27th Symposium on Biotechnology for Fuels and Chemicals

Hosted by the National Renewable Energy Laboratory

Program & Abstracts

Denver Marriott
City Center Hotel
Denver, Colorado
May 1-4, 2005

BIOTECHNOLOGY

Welcome
from the Organizing Committee

Improving the economics of producing fuels and chemicals is vital to many industrial sectors. We have designed the program for the 27th Symposium on Biotechnology for Fuels and Chemicals to deliver the latest research breakthroughs and results in biotechnology that stimulate such improvements. Whether you represent the industrial, academic, or government sector, we invite you to join us and participate in this exciting exchange of information and ideas. You will find valuable opportunities for productive interactions with your colleagues, both from a national and international perspective.

With the 27th Symposium, we continue the tradition of providing an informal, congenial atmosphere that our participants find conducive to discussing technical program topics. All this is made possible through the generosity of our sponsors, and we urge you to join us in thanking them. This year's sponsors include:

- Office of the Biomass Program, U.S. Department of Energy
- Agricultural Research Service, U.S. Department of Agriculture
- National Renewable Energy Laboratory
- Oak Ridge National Laboratory
- Idaho National Laboratory
- Abengoa Bioenergy R&D, Inc.
- Archer Daniels Midland
- Battelle Nanotechnology Innovation Network
- BBI International
- Cargill
- E.I. du Pont de Nemours & Co. Inc.
- Genencor International, Inc.
- Iogen Corporation
- Katzen International, Inc.
- Natural Resources, Canada
- Novozymes
- Purevision Technology, Inc.
- Tate & Lyle
- Wynkoop Brewing Company

This year we will augment our technical program with a stimulating banquet evening presentation by Dr. Gregory Stephanopoulos, Bayer Professor of Chemical Engineering and Director of the Laboratory of Metabolic Engineering and Bioinformatics at the Massachusetts Institute of Techology. The title of his presentation is *"Metabolic Engineering: A Core Technology for Fuels and Chemicals in the Post-Genomic Era."*

Each year at this Symposium we recognize an individual who has distinguished himself or herself in the application of biotechnology to produce fuels and chemicals. This award acknowledges contributions to the field as a whole or this symposium, particularly innovation in fundamental and applied biotechnology, insight into bioprocessing fundamentals, or commitment to facilitate commercialization of products from renewable resources. This award is named in honor of Dr. Charles D. Scott, founder of the Symposium on Biotechnology for Fuels and Chemicals and its chair for the first ten years. In his years of work at ORNL, Chuck performed research and development on many novel bioprocessing systems including high production bioreactors, immobilized microbes, enzymes in organic media, and a coal bioprocess to name a few. The award is presented annually at the Symposium on Biotechnology for Fuels and Chemicals to recognized persons who have distinguished themselves in the area of biotechnology to produce fuels and chemicals.

We also hope you will join us for the 28th Symposium in Chattanooga, Tennessee, at the Chatanooga Choo Choo Hotel, April 30-May 3, 2006. The Web link to the 28th Symposium is *www.simhq.org/html/meetings.html*

Charles D. Scott Award

Lee Lynd's interest in biotechnology for fuels and chemicals began as an undergraduate upon seeing clearing zones in cellulose agar. This observation, coupled with a desire to respond to the energy-related challenges of the late 1970s, prompted Lee to focus his senior thesis on using thermophilic bacteria to produce ethanol from cellulosic biomass. After receiving a B.S. degree in Biology from Bates College, he went on to obtain an M.S. degree in Bacteriology from the University of Wisconsin, Madison, and thereafter M.S. and D.E. degrees from the Thayer School of Engineering at Dartmouth. Currently a Professor of Engineering and an Adjunct Professor of Biology at Dartmouth, as well as a Professor Extraordinary of Microbiology at the University of Stellenbosch, South Africa, Lee has led an active research group at Dartmouth since 1987.

