in a continuous fluidized-bed reactor. A comparison of the cellulase production in an immobilized system versus that in free cell cultures will be made based on hydrolytic activity tests on carboxymethylcellulose and on filter paper.

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Poster 110

BIOMASS PRETREATMENT TO ETHANOL

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*Lab-scale conversion of high brix candy wastes to ethanol in a stirred reactor separator. *Lab-scale conversion of xylose to ethanol with various strains of yeast. Lab-scale conversion of cellulose materials to fermentable sugars. A reactor involving simultaneous enzymatic conversion of cellulose to glucose to ethanol will be operated. We are beginning a cooperation with Xylan Inc. which has developed a pretreatment process for wood chips/corn stalks/straw/wastepaper. *Lab-scale conversion of waste glucose to gluconic acid in a continuous immobilized cell reactor. *Plant cell culture conversion of waste glucose to antifungal agents. Enhanced production of the desired product is being attained by immobilizing, permeabilizing, and eliciting the generation of the antifungal compound from immobilized plant cells. *Development of a food processing unit operations computer modeling package for the design and economic comparisons of various food, fermentation, and waste stream utilization processes. This package, termed FOODS, has been worked on for the last several years and is used in teaching food process engineering at Purdue.

Poster 111

STUDIES ON MICROBIAL FILM FORMATION WITH RESPECT TO COAL WATER SLURRY EFFLUENT TREATMENT

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For separation and recovery of coal fines from the effluent discharge of coal washeries/power plants, microbial attached growth/film formation on coal particles has been attempted in the present study.

Aerobic attached microbial growth on coal particles of size (-)500 microns is possible within a pH range of 6.5–7.0 with an external substrate addition in the medium. While free cells, attached cells on coal, and total cells generally increase with substrate addition in the system following almost a similar growth pattern, the share of attached cells in the total cell yield remained quite high within 0.6–0.8. There existed a critical free cell concentration in the medium (2.8×10^9 per mililitre in the experimental system) for which a maximum attachment of 0.3 gm of cell mass per gm of coal was observed. Start-up and operational strategies of the process have been established in terms of coal to substrate concentration ratio and coal to free cell concentration ratio. The average cell aggregate diameter over a period of time ranged between 60–80 microns. The scanning electron micrographs indicated development of bio-film of uniform thickness. Reduction in the TSS from coal slurry effluents has been found to be the order of 10^5 ppm. Operational variables have been identified for their effect on attachment/bio-film thickness/reactor performance.

Poster 112

CELLULASE ACTIVITY OF *Trichoderma reesei* (RUT-C30) ON MUNICIPAL SOLID WASTE

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The loading of existing landfills toward capacity coupled with increasingly stringent environmental constraints invites considerable investigation into alternate methods for handling municipal solid wastes. The application of acid— and enzymatic—hydrolysis to the conversion of lignocellulosic materials including MSW has received a great deal of attention in recent years. It is likely that the growing constraints on landfilling and incineration will promote the application of an increasingly varied mix of these technologies to alleviate handling and disposal problems.

Enzymatic hydrolysis coupled with the fermentation of the resulting sugars presents a potentially strong alternative to landfilling and incineration for handling a sizeable portion of the MSW stream, with the added benefit of chemical production for feedstocks or fuels. The potential for MSW to serve as an inexpensive source for cellulase production for the hydrolysis step in the overall fermentation process further improves the attractiveness of integrating this technology into the broad scheme of solid waste management.

This paper focuses on the utilization of active microbial cultures, specifically *Trichoderma reesei* (strains *RUT-C30*), to hydrolyze the cellulosic fraction (45%–60%) of MSW.

A study is under way to evaluate the capability of *Trichoderma reesei* cultures to saccharify the cellulosic fraction of municipal solid waste. Results indicate the likelihood of using MSW as an inexpensive substrate for producing a "complete" cellulase enzyme system. Additionally, the resulting sugar solution may be used for fermentation to fuel ethanol.

Poster 113

RECOVERY AND PURIFICATION OF LACTIC ACID FROM FERMENTATION BROTH BY ADSORPTION

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Weak- (Reillex 425 and Riedel-de-Haen VI-15), moderate- (Dowex MWA-1, Dowex WGR-2, Dowex XUS-40283, and Dowex XUS-43432), and strong- (Dowex XUS 40196 and Amberlite IRA-958) base resins were evaluated for their sorption capacities of lactic acid from solutions with different pHs. Composite isotherms and fixed-bed sorption indicated that the sorption capacities of weak- and moderate-base resins decreased markedly as the pH of the feed exceeded the pK_8 of lactic acid. The strong-base sorbents exhibited significantly higher sorption capacities for free lactic acid than for lactate.

VI-15, MWA-1 and IRA-35 were employed in a lactic acid recovery scheme using model fermentation broth. The model broth was acidified by using cation exchange resin before passing through the basic sorbent column. The sorbed lactic acid in the column was eluted using methanol or 5% NH_4OH. Lactic acid was completely recovered from VI-15 column after 7 bed volume (BV) of methanol while only 64% was recovered from MWA-1 after 4.5 BV. The 5% NH_4OH eluted all lactic acid from MWA-1 column in 1.5 BV with a maximum effluent concentration of 115 mg/mL. High-purity, heat-stable lactic acid was recovered from Riedel-de-Haen VI-15 when the broth was treated with activated carbon before the acidification step.

Poster 114

PRODUCTION OF L-MALIC ACID FROM FUMARIC ACID BY RESTING CELLS OF *Brevibacterium sp.*

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L-Malic acid, a naturally occurring four-carbon dicarboxylic acid, is a normal intermediate in basic metabolism. It is commonly used as a food and beverage ingredient. L-Malic acid can be produced by the microbial fermentation of carbohydrates. It can also be produced through biological conversion of fumaric acid mediated by fumarase. Many species of microorganisms including bacteria, yeasts, and filamentous fungi are known to convert fumaric acid to malic acid. In this study, we used a fast-growing bacterium, *Brevibacterium sp.*, to produce malic acid from fumaric acid. The rate of malic acid production is influenced by the pH of the substrate, reaction temperature, initial substrate concentration, and the size of inoculum. Resting cells of *Brevibacterium* convert fumaric acid to malic acid at a rate of 50 mM/g/hr and about 85% of the fumaric acid was converted from an initial concentration of 60 g/L. The yield of malic acid is about 87% from fumaric acid. No succinic acid or other acids were detected as the by-product in the incubation mixture.

Poster 115

PRODUCTION OF ETHANOL FROM WASTEPAPER FIBERS USING CELLULASES AND A THERMOTOLERANT YEAST, *Kluveromyces marxianus*

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The production of ethanol by *Kluveromyces marxianus*, a thermotolerant yeast, from wastepaper fibers in the presence of fungal cellulases was studied. In this simultaneous saccharification and fermentation (SSF) process, cellulases performed an important role not only hydrolyzed cellulose to glucose, but also fluidized the wastepaper to form a slurry. The fluidization of wastepaper rendered the high-solid (up to 30% solid) fermentation possible. SSF with *K. marxianus* was evaluated at different temperature ranges with different substrate concentrations. At an elevated temperature of 43°C, about 4% (wt/vol) of ethanol was produced within 100 hrs of incubation. The rate of ethanol production at 43°C is about twice as fast as those conducted at 35°C. The advantages of using thermotolerant yeast for the production of ethanol from cellulose derived from wastepaper fibers are discussed.

Poster 116

SOLID-STATE PRODUCTION OF ETHANOL FROM SORGHUM

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The main goal of this research is to study the solid-state fermentation of sorghum-sudangrass, Grazex II, (F_1 hybrid of *Sorghum vulgare* × *Sorghum sudanese*) to ethanol. With the purpose of lowering production costs, our research focuses on using a modified method of ensiling to produce ethanol directly in the silo. Formic acid, +/- cellulase, and yeast were applied to fresh field-chopped sorghum and then packed tightly into five-gallon plastic silos. Countercurrent extraction methods were used as a means of biofuel separation. Thirty-eight liters of ethanol per metric ton (1/MT) on a wet weight basis (1,000 1/ha) were produced from sorghum receiving cellulase. Sorghum not receiving cellulase additives produced 23 1/MT (620 1/ha) of ethanol. In addition, lactic acid was produced as a by-product (5.2 and 4.6 kg lactic acid per MT sorghum on a wet weight basis for sorghum with or without cellulase, respectively). An average extraction efficiency of 0.64 for five separate extractions was obtained. Methods of improving extraction efficiencies will be discussed.

Poster 117

CONTINUOUS AND SIMULTANEOUS FERMENTATION AND PURIFICATION OF LACTIC ACID IN A BIPARTICLE FLUIDIZED BED BIOREACTOR

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A continuous biparticle fluidized bed reactor is utilized for the simultaneous fermentation and purification of lactic acid. In this processing scheme, bacteria are immobilized in gelatin beads and are fluidized in a columnar reactor. Solid particles with sorbent capacity for the product are introduced at the top of the reactor, and fall countercurrently to the biocatalyst, effecting *in situ* removal of the inhibitory product, while also controlling reactor pH at optimal levels. Long-term fermentation trials using immobilized *Lactobacillus delbreuckii* have demonstrated productivities of greater than 5 g/L*h and product concentrations upon resin regeneration in excess of 50 g/L. The benefits of this reactor system as opposed to conventional batch fermentation are discussed in terms of increased productivity, continuous operation, and decreased downstream processing costs. Potential impacts of improved resin regeneration, self-immobilizing biocatalyst, and decreased feed costs are also addressed.

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[1] Managed by Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

Poster 118

LOWER POWER AND IMPROVED MIXING OF HIGH BIOMASS CONCENTRATIONS WITH COUNTERCURRENT, DOUBLE HELICAL IMPELLER

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Bioconversion processes using biomass raw material require high biomass concentrations for reducing processing costs by minimizing bioreactor size, reducing material handling, and maximizing product concentrations. Biomass slurries containing more than 5%–10% solids form non-Newtonian suspensions that mix poorly using conventional devices. Small turbine or axial-flow impellers are not effective in mixing high viscosity pseudoplastic fluids distant from the impeller. In this case, larger diameter impellers extend the flow to the vessel wall. However, these large impellers, including the conventional anchor agitator, provide poor vertical mixing and require large power input. Helical ribbon impellers are more efficient than conventional, large diameter impellers, but biomass adheres to conventional helical ribbons at high solids concentrations without mixing. This results in unacceptably large temperature fluctuations for microbial processes. Such process instability can be lethal to microorganisms operating near their maximum temperature.