Lee has made many pioneering contributions to bioenergy and biomass conversion. Most impressively, his activities and accomplishments span the science, technology, and policy domains. Highlights include improving our fundamental understanding of microbial cellulose utilization, advancing the design and evaluation of biomass conversion processes, and providing a variety of critical analyses and input in support of biomass energy. Professional activities include serving as: the biofuels industry representative on an advisory committee to the Executive Office of President Clinton on reducing greenhouse gas emissions from personal vehicles (1994-1995); Associate Editor of the journal *Biotechnology and Bioengineering* (since 1995); Organizing Committee Member for this symposium (since 1996); Manager of the Link Foundation Energy Fellowship Program (since 1998); Member and R&D Area Coordinator for the Biomass and Agriculture Working Group of the Energy Future Coalition (2003-2004); and Steering Committee Member for an American Academy of Microbiology Symposium on Microbial Energy Production (ongoing). An active consultant and frequently invited presenter on technical and strategic aspects of biomass energy, Lee has twice testified before the United States Senate. He currently co-leads a large multi-institutional project entitled *The Role of Biomass in America's Energy Future*. The author of over sixty peer-reviewed papers and several comprehensive reviews, and the inventor of five patents, the field of biotechnology for fuels and chemicals would not be the same were it not for Lee's tireless and inspired efforts.

Registration and Editor's Desk

Registration — The Symposium registration fee includes admission to all technical sessions, continental breakfast Monday through Wednesday, refreshment breaks, the Sunday evening welcoming reception and poster session, the Monday evening poster session, the Wednesday evening banquet, and a copy of the published proceedings. Student and guest registrations include everything *except the published proceedings.*

On-site Registration — The registration and information desk, located on lower level 2, just downstairs from the hotel lobby, will be open Sunday, May 1, from 10:00 am to 5:00 pm and at convenient hours throughout the symposium.

Special Needs — The organizing committee wants to ensure your comfort and convenience at the symposium. If you have any special needs, please let us know at the registration desk.

Editor's Desk —The editor's desk will be open Sunday, May 1, from 10:00 am to 5:00 pm and at convenient hours throughout the symposium. Please turn manuscripts in immediately upon arrival at the symposium. We need to distribute many of them to reviewers who are attending.

Site Description

Denver — Downtown Denver offers a rich selection of culture, entertainment, restaurants, and shopping opportunities close to the symposium hotel. The greater Denver area and nearby Rocky Mountains provide incredible beauty and numerous recreational opportunities. For more information, ask at the hotel reception desk or check the Chamber of Commerce web site *www.denverchamber.org/visitor/index.asp* or the Convention and Visitor Bureau web site *www.denver.org*

Transportation — A free shuttle bus providing easy access to much of Downtown Denver runs along the 16th Street Pedestrian Mall, just one block from the hotel.

Social Program

Sunday, May 1	Evening welcoming reception, followed by Poster Session A which will include a light buffet
Monday, May 2	Continental breakfast on Monday
	Evening Poster Session B and light buffet
Tuesday, May 3	Continental breakfast on Tuesday
	Tours of NREL's National Bioenergy Center research facilities for those who preregistered and are approved for site access
Wednesday, May 1	Continental breakfast on Wednesday
	Reception and dinner banquet including guest speaker, award presentations, and entertainment

Lodging

The Denver Marriott City Center Hotel is a full-service hotel with more than 600 rooms on 20 floors in the heart of Denver. Check-in time is 3:00 pm. The hotel will make reasonable efforts to accommodate early arrivals. Check-out time is 12:00 noon.