This paper reports on the investigation of several improved mixing devices used in a high solids bench-scale bioreactor. Mixing is quantitated by measuring first order temperature control time constants. The improved mixing resulted in excellent temperature control. A double helix countercurrent device provides the best performance. This device mixed fed-batch or continuous feeding of dry, highly absorbent, pretreated fibrous biomass at initial or cumulative solids levels well in excess of 20% (w/w). The power reduction compared to conventional agitation is discussed.

Poster 119

IMPROVEMENT OF PRODUCTIVITY OF YEAST CELL WITH A NOVEL AIR-LIFT LOOP REACTOR

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The air-lift loop reactor has found wide applications in the biochemical industry because the energy consumption and the damage of shear force to cells can be decreased. But a traditional air-lift loop reactor is generally operated at steady mode, it leads that the velocity of the relative movement between cells and liquid medium is too slow to provide enough rate of mass transfer.

Based on the principle of forced periodic operation of the chemical reactor, a novel air-lift loop reactor was presented. Because of its special structure, while being dynamically run, the direction of circulation flow will be periodically changed so the relative movement between cells and liquid medium is significantly enhanced and the mass transfer coefficient is much increased.

In order to have a comparison, the baker's yeast cell was cultured with this new and traditional air-lift loop reactor, respectively. The experimental results show that both the specific growth rate of cell and the conversion efficiency from glucose to cells is much larger with the new air-lift reactor than with the traditional one.

Poster 120

BIODESULFURIZATION BIOREACTOR WHERE DIBENZOTHIOPHENE SULFUR IS REDUCED TO HYDROGEN SULFIDE BY SULFATE-REDUCING BACTERIA

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A biphasic liquid-liquid stirred-tank reactor was used to grow Sulfate-Reducing Bacteria (SRB) with the model organosulfur compound, dibenzothiophene (DBT) as the sole electron acceptor. DBT was disolved in kerosene, constituting an organic phase dispersed in aqueous microbiological media which was the continuous phase. The reaction mixture was run under a hydrogen atmosphere. Growth of SRBs was invariably associated with hydrogen sulfide production, which was quantified. Bacterial growth rates and H2S production rates were determined at various DBT concentrations and agitation regimes. SRB growth characteristics were influenced by the presence or absence of prior adaptation to growth on DBT. There was also a threshold lower limit on DBT concentration which supported SRB growth.

Poster 121

CELLULOSE DEGRADATION AND ETHANOL PRODUCTION BY THERMOPHILIC BACTERIA USING MINERAL GROWTH MEDIUM

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All studies known to us of ethanol production from cellulosic materials using thermophilic bacteria have used either complex growth medium and/or mixtures of vitamins. Using both previously-described strains of *Clostridium thermocellum* as well as newly-obtained thermophilic, cellulolytic isolates, we report demonstration of cellulase and ethanol production on completely mineral medium using both batch and continuous culture.

Poster 122

EVALUATION OF NITROGEN SUPPLEMENTS FOR BIOCONVERSION OF MUNICIPAL SOLID WASTE TO LACTIC ACID

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Previous studies have demonstrated the ability of *Lactobacillus pentosus* NRRL B-227 to ferment carbohydrates in acid-hydrolyzed municipal solid waste (MSW) to lactic acid. Optimized conditions for fermentation were reported to be nitrogen supplementation of the MSW substrate with tryptone and yeast extract, an initial pH of 7.6, addition of 5% calcium carbonate buffer, 1% v/v inoculation and static fermentation at 32°C. Under these conditions, sugar utilization, lactic acid production, and conversion of sugar to lactic acid were 78%, 65 mg/ml and 87%, respectively. The more expensive components of the MSW fermentation substrate are the nitrogen supplements, tryptone, and yeast extract. To find more economical sources of nitrogen, feather meal, fish meal, soybean meal, cottonseed meal, and meat and bone meal, were evaluated. At a soluble nitrogen concentration of 0.283%, which is equivalent to the concentration supplied by tryptone and yeast extract, soybean meal was the best alternative source of nitrogen and compared favorably to tryptone and yeast extract. After 3 days fermentation of soybean meal-supplemented substrate, sugar utilization, lactic acid production, and conversion of sugar to lactic acid were 82%, 64.8 mg/ml, and 88% respectively.

Poster 123

DYNAMICS OF THE IMMOBILIZATION OF ANAEROBIC MESOPHILIC BACTERIA ON A PLASTIC SUPPORT

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In this work a study on the dynamics of the immobilization of mesophilic anaerobic bacteria was made using as support high-density polyethylene (HDPE) grounded to fine particles and as immobilization system an inverse fluidized bed (IFB) similar to that of González et al. (1992)[1]. After 120 days of immobilization, the acetoclastic and methanogenic activities were stabilized, suggesting that the bioparticles were fully immobilized and the biomass concentration attained, expressed as protein, was 375 micrograms/g dry plastic support.

The specific rate for acetate breakdown and methane production were determined in a value near to 0.0008 millimol acetate/microgram protein/h.

The kinetic parameters of the bioparticles (Ks and μ_{max}) were determined by the initial rates method at different acetate concentrations and were compared to the values obtained by a direct parameter estimation method from the dynamic assays for acetate consumption at one initial concentration. At the same time, the particles were prepared for scanning electron microscopy (SEM) to evaluate quantitatively the biofilm. The SEM studies showed that the support has a furrowed surface with crevices, pleats, and cavities quite suitable for colonization. The same study for particles fully bioimmobilized showed that the surface and cavities were completely colonized and the bacterial structures found were bacillus similar to *Methanotrhix* and grouped *Methanosarcina*-like cocci.

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[1] González, G.; Ramírez, F., and Monroy, O. (1992). Development of biofilms in anaerobic reactor. *Biotech. Letters* **14**(2):149–154.

Poster 124

DEVELOPMENT OF A COST-EFFECTIVE MEDIUM FOR ETHANOL PRODUCTION FROM BIOMASS

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Minimization of nutrient costs is essential if the biomass-to-ethanol process is to be economical. Several industrially available nutrient sources were evaluated using *Saccharomyces cerevisiae* D_5A. Glucose fermentations were used to study the efficacy of nutrient sources at various concentrations. Based on glucose fermentations, a preliminary recipe of 0.1% CSL (corn steep liquor), and 2.5 mM $MgSO_4$ appeared to meet all the requirements needed by D_5A to carry out the fermentation. SSF studies with washed wood solids from dilute-acid pretreated poplar showed that the low-cost medium was similar in performance to a nutrient-rich medium. Upon adaptation of yeast to the hydrolyzate, comparable fermentation performance was also obtained using the whole slurry (wood solids + liquor) as a substrate. This cost-effective medium represents progress toward developing an industrially feasible fermentation for ethanol production from biomass.

Poster 125

CONTINUOUS ALCOHOLIC FERMENTATION PROCESS IN A TOWER REACTOR WITH RECYCLING OF FLOCCULATING YEAST

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The influence of different substrate concentrations on the performance of a continuous system of alcohol fermentation using a tower reactor with recycling of flocculating yeasts was investigated. All of the experiments were carried out using a flocculating yeast strain IR-2, isolated from a fermented food, and identified as *Saccharomyces cerevisiae*. A satisfactory operation was maintained in prolonged runs with yeast concentrations about 100 $g.L^{-1}$. Sugar cane juice, at concentrations from 160 to 200 $g.L^{-1}$, was rapidly fermented to ethanol in a single stage. A dilution rate of 0.20 h^{-1}, a temperature of 30°C, and a pH of 3.3 were used. The performance of the reactor was observed to be efficient with high substrate concentrations. The maximum ethanol concentration and productivity were 90 $g.L^{-1}$ and 18 $g.L^{-1}$, respectively, using 200 $g.L^{-1}$ of sugar in the feedstock. The substrate consumption was 99% for all concentrations of the sugar cane juice used. For substrate concentrations of 160 $g.L^{-1}$, a maximum yield of 0.45 g of ethanol per g of sugar was observed, which is equivalent to 90% of the theoretical value.

Poster 126

OPTIMUM CONDITIONS OF A BIOSURFACTANT PRODUCT BY *Torulopsis bombicola*

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The present study was aimed at developing optimum experimental conditions of a biosurfactant product by *Torulopsis bombicola*. *Torulopsis bombicola* produced Sophorolipid as a biosurfactant. In this study, the effects of carbon source, nitrogen source, and agitation speed were investigated in batch cultures using the flask and bioreactor.

In conclusion, the maximum Sophorolipid product was 130 g/L or 58% of the weight of the total substrate as experimental conditions were agitation speed 600 rpm, temperature 30°±1°C, aeration 1.0 vvm, carbon source of 11% (wt./v.) glucose and 10% (wt./v.) corn oil and nitrogen sources of 0.5% (wt./v.) yeast extract, and 0.1% (wt./v.) urea.

Poster 127

BIOCHEMICAL PROCESSING OF HEAVY OILS AND RESIDUUM

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Heavy oils, residuum and oil wastes represent a substantial resource if a low cost technology for their processing could be developed. In terms of reserves, 50%–70% of original oil in place is still available. However, it is heavy and requires extensive secondary and tertiary recovery technology. Similarly, wastes from oil processing; e.g., refineries, etc., amount to 400 million gallons annually. At the Brookhaven National Laboratory (BNL), we have been over the past few years investigating biochemical processes for the treatment of heavy crude oils and heavy fractions of crude oils. Particular attention has been given to the interactions between extremophilic microorganisms (i.e., high temperature, pressure, salinity) and selected heavy oils. Significant biochemical conversions occur, leading to lighter oils. Recent advances in these studies will be presented and their significance discussed.

Poster 128

CONTINUOUS FERMENTATION BY *S. cerevisiae* IMMOBILIZED IN Ca-ALGINATE BEADS HARDENED WITH TRIVALENT ION

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It is well known that bioreactors' productivities are maximized by increasing the concentration of viable microorganisms by using immobilization techniques. Among others, Ca-alginate is the most common immobilization support applied for this purpose. Mechanical strength of Ca-alginate beads can be increased by a hardening treatment with a trivalent ion. However, there is a lack of knowledge about the influence that such treatment has on the fermentation parameters.

In this work, a repeated batch technique, using a 2^3 experimental design, has been carried out to investigate the influence that the variables involved in a hardening process with a trivalent ion (hardener concentration 0.1–0.3M, time of treatment 5–15 min. and initial biomass concentration in the gel 1–3 g d.w./L) have on fermentation parameters. The contribution of free cells was considered in the final results. The best treatment conditions, considering the specific productivity in ethanol based on viable cells, were 0.3 M, 5 min, and 3 g. d.w./L, respectively.

The biocatalyst obtained by this procedure was employed to carry out an ethanolic fermentation process in a semi-pilot packed bed bioreactor, prolonged during seventy days. Ethanol productivities up to 30.7 g/l-h were obtained. The activity of the biocatalyst and viable cells were evaluated in different zones of the bioreactor remaining quite stable during the continuous process. However, the $Y_{p/s}$ obtained in batch experiments with the beads after the continuous process was lower than the corresponding one with the beads before the process due to the cell leakage that induces the free cells growth reducing the yield observed. A reduction in the overall volume of the bed of about 10% was also observed after the fermentation process.

Poster 129

AEROBIC IMMOBILIZED CELLS IN ALGINATE GEL PARTICLES OF VARIABLE DENSITY

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The use of gel particles of higher density than the conventional gel particles is of particular interest for the application of gel immobilized biocatalyst in fluidized bed bioreactors. The objective of this study is to show a method of preparation of variable density particles of alginate gel containing aerobic immobilized viable cells. It also aims to examine the behavior of these biocatalysts in a fermentation process. A strain of *Acetobacter aceti* sp cells was used as a model of aerobic cells in the oxidation of ethanol to acetic acid process. Particles of gel of variable density were prepared adding different concentrations of α-alumina in the gel matrix. In order to prevent the influence of α-alumina concentration on the cell distribution in gel matrix, the particles were composed of a nucleus containing gel plus α-alumina covered by a layer of gel containing viable cells. Particles of biocatalyst were prepared using an ejector constructed with internal and external capilar tubes. Two solutions—one of them composed of α-alumina plus alginate and the other containing alginate plus cells—were pumped through the capilars and dropped in a calcium chloride solution to gelification. The density of particles was characterized by their terminal velocity and the biocatalyst characterized by the initial respiration rate and by photographs of the cross-section of particles. Profiles of acetic acid concentration and the gas-liquid oxygen transfer volumetric coefficient $K_L a$, were obtained as a function of dilution rate in continuous fermentation. Denser particles of biocatalyst improved the oxygen transfer to the broth, resulting in higher productivity of acetic acid compared with conventional gel particles. This effect increased with the density of the particles, and could be observed at high dilution rates. An empirical correlation among $K_L a$, the fraction solid-liquid in the bioreactor, the terminal velocity, and diameter of particles has been proposed.

Poster 130

PROTEIN-ENRICHED FEED PRODUCTION BY FUNGAL BIOTECHNOLOGY

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Cereal straw (4% urea treated) was inoculated with *Coprinus fimetarius* 386 (3% millet based) at 60%–65% moisture content and incubated for 7 days at room temperature. During the solid substrate fermentation, crude-protein and amino acid contents were increased. The level of contamination was as low as 30%–35%. No mortality was observed when rabbits were fed on concentrate diet containing fungal treated straws. The *in vitro* dry matter digestibility of fermented cereal straw was also increased from the original value. The dry matter intake of goats was higher on fungal treated straw but total digestible nutrients were lower. In a subsequent trial, addition of molasses increased the dry matter intake of goats on fungal treated straws and improved total digestible nutrients also. The dry matter loss was 6.60, 7.56, and 23.36% after 3, 5, and 7 days of fermentation, respectively. The untreated cereal straws had a very wide *in vitro* dry matter digestibility and crude protein ratios that narrowed down during the fermentation which was very similar to oat fodder.

Poster 131

EFFECT OF CORN STEEP LIQUOR ON FERMENTATION OF MIXED SUGARS BY *Candida shehatae* FPL 807

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Candida shehatae FPL 807 is a mutant strain obtained from *C. shehatae* ATCC 22984 by selection for rapid growth on L-xylose and xylitol in the presence of respiratory inhibitors. This mutant strain produced more ethanol from xylose than the parental strain and other wild or mutant strains of *Pichia stipitis* CBS 6054 or *Pachysolen tannophilus* NRRL Y-2460. The growth of FPL 807 on xylose was initially faster but leveled off. Its growth on arabinose continued for 4 to 5 days. Neither ATCC 22984 or FPL 807 fermented arabinose. During fed batch fermentation of glucose alone with FPL 807, ethanol production amounted to 3.5% (w/v). Ethanol production from glucose increased from 3.5% to 5.0% (w/v) when we included corn steep liquor (CSL) in the fermentation medium, but CSL reduced xylose utilization, ethanol, and xylitol formation from xylose. CSL similarly affected glucose and xylose utilization during fermentation of mixed sugars (containing 40 glucose; 40 xylose; 20 arabinose). The maximum ethanol production from mixed sugar fermentation was 3.25% (w/v), and the rate of ethanol production did not change with the addition of CSL. Arabinose was not utilized during mixed sugar fermentation in the presence or absence of CSL. Similar rates of ethanol were obtained with mixed sugar fermentation in 2-L batch fermentor. However, arabinose was utilized when present alone and it promoted arabitol and xylitol formation. When the *C. shehatae* mutant 807 was adapted by serial transfer on arabinose, we observed conversion of arabinose to arabitol and other polyols.

Poster 132

BIOPROCESSING OF SWEET SORGHUM WITH *In Situ* PRODUCED ENZYMES

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Enzyme-assisted ensiling (ENLAC) with *in situ* produced enzymes was developed for a novel bioprocessing of sweet sorghum to ethanol and protein enriched animal feed. The process is based on the ensiling of freshly harvested sweet sorghum with *in situ* produced enzymes and lactic acid bacteria additives. ENLAC increases the sugar yield and the storability of the crop for year-round continuous processing. The ensiled material was extracted in sugar industry type countercurrent diffusers, and the extract was fermented with hexose and pentose fermenting yeasts to produce ethanol. The extracted pulp was recycled as *in situ* enzyme source, after solid substrate fermentation (SSF) with cellulolytic fungi, such as *Gliocladium* and *Trichoderma* ssp. In a 96h SSF, the fermented pulp had 4–5 IU/g DW cellulase and 200–300 IU/g DW hemicellulose activity. About 2% of this fermented substrate was used for inoculating the fresh sorghum, equivalent to 20,000 IU cellulase/MT green material. The use of *in situ* enzymes results in about 30% saving of processing costs, compared to the use of commercial enzymes. The rest of the enzyme-enriched, partially digested substrate was used as animal feed.

Poster 133

INFLUENCE OF LIPOPEPTIDE PRODUCTION ON OXYGEN TRANSFER DURING FERMENTATION OF *Bacillus subtilis*

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Bacillus subtilis produces three types of cyclic lipopeptides named: Iturins, Fengycins and Surfactin. Peptidic moiety of these compounds is constituted of 7 L and Dα amino acids for Iturins and Surfactin and 10 L and D residues for Fengycins. Lipidic moiety is a fatty acid with an amino group in Iturin and a hydroxylic group in Surfactin. Amphophilic structure of these molecules gives them a surfactant behavior. Surfactin is the most powerful biosurfactant so far known. Iturins are also well known for their antifungal activities.

This work deals with the influence of the lipopeptide production on the oxygen transfer during fermentation. Different *Bacillus subtilis* strains have been selected in function of their pattern of produced lipopeptides. Two of them produce only Iturin A or Surfactin, two others coproduce Iturin and Fengycin or Iturin and Surfactin and a fifth strain is able to synthesize Iturin, Fengycin, and Surfactin together. $K_L a$ values have been evaluated with the fermentation supernatant of these different strains.

$K_L a$ values were two or three times lower when the three lipopeptides were coproduced. We also studied the influence of an oxygen vector (n-dodecane) on this $K_L a$.

Poster 134

HIGH ETHANOL TOLERANCE YEAST FOR MANUFACTURE OF ETHANOL

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The subject of ethanol tolerance in yeasts has been receiving significant attention as a result of renewed interest in ethanol as a fuel source. Fermentation of sugars to ethanol using a high ethanol tolerance yeast strain 1400 (fusion product of *Saccharomyces avarum* and *Saccharomyces diastaticus*) has been studied in our laboratory. Inhibitory effects of ethanol and glucose have been studied in batch cultures. A model incorporating substrate and product inhibition has been developed which fits the experimental data well. A maximum ethanol concentration of 13.6% (w/v) was obtained by fed-batch fermentation using glucose as a substrate.

This high ethanol tolerance yeast strain 1400 is unable to ferment xylose. However, genetic engineering of the strain 1400 has made it possible to develop a xylose-fermenting yeast. Preliminary studies are being carried out to investigate the growth and fermentation behavior of this strain. The model characterizing glucose fermentation by the fusion product is being extended to xylose fermentation by the genetically engineered strain.

Poster 135

LIQUID-LIQUID BIOREACTOR SYSTEMS FOR REDUCTIVE DESULFURIZATION OF DIBENZOTHIOPHENE

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Two liquid-liquid bioreactors were used to investigate disulfurization of dibenzothiophene (DBT) by Sulfate-Reducing Bacteria (SRB). Bacteria were suspended in an aqueous phase while DBT was dissolved in an organic phase (kerosene). Studies were initially conducted in a batch stirred-tank reactor, progressing to a system based on electrostatic dispersion. The hydrodynamic behavior of both bioreactors under various conditions was investigated. For the stirred-tank system, total liquid volume wis 1 L in a 1.5-L cylindrical tank equipped with two baffles. Mixing was provided with two Rushton-type six-blade impellers. The steady-state average drop size (d32) was determined by a video technique and correlated with operating conditions, physical properties, and geometry. For the electrostatic dispersion system, a column was used with kerosene as the continuous phase and water containing SRB as the dispersed phase. Micro-droplets were obtained by the breakup of the aqueous phase meniscus at the tip of a capillary using direct current and pulsed-direct-current electric fields. The size of the drops ejected from the capillary was measured as a function of the intensity and the pulsation frequency of the applied voltage.

Poster 136

FERMENTATION OF XYLOSE AND CELLULOSE MIXTURES BY THE SFIX/SSF PROCESS

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The polymers cellulose and hemicellulose can comprise up to 70% of the biomass from plants on a dry weight basis. The ability to ferment the xylose and glucose obtained from hydrolysis of hemicellulose and cellulose in a combined process without having to separate the xylose and cellulose streams would have a significant beneficial impact on the costs of producing ethanol from renewable biomass. It is possible to ferment mixtures of 5% (w/v) xylose and 10% (w/v) cellulose by combining the simultaneous fermentation and isomerization of xylose (SFIX) process with the simultaneous saccharification and fermentation (SSF) of cellulose. The yeast *Schizosaccharomyces pombe* ATCC 2476 (NRRL Y-164) can anaerobically ferment the above mixtures in an enzyme mediated process containing the enzymes xylose isomerase (NOVO Sweetzyme Q), cellulase (Genencor Laminex) and ß-glucosidase (NOVO SP 188). The xylose is fermented within 40 hours and the cellulose within six days at pH 5.75 and 37°C. Lower yields of ethanol are found in control fermentations without ß-glucosidase. Cobalt addition was not found to be necessary for stability of the Sweetzyme Q enzyme. The yeast cells were capable of being recycled into fresh media to produce similar yields of ethanol.

Poster 137

MODELING OF A SMALL PILOT-SCALE, FLUIDIZED-BED REACTOR FOR FUEL ETHANOL PRODUCTION[1]

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A developed mathematical model of a three-phase, fluidized-bed reactor (FBR) for fuel ethanol production is compared to the performance of a scaled-up FBR. This small pilot-scale FBR was 2 to 5 m tall with a 10.2-cm ID and had an approximate capacity of 6,000 gallons per month. The FBR demonstrated significant potential for reducing production costs of ethanol (5% to 10% per gallon). Conversion and productivity are compared under a variety of conditions, including differing feedstocks, flow rates, bed loadings, and feed concentrations. The bacterium, *Zymomonas mobilis* was used as the biocatalyst and was immobilized in small beads (1 to 1.5-mm-diam.) of κ-carrageenan (4 wt %) at cell loading of 15 to 80 g (dry wt) L^{-1}. Significant improvements in volumetric productivities (50 to 200 g EtOH h^{-1} L^{-1} compared to 40 to 110 for bench-scale experiments and 2 to 10 for reported industrial benchmarks) as well as superior yield (96%, whereas industrial yeast systems typically demonstrate 92% to 95% of theoretical yield) and good operability were demonstrated. These results suggest that further improvements in performance (productivity, conversion, and operability) may be possible.

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Poster 138

OPTIMIZATION OF AN ATTRITION BIOREACTOR FOR HYDROLYSIS OF WASTEPAPER BY A *Pseudomonas* Strain[1]

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Newsprint makes up an estimated 14% of the 180 million tons/yr of municipal solid waste. Conversion of a fraction of the available newsprint represents a potential for several times the present national production of ethanol. A *Pseudomonas* strain has been subjected to a mutagenesis program to select for high cellulase activity. The cellulase is constitutively excreted from the cells both during growth and resting states. The bacterium has been grown in batch culture with cellulase activity being recovered from the supernatant. Fractions containing cellulase activity were recovered via ultrafiltration. The recovered enzyme was added to a batch attrition bioreactor in order to study the kinetics of wastepaper hydrolysis. Optimized growth media and attrition bioreactor conditions are discussed.

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[1] Managed by Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

Poster 139

FUMARIC ACID PRODUCTION BY *Rhizopus* STRAINS

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Fumaric acid, a 4-carbon dicarboxylic acid, is a metabolic intermediate in basic metabolism. Due to its double bond and two carboxylic groups, fumaric acid has many industrial applications. Fumaric acid can be produced either chemically or biologically. Many strains of mycelial fungi, especially those belonging to the genus *Rhizopus,* are known to produce appreciable quantities of fumaric acid and are reported to yield 1.45 moles per mole of glucose utilized. This high yield is due to the ability of organism to fix carbon dioxide that takes place to carboxylate pyruvic acid to generate a 4-carbon intermediate, then lead to the formation of fumaric acid. With the potential high yield, fumaric acid is a desirable intermediate in the development of biomass-based chemical synthesis route. In this study, we evaluate the ability of several strains of *Rhizopus* to produce fumaric acid from both glucose and xylose. There is a major problem for most fungal fermentation in that cells tend to grow into large mycelial pellets or clumps. This may cause oxygen and/or nutrient limitation inside the mycelial pellets. As part of this study, we also devise a cultural medium that limits the growth of mycelial pellets without affecting fermentation activity. After the growth period, mycelial pellets formed are transferred into the fermentation medium with limited nitrogen source for fumaric acid production with calcium carbonate as a neutralizing agent. Fumaric acid produced is analyzed using liquid chromatography. *R. thailandensis* ATCC 20344 produces fumaric acid at a specific productivity of about 0.075 g/hr×g-biomass.

Poster 140

STARCH WASTEWATER TREATMENT BY PHOTOSYNTHETIC BACTERIUM IMMOBILIZED ON THE FIBER IN A COLUMNAR BIOREACTOR

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Several strains of photosynthetic bacterium which can reduce COD of wastewater rapidly were isolated in the pollution mud. The starch wastewater was continuously treated by immobilizing photosynthetic bacterium on the fiber in a columnar bioreactor. High COD volume load can be obtained under the optimum operation condition: when the dilution rate was 0.33 h^{-1}, the maximal COD volume load is 15.85 KgCOD/m^3.d, 60% of COD was removed. When the backwash rate is 0.2, the dilution rate is 0.35 h^{-1}, the maximal COD volume load is 21.80 KgCOD/m^3.d, 75% of COD was removed. Not only can the pollution of wastewater be eliminated effectively, but the bacterial mud is also an important source of feed additive. The results presented in this paper provide a useful basis for the further scale-up and operation.

Poster 141

MODELING CASSAVA STARCH SACCHARIFICATION WITH AMYLOGLUCOSIDASE

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A solution of α-amylase liquefied cassava starch, 30% w/v, was saccharified with amyloglucosidase at 45°C pH 4.5 in a batch reactor in the presence and absence of added glucose. Reactor conversion results were modeled with a multisubstrate model which considers intermediate dextrins of starch hydrolysis, reversibility of reactions, substrate and product inhibition, competition among dextrins, and isomaltose formation.

The model is of intermediate complexity because instead of considering every single dextrin in the reaction medium, it lumps them together in two classes. The first class, considered more susceptible to hydrolysis, makes up 77% of the molecules and contains α-1,4 bonds. The second class, more resistant to hydrolysis, comprises the remaining 23% of the molecules and contains α-1,6 bonds associated with branching.

Kinetic parameters were obtained from initial velocity saccharification tests at different starch concentrations and a few from literature. The model can represent well the saccharification of cassava starch even in the presence of a great excess of glucose (100 g/l), added to test its capability.

Poster 142

LACTIC ACID FERMENTATION AND ADSORPTION SEPARATION USING A POLY(4-VINYLPYRIDINE) COLUMN

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Lactic acid was produced by immobilized *Lactobacillus delbrueckii* cells in a fixed-bed reactor and separated in a column packed with poly(4-vinylpyridine) as an adsorbent. Poly(4-vinylpyridine) is a polymer that selectively adsorbs organic acids, and does not bind with most inorganic salts. This kind of selective adsorption provides a good method of separation of lactic acid from fermentation broths. Poly(4-vinylpyridine) adsorbs lactic acid from a fermentation broth, thus increasing the pH value of the broth. This eliminates the inhibition effect due to low pH in the process. Theoretically, satisfactory simulation results were obtained by describing the fermentation process using a fermentation kinetics model with axial dispersion, and a linear-driving-force model for *in situ* column adsorption process.

Poster 143

SIMULTANEOUS HYDROLYSIS AND FERMENTATION OF PULP MILL PRIMARY CLARIFIER SLUDGE

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Bioconversion of sludge from the primary clarifier of a sulphite pulping operation to ethanol offers a number of advantages over conventional disposal options. The amount of material which must be disposed of is reduced while, at the same time, salable and environmentally friendly fuel-ethanol is produced. The option of fortifying existing spent sulphite liquor (SSL) fermenting processes with the sugars produced via the enzymatic hydrolysis of sulphite primary clarifier sludge (PCS) has been explored. In this study, PCS hydrolysis rates as high as 12.6 g reducing sugar/(L-h) were observed at an initial enzyme loading of 10 filter paper units (FPU)/g. Hydrolysis was inhibited by SSL, an inhibition that could be completely overcome by fermenting the SSL to remove sugars. Lower (1–5 FPU/g) enzyme loading could be used in pre-fermented SSL. To reduce the deleterious effects of end-product inhibition, single-stage simultaneous hydrolysis and fermentation (SHF) was carried out using cellulase enzymes and *Saccharomyces cerevisiae*. The addition of an organic extracting solvent (oleyl alcohol) *in situ* increased the ethanol productivity by 41% through the reduction of yeast inhibition by ethanol.

Poster 144

USED FRYING OIL AS A FUEL OIL ALTERNATIVE

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Supply-demand imbalances in the energy sector and predicted depletion of conventional fossil derived fuels stimulated interest and research in the field of fuel alternatives of new and renewable origin. Among the new, clean, and renewable energy sources, biomass potential is particularly attractive since its availability is unlimited compared to fossil resources. The majority of investigations have concentrated on vegetable oils. Crude, refined, waste, residual vegetable oils have been considered as an attractive alternate to conventional ones for the last decade. Vegetable oils in particular have an exceptional importance since they can be used as a fuel oil alternative. In this research, evaluation possibilities of a waste product, namely used frying oil, have been investigated as a fuel oil alternative. In addition, the mixtures consisting of 25, 50, 75 (vol)% used frying oil and a balance of commercial fuel oil have been examined. Some technological characteristics of used frying oil have been determined according to standard methods for fat and oil analysis. The fuel oil property tests of used frying oil, mixtures of used frying oil-fuel oil, and reference fuel oil supplied by TÜPRAŞ Petroleum Refinery (İzmit-Turkey) were performed according to ASTM standard test methods for fuel oil. An overall evaluation of the experimental data indicates that used frying oil and its mixtures can be proposed as possible substitutes for fuel oil.

Poster 145

USE OF K_La AS A CRITERION FOR SCALING UP THE INULINASE FERMENTATION PROCESS

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The scale-up of the inulinase production in aerated cultures of *Candida kefyr* DSM 70106 was studied, taking into account the criterion based on maintaining constant K_La (volumetric oxygen transfer coefficient). This microorganism was grown on a bench scale in a 15-L fermenter containing 10 L of the culture medium: $MgSO_4.6H_2O$ (0.05 $g.L^{-1}$), urea (2.25 $g.L^{-1}$),KH_2PO_4 (0.30 $g.l^{-1}$), $CaCO_3$ (0.01 $g.L^{-1}$), peptone (6.5 g.L), yeast extract (2.8 $g.L^{-1}$) and inulin (10.0 $g.L^{-1}$). The culture was carried out batchwise, as follows: 30°C, pH 5.0, and K_La varying from 25 to 199 h^{-1}. The highest inulinase production was attained at initial K_La value of 46 h^{-1} (agitation = 120 rpm and aeration = 1.0 vvm) under which the dissolved oxygen was the limiting factor. A large-scale fermentation (300-L fermenter containing 200 L of medium) was performed using identical culture medium conditions (K_La of 46 h^{-1} was attained at agitation = 100 rpm and aeration = 0.8 vvm). The obtained responses for the bench and scaled-up experiments (i.e., in relation to cell growth, substrate consumption, and inulinase activity) showed similar behaviors and the results were, respectively: 0.60 and 0.58 $U.mL^{-1}.h^{-1}$ for productivity and 43.0 and 41.5 $U.mL^{-1}$ for activities. The results allowed us to conclude that the criterion adopted for the twenty-fold scale-up was appropriate.

Poster 146

THE USE OF A TECHNOECONOMIC MODEL TO ASSESS THE FLEXIBILITY OF A BIOMASS-TO-ETHANOL PROCESS WHEN VARIOUS FEEDSTOCKS AND PROCESS OPTIONS ARE CONSIDERED

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It is generally recognized that the front-end (pretreatment, fractionation, enzymatic hydrolysis) steps of a lignocellulose-to-ethanol process are both technologically immature and represent a large component (≈60%) of the total product cost. In the past we have tried to itemize the process steps and equipment from a complete plant. It was evident that, due to the complexity and interrelated nature of this process, it was difficult to determine the influence of even minor changes to the process on the overall production cost of the product. As a result we originally developed a technoeconomic model, based on spreadsheets, as a computational and assessment tool. However, our more recent work, which has looked at various process options such as hardwood vs. softwoods, SO_2 pretreatment of softwoods, enzyme recycle, indicated that the model required greater flexibility if it was to assess a "generic" biomass-to-ethanol process. We will discuss some of these issues through the presentation of an updated model which incorporates flowsheet modeling concepts.

Poster 147

DESIGN AND INSTALLATION OF A LIGNOCELLULOSIC BIOMASS TO ETHANOL PILOT PLANT

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One popular alternative transportation fuel that is both an octane enhancer and a fuel extender is ethanol, and if used in the neat form could replace a large fraction of our gasoline consumption, However, the supply must be both plentiful and inexpensive. A potential resource that could meet these criteria is the conversion of lignocellulosic biomass to ethanol. In support of the effort to develop this process, the National Renewable Energy Laboratory (NREL) built a pilot plant based on the enzymatic conversion of lignocellulose to ethanol. The plant incorporates operations for feed handling, size reduction, pretreatment, fermentation, distillation, and solid separation. The plant was designed to continuously process 900 kg of dry feedstock per day to ethanol. The equipment currently installed in the plant includes 150-, 1500-, and 9000-L fermenters, a vertical pulp digester, an impact mill, feeding and conveying equipment, a 10 m-high ethanol stripping column, and miscellaneous tanks. The pilot plant was designed and installed by John Brown Engineering and Construction and has begun startup operations.

Poster 148

RESEARCH AND DEVELOPMENT NEEDS OF FLAX PROCESSING AND STRATEGY FOR THE FLAX UTILIZATION AT THE 1990s

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The aim of this study was to highlight the research and development needs of the flax processing in the short and long time scale and to plan the strategy for the flax cultivation and utilization in Finland at the 1990s

The first part of the study was to determine the environment which the strategy will plan for. The main factors in the function environment are:

- Customers and their needs
- Historical background of the flax processing Finland
- Economical factors, marketing trends and legislation, environmental and technological factors.

According to the functional environment and results of the marketing study, the competition strategy of the Finnish flax processing has been planned. The main competition advances are:

- Unique quality of the arctic fiber, non-food integrated flax processing, utilization of the bio- and new process technology and by-products of the flax processing.

Poster 149

BIODEGRADATION OF DILUTE VOLATILE ORGANIC CONTAMINANTS IN GASEOUS WASTE STREAMS

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Treatment of dilute gaseous hydrocarbon waste streams remains a current need for many industries, particularly as increasingly stringent environment regulations and oversight force emission reduction. Advances in this technology hold promise for providing low-cost alternatives to more traditional, energy-intensive treatment methods such as incineration and adsorption. Elucidation of engineering principles governing the behavior of such systems, such as mass-transfer limitations, will broaden their applicability.

Our processes exploit a microbial consortia to treat a mixture of 0.5% n-pentane and 0.5% isobutane in air. Since hydrocarbon gases are sparingly soluble in water, good mixing and high surface area between the gas and liquid phases are essential for biodegradation to be effective. One liquid-continuous columnar bioreactor has been operating for more than 30 months with continued degradation of n-pentane and isobutane as sole carbon and energy sources. The maximum degradation rates observed so far in this gas-recycle system are 2 g VOC/h/m^3.

A trickle-bed bioreactor (or trickling bioreactor) has been operated continuously for over ten months to provide a higher surface area (using a structured packing) with increased rates. Maximum degradation rates achieved thus far have been ~60 g VOC/h/m^3 in a single pass in this gas-continuous columnar system. Mass transfer coefficients comparable to literature values have also been measured for this reactor, with values being two to three times higher than those found in the gas-recycle reactor.

Poster 150

PCR CHARACTERIZATION OF THE MICROBIAL RESPONSE TO BIOVENTING AT A JP5-CONTAMINATED SITE

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Bioventing is commonly used to bioremediate sites contaminated with gasoline and other easily degraded fuels. JP-5 is more difficult to biodegrade and is composed primarily of C10–C15 compounds, but also contains a small fraction of polyaromatic hydrocarbons. Because bioremediation was progressing slowly at the site, a detailed microbial characterization was conducted before the placement of additional vent wells and after bioventing had been conducted. Assays included direct counts, aerobic and anaerobic acetate mineralization activity and dodecane mineralization activity, and extraction of microbial community nucleic acid followed by PCR analysis of the relative abundance of specific biodegradative genes. Conserved PCR primers which targeted the naphthalene dioxygenase/polyaromatic hydrocarbon dioxygenase genes (nahA/pahA, 4 sequences) and the catechol 2,3-dioxygenase genes (xylE, 8 sequences) were constructed. Primers were also constructed for the alkane hydroxylase gene (alkB). Direct cell counts were greater than 10^{10}/g in all samples. Anaerobic activity was very high in samples before and also after bioventing, suggesting that oxygen was not being effectively delivered to large volumes of the contaminated subsurface. The nahA/pahA genes were present at densities of at least 10^{7}/g in some samples, but densities were highly variable. High nahA/pahA gene densities were correlated with high aerobic activity. Densities of the xylE gene were generally lower and less variable than for the nahA/pahA genes. The alkB gene was rarely detected (although the enzyme oxidizes C6–C12 alkanes), indicating that other alkane biodegradative genes were responsible for JP-5 degradation at the site.

Poster 151

START-UP OF A NOVEL ANAEROBIC FILTER FOR TREATING FOOD-PROCESSING WASTEWATER

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An anaerobic filter, packed with fire-expanded clay spheres (FECSs), was started up to treat food-processing wastewater. The filter was seeded with mesophilic anaerobic sludge and continuously fed with a 1,800 mg COD/L simulated food-processing wastewater at 10-day hydraulic retention time for a period of 45 days. The filter liquor was recirculated and the process temperature was maintained at 35°–40°C, which was near the upper end of the mesophilic temperature range. The porous FECSs were excellent media for biofilm attachment. The biofilm examined was dominated by *Methanococcus*-like and *Methanothrix*-like cells. The transient response of the filter to the feed was closely monitored. Adsorption of organic matters on the packing medium with immobilized biofilms provided a long retention time for these compounds, which were otherwise difficult to degrade. These rendered the filter more easily started up and more stable at high loading rates.

Poster 152

EFFECTS OF HEAVY METAL ON ACTIVATED SLUDGE

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An 18-L sequencing batch reactor (SBR) treating a synthetic wastewater was used to study the effects of a common heavy metal on activated sludge. Removal of COD in the absence of zinc proceeded by a rapid adsorption of organic matters on the biomass, followed by a much slower metabolic assimilation mechanism. The daily COD removal efficiency maintained at 90%. When zinc-laden wastewater was introduced, SBR performance was affected in varying degrees depending on the zinc concentration. The COD removal efficiency fell sharply to 27% when the reactor was dosed with 30 mg-Zn/L.

Isothermal adsorption tests showed that zinc adhered on biomass through a combination of physical and chemical adsorption, and at a relatively high adsorption rate. Zinc acted as a strong competitor for active sites and hampered the natural adsorption of organic matters on the biomass, and hence the SBR performance.

Poster 153

FILAMENTOUS GROWTH IN ACTIVATED SLUDGE

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The activated sludge plants of major municipal sewage treatment works in Hong Kong frequently experience bacterial foaming, which often results in difficult operational problems. An actinomycetes was identified as the dominant species in foaming sludges. Physiological and morphological characterization of the actinomycetes showed consistency with the *Nocardia amarae* type strain (ATCC 27808). *N. amarae* was cultured in shake-flasks with common long-chain fatty acids as the sole carbon source. Growth yields reached as high as 0.18 g dry cell mass/g stearic acid. *N. amarae* is competitive among other microorganisms in activated sludge processes that receive sewage containing a high concentration of long-chain fatty acids, which is typical for greasy food-processing effluents.

Poster 154

PYROLYSIS OF SEWAGE SLUDGE WITH OBTAINING OF SORBENTS

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Sewage sludge from wastewater biological cleaning stations are multitonnage solid industrial waste. The main part of these wastes is never used and makes ecological difficulties. Nowadays significant attention is paid to searches connected with the burning of sewage sludge. In the present work the possibility of sorbents obtaining by partial gasification; i.e. pyrolysis, of composition of sewage sludge with biomass has been shown. The obtained organo-mineral sorbents are characterized by sufficient sorption activity and the value of specific surface more than 1200 m^2g^{-1}. Co-pyrolysis within temperatures range 700°–900°C of sewage sludge and biomass waste, having initial porous structure, allowed to regulate properties of sorbents obtained owing to variation of porous size distribution. The efficiency of the sorbents has been demonstrated on model water effluents of pulp and paper industry under conditions of their application in aeration system.

Poster 155

URANIUM BIOSORPTION BY *Pseudomonas aeruginosa* CSU IN BATCH AND CONTINUOUS-FLOW SYSTEMS[1]

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Pseudomonas aeruginosa CSU is a naturally occurring, non-genetically engineered bacterial strain shown in previous work to bind dissolved hexavalent uranium as UO_2^{2+} and/or its cationic hydroxo complexes, with extremely rapid kinetics. Uranium sorption was enhanced by treatment with heat or organic solvents, but was inhibited in the presence of iron. *P. aeruginosa* CSU was chosen as the basis of a novel process for the removal of uranium from acidic aqueous washes (pH 4.0). Immobilization of *P. aeruginosa* biomass within various inert polymers, such as polyurethane, did not affect the cells' sorptive behavior. Immobilized biomass was found to be superior to conventional sorbents, including commercial ion-exchange resins, with respect to uranium binding kinetics, loading capacity, specificity, and cost. This biosorbent could, however, be deployed within conventional batch or flow-through reactor systems. Methods for large-scale production and storage of stable (heat-killed), optimized (acetone-treated), immobilized biosorbent were developed and validated.

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[1] Research sponsored by the U.S. Department of Energy, Office of Environmental Restoration/Waste Management.

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

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THE ANTIBIOTIC EFFECT ON THE NITRIFICATION PROCESS

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The nitrifying microbial population is very sensitive to inhibition by many chemicals. The organic matter in the wastewater is the main nitrifying inhibitor. There is wastewater as the food industry where toxic compounds like the antibiotics are present. Evidence indicates that the anaerobic digestion is strongly inhibited by the antibiotics. As the nitrification is one of the steps in organic matter removal from wastewater, it is important to know which is the nitrification behavior if antibiotics are present. The work presents the result of the evaluation of five antibiotics and six concentrations on the nitrification. The antibiotics were benzyl-penicillin, novobiocin, chlortetracycline, chloramphenicol, and ampicillin. The size range for each antibiotic was different. The effect was studied in batch culture using a stabilized nitrifying microflota and basal litoautotrophic medium. The inhibition effect was followed through the measure of microbial growth, nitrite, and nitrate formation. The results showed that none antibiotic were the nitrifying inhibitor. One possible explanation could be around the environmental culture condition of the nitrification.

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FREQUENCY RESPONSE OF GLUCOSE SENSOR BASED ON IMMOBILIZED GLUCOSE OXIDASE MEMBRANE

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For the purpose of studying dynamic behaviors of biosensors as one of their practical characteristics, the generator of sinusoidal substrate concentration signals was made with four plungers whose strokes were controlled by computers so as to generate sinusoidal change of flow rate. The frequency response of the glucose sensor based on the immobilized glucose oxidase collagen membrane was studied with this generator as signal input and output signals were measured to be found to follow the scheme of the first-order response with time delay. This generator will be applicable to other types of biosensors for the study of their dynamic response characteristics.

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SOME ASPECTS OF BIOLOGICAL METHODS OF OIL REFINEMENT OF SEWAGE

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The biological refinement of sewage at oil-processing plants as well as the oil refinement of reservoirs, rivers, and seas is one of the most pressing problems nowadays.

The microorganism cultures which assimilate oil products and which have the oil coalescence ability in "oil-in-the water" system have been tested. The strains of *Rhodococcus erythropolis* and *Acinetobacter calcoaceticus* which assimilate a wide spectrum of hydrocarbons and which produce oil-products emulsifying or de-emulsifying biological surface-active agents have been selected.

Cells immobilization of *Rhodococcus erythropolis* intensified the refinement of oil-containing sewage. Con-centration of oil products decreases from 200 mg/1 to 2,0-2,5 mg/1 at a constant polluted water feeding (at the rate of 4 m^3/h).

High rate of sewage refinement is achieved not only by assimilation of hydrocarbons, but also by the synthesis of biological surface-active agents synthesized by *Acinetobacter calcoaceticus* culture, which intensifies the coalescence of non-assimilated oil. The enlarged oil drops are removed from the water surface. Such an approach enacts all metabolic potential of the microorganisms oxidizing hydrocarbons and also provides full water refinement.

Poster 159

TREATMENT OF ACID MINE DRAINAGE WATERS BY MEANS OF NATURAL WETLANDS

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Acid drainage waters from a copper mine in Central Bulgaria were directed to flow through natural wetlands located in the proximity of the mine. A biocenose consisting of various water plants, microorganisms, protozoa, insects, mollusks, and other invertebrate organisms was established in the wetlands. The flow rate of the mine effluents was in the range of about 10–35 m^3/day, their pH was about 2–2.5, and sulphate, iron, and copper ions were the main pollutants. These pollutants were efficiently removed from waters being treated mainly as a result of the activity of sulphate-reducing bacteria growing in the anaerobic bottom zone of the wetlands. These bacteria used organic carbon and sulphate in the process of anaerobic respiration and the by-products of the reaction, hydrogen sulphide, and bicarbonate precipitated the heavy metals as the relevant sulphides and raised the pH of the effluents, respectively. In the near future a system of several constructed wetlands will be created in this area to treat the acid drainage effluents from different mines.

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SEQUENTIAL ANAEROBIC-AEROBIC BIODEGRADATION OF PCBs IN SOIL SLURRY REACTORS

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Many industrial locations have identified needs for treatment of polychlorinated biphenyl (PCB) wastes and remediation of PCB-contaminated sites. Biodegradation of PCBs is a potentially effective technology for the treatment of PCB-contaminated soils and sludges; however, a practical remediation technology has not yet been demonstrated.

In laboratory experiments, soil slurry bioreactors inoculated with microorganisms extracted from PCB-contaminated sediments from the Hudson River have been used to obtain anaerobic dechlorination of PCBs. The anaerobic dechlorination was followed by aeration, addition of biphenyl, and inoculation with aerobic PCB degraders. The sequential anaerobic-aerobic treatment constituted an improvement compared to the anaerobic treatment alone by reducing the total amount of PCBs remaining and decreasing the tendency for the end products to accumulate in humans.

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

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PERCHLOROETHYLENE UTILIZATION BY METHANOGENIC FED-BATCH CULTURES: ACCLIMATION AND DEGRADATION

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Anaerobic sludge granules from two different sources were maintained in serum bottles and fed at three-day intervals in the presence of 0.2 ppm PCE. Following acclimation periods ranging from 48 to 79 days, PCE degradation was observed with 95% utilization in three days. Only granules amended with formate as a co-substrate showed PCE degrading activity, while those utilizing acetate, lactate, ethanol, and methanol remained PCE inactive after 90 days. Both co-substrate consumption and PCE degradation ceased when acetate replaced formate as the co-substrate for formate-acclimated cultures. The total moles of chlorinated and non-chlorinated ethene moieties were found to remain constant over time, demonstrating conservation of mass. Trichloroethylene (TCE) and cis 1,2 dichloroethylene (cis 1,2 DCE) were identified as the major dechlorination end-products. The absence of further dechlorination in the presence of a great excess of reducing equivalents (13,000 times that needed) supports the hypothesis that complete degradation of PCE to ethene is not solely dependent on excess reducing equivalents, but may require the presence of an appropriate microbial consortia.

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INFLUENCE OF MEDIA ON MEASUREMENT OF BACTERIAL POPULATIONS: NUMBERS AND DIVERSITY

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Heterogeneity in the subsurface may play an important role in the success of bioremediation activities. To examine the extent of and factors contributing to microbial heterogeneity, the DOE Subsurface Science Program has instituted a study at a coastal plain site near Oyster, Virginia. Sediments at the site are unconsolidated, fine to coarse beach sands and gravel. The influence of media on the measurement of colony forming units (CFU) and diversity is the subject of this portion of the study. Although low nutrient media is often selected to maximize bacterial counts, its use could limit the ability to distinguish among colony types and reduce the measured diversity. Two low nutrient media formulations, 1% PTYG and soil extract media, gave equivalent estimates of CFU which decrease logarithmically with depth from a high of about 6.5×10^5 CFU/g at about 0.4 m below the surface. However, a high nutrient media (N/BHI) gave slightly lower numbers than the PTYG. Although there were highly significant correlations among CFU measured with the different types of media, there were no significant correlations in several indices of microbial diversity. Thus, CFU was a relatively robust measurement, but measured microbial diversity was not as consistent when assayed with different media.

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EFFECT OF EXPOSURE TO OXYGEN ON GROWTH AND METABOLISM OF A METHANOGENIC CONSORTIUM

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To achieve complete destruction of the highly chlorinated pollutants to nontoxic degradation products in *in situ* bioremediation systems, it may be necessary to develop nutrient addition strategies which would allow space and time variations of redox potential such that both anaerobic and aerobic microbes can be employed. One of the factors that must be determined before such strategies could be developed, however, is the effect of oxygen on anaerobic microbes. In this study an exponentially growing, methanogenic consortium was exposed to several different oxygen concentrations for several different time intervals. The exposed culture was then transferred to an anaerobic medium which did not contain reducing agents and was incubated at 30°C. The response of the consortia to exposure to oxygen was assessed by monitoring the amount of gaseous product produced by the consortia as a function of time. These data provide information on the effect of oxygen on the length of lag period and the rate of product formation. Additionally, the rate of substrate consumption and identification of dominant type of bacteria in the culture was studied. Based on these results, a discussion of the reason for the loss of methanogenic activity is presented.

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EFFECT OF PERCHLOROETHYLENE (PCE) ON GROWTH AND METABOLISM OF A METHANOGENIC CONSORTIUM

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Perchloroethylene (PCE) is one of the priority pollutants found in groundwater. Many researchers have previously reported that this contaminant can be transformed to less chlorinated compounds by active anaerobic microbial consortia. This work has also demonstrated that there is a strong correlation between culure catabolic activity and contaminant degradation. In addition, it has often been observed that the activity of the microbes may be inhibited by the presence of the chlorinated solvent. However, there is no published information that quantifies the effects of PCE concentration on growth and activity of a dechlorinating culture. Such information is needed to help develop a numerical description for contaminant fate and transport in the subsurface. In this work, the effect of PCE concentration on growth and methane production by a methanogenic consortia is investigated by exposing the organisms to PCE concentrations of 0, 10,50 and 100 mg/L at 17°C. Results indicate that PCE inhibits the growth of the culture even at low concentrations. At 10 mg/L the lag phase is extended, while at 100 mg/L growth is completely inhibited.

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PRETREATMENT TECHNOLOGY FOR THE BENEFICIAL BIOLOGICAL REUSE OF MUNICIPAL SEWAGE SLUDGES

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Modern municipal sewage waste treatment plants use conventional mechanical and biological processes to reclaim wastewaters. This process has the overall effect of converting a water pollution problem into a solid waste disposal problem (sludges). The costs for conventional disposal of sewage sludges have risen dramatically because of increased environmental mandates, which restrict their disposal, as well as a dwindling number of landfills.

Previously, we determined that secondary bioprocessing (specifically anaerobic digestion) was not effective in reducing the organic content and bulk of the sludge waste. Therefore, we have examined the potential of a variety of pretreatment technologies designed to disrupt the macro-structure of the sludge and thereby enhance its subsequent biodegradation.

Two thermal/mechanical pretreatments tested were found to have a dramatic effect on the subsequent bioconversion of the microbial sludges. Both technologies evaluated, sonication and shear, were found to be affected by sludge solids levels, duration of treatment, and treatment temperature.

Optimum sonication pretreatment occurred with sludge solids of 1% and treatment times of 4–8 minutes. The most effective treatment temperature tested was 55°C. The optimum enhancement in bioconversion potential for the sonication pretreatment was 80%–83% of the material's carbon oxygen demand (COD) content.

The optimum shear pretreatment occurred with sludge solids of 1%–2% and treatment times of 6–10 minutes. The most effective pretreatment temperature tested was 87°C. The optimum enhancement in bioconversion potential for the shear pretreatment was 88%–90% of the materials COD content.

These data form the basis for a pending U.S. patent.

Poster 166

MICROBIAL REDUCTION OF SULFUR DIOXIDE IN MIXED CULTURES OF SULFATE-REDUCING BACTERIA

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In an effort to reduce emissions of sulfur dioxide (SO_2), microbial reduction of SO_2 to hydrogen sulfide (H_2S) using mixed cultures of sulfate reducing bacteria (SRB) has been considered as an economically feasible process compared to conventional SO_2 hydrogenation. The sulfur compounds produced microbiologically could be recovered as elemental sulfur using the Claus process or by additional microbial action. The feed cost and the biomass concentration in the bioreactor were considered to have significant impact on the economics of the SO_2-reducing bioprocess. Anaerobically digested municipal sewage solids medium (AD-MSS) was previously identified as a viable feedstock. A continuous process of making AD-MSS medium with high soluble organic content has been investigated in order to further reduce the cost of feed stock preparation. Microbial SO_2-reduction in various immobilized cell bioreactors has been addressed to achieve maximum cell density in the reactor. The initial economic assessment of the microbial process has been discussed based on the results from bench scale experiments.

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STIMULATORY EFFECT OF DAIRY EFFLUENT SLUDGE ON ANAEROBIC DIGESTION OF CATTLE DUNG

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The stimulatory effect of dairy effluent sludge was studied during the acidogenic phase of the anaerobic digestion of cattle dung. The volatile fatty acids increased during the early periods of digestion. Acetic acid was the main VFA produced. Propionic acid accumulation occurred after 20 days of the digestion. Methane content of biogas increased with the progress of the digestion. Cattle dung supplemented with dairy effluent sludge showed higher gas production. Maximum methane content (48.95%) was recorded in the biogas produced from dairy effluent sludge, whereas it was minimum (36.32%) in cattle dung digestion. Total anaerobic counts were in the range of 10^8–10^{10} cfu/ml. The cellulolytic, amylolytic, proteolytic, and lipolytic bacteria were present in the range of 10^6–10^7 cfu/ml. The acid formers were less in number (10^5–10^6 cfu/ml) as compared to other groups.

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MECHANISMS OF INTRINSIC BIOREMEDIATION OF GAS CONDENSATE HYDROCARBONS UNDER HYDROCARBON LIMITING CONDITIONS

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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 Production 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 suggests 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 microcosms 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 experiments, 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 soil or soil 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 either nitrate, Fe(III), or sulfate and carbon dioxide were added as electron acceptors. 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.

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BIOREMEDIATION OF BTEX HYDROCARBONS UNDER MICROAEROPHILIC CONDITIONS

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Inadvertent release of petroleum hydrocarbons such as crude oil or gasoline into the subsurface can cause groundwater contamination, mainly by the relatively water-soluble and mobile components: benzene, toluene, ethylbenzene, and xylenes (collectively referred to as BTEX). These BTEX components are known health hazards and are regulated by the Environmental Protection Agency (EPA). Bioremediation—stimulation of natural biodegradation processes—is an attractive alternative/complement to the conventional pump-and-treat technology for restoration of contaminated aquifers. Aerobic metabolism is known to be the major mechanism for attenuation of BTEX under aquifer conditions. Natural decay process of dead plants and organisms can exert some oxygen demand in the subsurface soil. This oxygen demand frequently results in microaerophilic or anaerobic environment in the subsurface. It is also known that the dissolved oxygen (DO) concentration in the subsurface is an important factor limiting the rates and extent of biodegradation of BTEX components. The primary objective of this study is to determine the threshold limit of DO for biodegradation of BTEX and assess the effect of different DO concentrations on the rates of biodegradation mediated by alternate electron acceptors.

Microcosm studies will be carried out under various microaerophilic conditions viz. 0, 0.5, 1 and 2 mg/L DO. Nutrient medium saturated with gas condensate obtained from a natural gas production site will be used as the growth medium. Soil obtained from the same site will be used as a source of native microorganisms. The microcosm will be a 1600-mL capacity, glass reactor employed with an aerator and a basket to hold the soil. In order to eliminate loss of BTEX by volatilization into bubbles, a novel aerator design will be employed. The aerator consists of an end-sealed bundle of oxygen -permeable hollow fibers. The fibers are pressurized by oxygen-nitrogen mixture (a suitable composition to attain the desired DO); oxygen diffuses across the membrane and dissolves in the bulk liquid phase. In order to prevent bubble formation on the surface of fibers, it is essential to maintain high shear velocity across the fibers. This will be attained by using a magnetic stirrer inside the basket at the bottom of the reactor. The basket will have an annular construction with the inner wall comprised of ~100 micron SS mesh and an outer SS wall. The inoculum will be held in the annular space of ~5 mm. The basket will allow diffusive transport of liquid phase components across the inoculum but minimize the contact between soil particles and the fibers, thereby minimizing abrasion of the hollow fibers. A Clark type DO probe installed in a flow loop external to the reactor will be employed to measure DO in combination with a picoammeter (sensitivity of 0.1 pA). Gas pressure inside the fibers will be adjusted to maintain a constant DO.

The entire experimental set will be comprised of duplicate microcosms for each level of DO and two sterile, anaerobic controls (to account for abiotic mechanisms of BTEX loss). Inocula from contaminated as well as pristine soil from the same site will be used. A number of alternate electron acceptors will be used viz. NO_3^-, SO_4^{--}, Fe(III). Aqueous concentrations of BTEX and these electron acceptors will be monitored as a function of time. The kinetic data thus obtained will be used to estimate the critical DO level limiting BTEX biodegradation.

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NATURAL ATTENUATION OF HYDROCARBONS IN SOIL AND GROUNDWATER CONTAMINATED WITH GAS CONDENSATE

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A field scale study was performed to assess the rate and extent of natural hydrocarbon biodegradation in soils and groundwater at two gas condensate production sites in the Denver Basin, Colorado. Soils and groundwater were contaminated with petroleum condensate which had leaked from concrete sumps used to store produced water and petroleum condensate during production operations. Subsurface contaminant plumes were defined with soil gas surveys in which real-time measurements of VOCs, CO_2, and O_2 were used to construct soil gas contour maps. Based on these data, 92 monitor wells were installed at the two sites.

At each site, 15 nested monitor wells were installed in a five-spot pattern along the major axis of the plume. Each well nest consisted of three monitor wells with 18-in. long screens. The screens were installed at the water table interface and 5 and 10 ft below the top of the water table. An identical array of nested monitor wells was placed along the axis of the plume upgradient of the source in an area that had not been impacted by hydrocarbons. Additional monitor wells, screened over 10-ft intervals, were installed along the axis of the plume in the control area and in the plume. Five hydraulic monitor wells with pressure transducers were installed at each site to define the groundwater gradient and record daily water table fluctuations.

Groundwater samples were analyzed in the field for dissolved oxygen (DO), sulfate (SO_4^{2-}), nitrate (NO_3^-), Iron (II), and pH. BTEX, TPH, and semi-volatile TPH were measured in the laboratory using EPA Method 8015 (modified). Results suggest that natural aerobic and anaerobic biodegradation is occurring at the two sites. In the vadose zone, soil gas surveys and soil cores revealed that elevated CO_2 and depressed O_2 soil gas levels are associated with elevated soil gas VOC levels and hydrocarbon-impacted soils. This suggests the presence of aerobic hydrocarbon-degrading bacteria which are actively consuming O_2 and producing CO_2. In the saturated zone, groundwater samples suggest that the aerobic biodegradation potential is limited due to uniformly low DO levels (1.4 mg/L or less) within the plume and in upgradient groundwater. However, utilization of sulfate is significant within the BTEX plume and appears to be a primary means of biodegradation. The presence of iron sulfide (FeS) in groundwater samples collected from the contaminant plume is consistent with groundwater, nitrate has limited potential as an alternate electron acceptor at the two sites. Analysis of leachates from soil cores showed higher Iron(II)/Iron(III) ratios in soil cores which had been impacted by hydrocarbons. Elevated Iron(II) levels are consistent with hydrocarbon biodegradation by iron reduction. In total, the data suggest that hydrocarbon biodegradation is proceeding along multiple pathways at these sites.

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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.

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MICROBIAL CONTROL OF HYDROGEN SULFIDE PRODUCTION IN A POROUS MEDIUM

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The ability of a sulfide- and glutaraldehyde-resistant strain of *Thiobacillus denitrificans* (strain F) to control sulfide production in an experimental system of cores and formation water from the Redfield natural gas storage facility was investigated. *Thiobacillus denitrificans* is an obligate autotroph and facultative anaerobe that oxidizes reduced inorganic sulfur compounds (i.e., sulfide) to sulfate using nitrate as the terminal electron acceptor. A stable, sulfide-producing biofilm was established in two separate core systems. One core system was inoculated with strain F; the other core system (control) was treated in an identical matter, but was not inoculated with strain F. First, formation water with 10 mM acetate and 5 mM nitrate was injected into both core systems. Effluent sulfide concentrations in the control core system ranged from 200 to 460 μM. In the test core system inoculated with strain F, the effluent sulfide concentrations were lower, ranging from 70 to 110 μM. In order to determine whether strain F could control sulfide production under optimal conditions for sulfate-reducing bacteria, the electron donor was changed to lactate and inorganic nutrients (nitrogen and phosphate sources) were added to the formation water. When nutrient-supplemented formation water with 3.1 mM lactate and 10 mM nitrate was used, the effluent sulfide concentrations of the control core system initially increased to about 3800 μM, and then decreased to about 1100 μM after 5 weeks. However, in the test core system inoculated with strain F, the effluent sulfide concentrations were much lower, 160 to 330 μM. Increasing the nitrate concentration to 20 mM did not affect the effluent sulfide concentration in either core system. Nitrate consumption (5 mM) and high concentrations (10^7 to 10^8 cells per mL) of strain F were detected in the test core system. The accumulation of biomass occurred in the influent lines during two months of continuous operation, but only a small increase in injection pressure was observed. These studies showed that inoculation with strain F was needed for effective control of sulfide production, and that significant plugging or loss of injectivity due to microbial inoculation did not occur.

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AN ECONOMIC ANALYSIS OF MICROBIAL REDUCTION OF SULFUR DIOXIDE WITH ANAEROBICALLY DIGESTED SEWAGE SLUDGE BIOSOLIDS AS ELECTRON DONOR

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A concentrated stream of sulfur dioxide (SO_2) is produced by regeneration of the sorbent in certain new regenerable processes for the desulfurization of flue gas. We have previously proposed that this SO_2 can be converted to elemental sulfur for disposal or by-product recovery using a microbial/Claus process. In this process, two-thirds of the SO_2-reducing gas stream would be contacted with a mixed culture containing sulfate-reducing bacteria (SRB) where SO_2 would act as an electron acceptor with reduction to hydrogen sulfide (H_2S). This H_2S could then be recombined with the remaining SO_2 and sent to a Claus unit to produce elemental sulfur.

In work reported previously, the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, was immobilized by co-culture with flock-forming heterotrophs from an anaerobic digester resulting in a SO_2-reducing floc which could be collected from the effluent of a continuous reactor for recycle by gravity sedimentation. The carbon and energy source for these cultures was anaerobically digested municipal sewage solids. The maximum specific activity for SO_2 reduction in these cultures, in terms of dry weight of *D. desulfuricans* biomass, was 9.1 mmoles SO_2/hr-g. The stoichiometry with respect to the electron donor was 15.5 mg soluble COD per mmole SO_2 reduced.

In this paper, we report the results of an economic analysis of the microbial reduction of SO_2 with anaerobically digested municipal sewage solids serving as the electron donor and carbon source for a SO_2-reducing culture of *D. desulfuricans*. The design basis was a regenerated SO_2 gas stream produced by processing raw flue gas generated by coal-fired utility boilers (1000 MW_e) through a dry, regenerative fluidized-bed, copper-oxide type flue gas desulfurization system. The flue gas composition was based on burning coal containing 3.5 wt % sulfur.

Poster 174

BIOTREATMENT OF SPENT SULFIDIC CAUSTIC BY SPECIALIZED CULTURES AND ACCLIMATED ACTIVATED SLUDGE

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Sodium hydroxide (NaOH) solutions are used in petroleum refining to remove hydrogen sulfide (H_2S) from various hydrocarbon streams. Once H_2S reacts with the majority of the NaOH in the solution, the solution becomes known as a spent sulfidic caustic. Spent caustics typically have a pH greater than 13, sulfide concentrations exceeding 2–3 wt %, and a large amount of residual alkalinity. Depending on the source, a spent caustic may also contain phenols, mercaptans, amines, and other organic compounds that are soluble or emulsified in the caustic.

We have previously demonstrated that caustics containing amines, but free of mercaptans, can be biotreated without prior neutralization using a flocculated culture of a sulfide-tolerant strain (strain F) of the chemoautotroph, *Thiobacillus denitrificans*. Sulfides were completely oxidized to sulfate and microbial oxidation of sulfides produced acid which at least partially neutralized the caustic.

The objective of the work described in this paper was to compare the performance of the specialized culture of *T. denitrificans* (strain F) with an acclimated culture that results from supplementing the feed to a refinery activated sludge system with spent sulfidic caustic. Acclimated activated sludge was obtained from a local refinery that occasionally treats spent caustic in the activated sludge system. The two reactors were operated in parallel with two refinery caustic feeds, one with no mercaptan and one containing 3 wt % mercaptan (primarily methyl and ethyl mercaptan).

The two cultures were shown to be comparable in treating the mercaptan-free caustic in terms of the specific activity of the cultures for sulfide oxidation. However, the acclimated activated sludge exhibited less stable operation in terms of H_2S emissions and was less tolerant of reduced temperatures (20°C). In both cultures sulfide oxidation was shown to be inhibited by mercaptans; however, mercaptans were primarily absorbed by the cultures and modified in some way to prevent total stripping from the reactors.

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IMMOBILIZATION OF SULFIDE-OXIDIZING BACTERIUM IN A NOVEL ADSORBENT BIOCATALYST SUPPORT

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Thiobacillus denitrificans is a chemoautotroph and facultative anaerobe which may use reduced sulfur compounds, such as H_2S, and salts of HS^- and S^{-2}, as energy sources with oxidation to sulfate. Under anaerobic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen. Carbon dioxide or carbonates are used as carbon sources.

We have previously demonstrated that suspended cultures of *T. denitrificans* may be used to remove H_2S from sour gases, remove sulfides from sour water, and treat refinery spent sulfidic caustics. Each process results in complete oxidation of sulfides to sulfate. Recently, *T. denitrificans* has been immobilized in a novel adsorbent biocatalyst support material manufactured by DuPont. The support material consisted of 3–4 mm beads which were a composite of a polymer (25 wt%) and powdered activated carbon (75 wt%). The beads had a porosity of 75% and a very favorable macroporous structure which allow microorganisms to colonize throughout the entire internal volume of the beads.

Inoculation of the support material was accomplished by circulating *T. denitrificans* in a thiosulfate mineral salts medium through a packed bed containing the beads. After approximately 60 days the beads contained dense cultures of the organism as demonstrated by scanning electron microscopy. It is anticipated that this sulfide-active biocatalyst will greatly simplify reactor design for bioreactors treating sour gases, sour water, and refinery caustic. The powdered activated carbon is also expected to enhance reactor stability as a sink for potentially inhibitory components of these waste streams.

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PRODUCTION OF SULFUR FROM GYPSUM AS AN INDUSTRIAL BY-PRODUCT

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More and more industrial processes lead to gypsum formation. However, there is no really ecological way to dispose of it. Our goal is to transform gypsum by means of a two-stage process involving a biological step, leading to H_2S and $CaCO_3$, and a chemical step oxidizing H_2S into sulfur.

In this work efficient strains were selected and continuous pilot-scale bioreactors were performed to find optimum culture conditions.

Two types of sulfate-reducing bacteria were studied: incompletely lactate oxidizing (into acetate) once and completely acetate oxidizing ones. Highly sulfate-reducing strains were isolated after chemical mutagenesis.

Two strategies were adopted: culture in single mixed bioreactor and in two-stage immobilized cell bioreactor. As acetate oxidizing metabolism is slower, large amounts of acetate appear in the single bioreactor effluent. The second stage of the immobilized cell bioreactor (predominating acetate oxidizing bacteria) raises DOC conversion from 45% to 65%. An overall bioconversion capacity of 11 g/L.d of gypsum and 1.2 g/L.d of DOC was achieved.

The prospective works intend to improve bioconversion capacity using acetate oxidizing bacteria. New strains have been specifically isolated. They will be studied in bench-scale bioconversions. The most suitable bioreactor will be determined.

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ENHANCEMENT OF Cr (VI) AND Co (III) REDUCTION AT ELEVATED TEMPERATURES AND BY THERMOPHILIC MICROORGANISMS

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Reduction of toxic Cr (VI) and Co (III) to less toxic lower valence states represents an important mechanism for detoxification of contaminated soil and water. We examined Cr (VI) and Co (III) reduction at temperatures from 25°C to 75°C in media containing up to 7% salts. The initial concentration was 2.5 mM for Cr (VI) and 10.0 mM for Co (III). Thermophilic bacteria from >2500 m beneath land surface were isolated for biological experiments. Glucose (10 mM) served as a chemical reductant and an organic substrate for bacterial growth. Initial chemical reduction rates for Co (III) increased from 0.13 mM/hour at 25°C to 0.67 mM/hour at 75°C. The addition of thermophilic bacteria further enhanced the reduction by as much as 33%. Chemical reduction of Cr (VI) was not detected at 25°C, but had an initial rate of 0.08 mM/hour at 75°C. The addition of bacteria enhanced Cr (VI) reduction by 24% at 55°C, but did not enhance the reduction at 65°C. This study suggests that thermally- and biologically-enhanced metal reduction by nontoxic organic reductants may provide an alternative for remediating toxic metal contamination.

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