Conference Program

27th Symposium on Biotechnology for Fuels and Chemicals
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Sunday, May 1, 2005		
10:00 am - 5:00 pm	Registration/Editor's Desk Open	Ballroom Foyer
11:00 am - 5:00 pm	Poster Session Set-up	Denver Ballroom
1:00 pm - 5:00 pm	**Opening Remarks**	**Colorado Ballroom, E-J**
	Session 1A (Concurrent)	**Colorado Ballroom, E**
	Session 1B (Concurrent)	**Colorado Ballroom, F-J**
5:45 pm - 6:45 pm	Welcoming Reception	Denver Ballroom
6:45 pm - 9:30 pm	**Poster Session A (Sessions 1A, 1B, 2, 6)**	**Denver Ballroom**
Monday, May 2, 2005		
7:30 am - 5:00 pm	Registration/Editor's Desk Open	Ballroom Foyer
7:15 am - 8:00 am	Continental Breakfast	Ballroom Foyer
7:15 am - 8:00 am	Speakers' Breakfast	Gold Coin
8:00 am - 12:00 pm	**Session 2**	**Colorado Ballroom, E-J**
12:00 pm - 2:00 pm	Lunch On Your Own	
2:00 pm - 5:00 pm	**Special Topic A (Concurrent)**	**Colorado Ballroom, E**
2:00 pm - 4:00 pm	**Special Topic B (Concurrent)**	**Colorado Ballroom, F-J**
6:45 pm - 9:30 pm	**Poster Session B (Sessions 3A, 3B, 4, 5)**	**Denver Ballroom**
Tuesday, May 3, 2005		
7:30 am - 1:00 pm	Registration/Editor's Desk Open	Ballroom Foyer
7:15 am - 8:00 am	Continental Breakfast	Ballroom Foyer
7:15 am - 8:00 am	Speakers' Breakfast	Gold Coin
8:00 am - 11:45 am	**Session 3A (Concurrent)**	**Colorado Ballroom, E**
	Session 3B (Concurrent)	**Colorado Ballroom, F-J**
12:00 pm - 1:30 pm	Organizing Committee Luncheon and Meeting	Gold Coin
	Free Afternoon/NREL National Bioenergy Center Tours	
3:00 pm - 10:00 pm	Poster Removal	Denver Ballroom
7:00 pm - 9:40 pm	**Session 4**	**Colorado Ballroom, E-J**
Wednesday, May 4, 2005		
7:30 am - 3:00 pm	Registration/Editor's Desk Open	Ballroom Foyer
7:15 am - 8:00 am	Continental Breakfast	Ballroom Foyer
7:15 am - 8:00 am	Speakers' Breakfast	Gold Coin
8:00 am - 11:45 am	**Session 5**	**Colorado Ballroom, E-J**
8:00 am - 11:00 am	Final Poster Removal	Denver Ballroom
11:45 pm - 1:15 pm	Lunch On Your Own	
1:15 pm - 5:00 pm	**Session 6**	**Colorado Ballroom, E-J**
6:00 pm - 7:00 pm	Pre-Banquet Social	Ballroom Foyer
7:00 pm - 10:00 pm	Banquet/Speaker/Awards/Entertainment	Colorado Ballroom, E-F

Sunday, May 1, 2005

Welcome

1:00 p.m. Opening Remarks—Conference Chairs

Session 1A: Feedstock Supply and Logistics

Chair: Peter Flynn, University of Alberta
Co-Chair: Foster Agblevor, Virginia Polytechnic Institute and State University

1:15 pm Opening Remarks—Session Chair/Co-Chair

1:25 pm Oral Presentation 1A-01. **Agricultural Residue Availability in the United States,** *Zia Haq*, Energy Information Administration, Washington, DC and *James Easterly*, Easterly Consulting, Fairfax, VA

1:50 pm Oral Presentation 1A-02. **New Technology for Reed Canary Grass Production,** *Arvo Leinonen*, Samuli Rinne, and Tuulikki Lindh, Technical Research Centre of Finland (VTT), Jyväskylä, Finland

2:15 pm Oral Presentation 1A-03. **Corn Stover Fractions Related to Bioenergy: Chemical Composition and Structure,** *Danny E. Akin,* W. Herbert Morrison III, Franklin E. Barton, II, and David S. Himmelsbach, R.B. Russell Agricultural Research Center, U.S. Department of Agriculture Agricultural Research Service, Athens, GA; and Kevin B. Hicks, Eastern Regional Research Center, U.S. Department of Agriculture Agricultural Research Service, Wyndmoor, PA

2:40 pm Oral Presentation 1A-04. **The BTL2 Process of Biomass Utilisation: Entrained Flow Gasification of Pyrolysed Biomass Slurries,** *Klaus Raffelt,* Edmund Henrich, Andrea Koegel, Ralph Stahl, Joachim Steinhardt, and Friedhelm Weirich, Forschungszentrum Karlsruhe, Institut für Technische Chemie (ITC-CPV), Eggenstein-Leopoldshafen, Germany

3:05 pm Break

3:35 pm Oral Presentation 1A-05. **Ethanol Production from Wet-Oxidized Wheat Straw by Different Recombinant *Saccharomyces cerevisiae* Strains,** *Gianni Panagiotou* and Lisbeth Olsson, Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark

4:00 pm Oral Presentation 1A-06. **Under What Conditions is Ethanol from Corn Stover Economically Viable?** *Burton C. English* and R. Jamey Menard, Agri-Industry Modeling & Analysis Group, Department of Agricultural Economics, University of Tennessee, Knoxville, TN; Daniel De La Torre Ugarte, Agricultural Policy Analysis Center, Department of Agricultural Economics, University of Tennessee, Knoxville, TN; and Marie E. Walsh, Department of Agricultural Economics, University of Tennessee, Knoxville, TN

4:25 pm Oral Presentation 1A-07. **Modeling Cellulosic Ethanol Production and Distribution in the United States,** William R. Morrow III, *Michael Griffin,* and Scott Mathews, Carnegie Mellon University, Pittsburgh, PA

4:50 pm Closing Remarks—Session Chair/Co-Chair

Session 1B: Enzyme Catalysis and Engineering

Chair: Joel Cherry, Novozymes, Inc.
Co-Chair: Kevin Gray, Diversa Corporation

1:15 pm Opening Remarks—Session Chair/Co-Chair

1:25 pm Oral Presentation 1B-01. **Chemical Complementation: A Potentially General Method of Engineering Enzymes through Genetic Selection,** Bahareh Azizi, Lauren J. Schwimmer, and *Donald F. Doyle,* School of Chemistry and Biochemistry, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA

1:50 pm Oral Presentation 1B-02. **Effect of Cellulase Supplementation on Cookline Operation in a Dry Mill Ethanol Plant,** *Bradley A. Saville* and Vince Yacyshyn, Dept. of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada

2:15 pm Oral Presentation 1B-03. **Improving Cellulose Hydrolysis with New Cellulase Compositions,** *Elena Vlasenko* and Joel Cherry, Novozymes, Inc., Davis, CA

2:40 pm Oral Presentation 1B-04. **Production of Granular Starch Hydrolyzing Enzymes for Low Energy Grain Ethanol Production,** *Nigel Dunn-Coleman*, Suzanne Lantz, Michael Pepsin, Tim Dodge, Bradley Kelemen, and Jayarama K. Shetty, Genencor International, Inc., Palo Alto, CA; and O. J. Lantero, James Miers, and Craig Pilgrim, Genencor International, Inc., Beloit, WI

3:05 pm Break

3:35 pm Oral Presentation 1B-05. **A High Throughput Micro-Assay to Evaluate Enzymatic Hydrolysis of Lignocellulosic Substrates from Agricultural and Forest Residues,** *Alex Berlin,* Vera Maximenko, Renata Bura, Neil Gilkes, Douglas Kilburn, and Jack Saddler, Biotechnology and Biomaterials Group, Faculty of Forestry, University of British Columbia, Vancouver, BC, Canada

4:00 pm Oral Presentation 1B-06. **Development of Enzyme Cocktails for Feedstock Conversion to Fermentable Sugars,** *Lishan Zhao*, Kelvin Wong, Myoung Kim, Lori Preston, Flash Bartnek, Yoko Philips, Chris Lyon, Uvini Gunawardena, Charles Tweedy, Olen Yoder, Mike Lafferty, and Kevin Gray, Diversa Corporation, San Diego, CA

4:25 pm Oral Presentation 1B-07. **Poly (Ethylene Glycol) as Additive for Increased Conversion of Lignocellulose,** *Johan Börjesson* and Folke Tjerneld, Department of Biochemistry, Lund University Center for Chemistry and Chemical Engineering, Lund, Sweden

4:50 pm Closing Remarks—Session Chair/Co-Chair

6:45-9:30 pm Poster Session A (1A, 1B, 2, 6)

Monday, May 2, 2005

Session 2: Today's Biorefineries

Chair: Robert Benson, Tembec Chemical Products Group
Co-Chair: Paris Tsobanakis, Cargill, Inc.

8:00 am Opening Remarks—Session Chair/Co-Chair

8:10 am Oral Presentation 2-01. **Tomorrow's Biomass Refineries,** *Lee R. Lynd,* Mark Laser, Haiming Jin, and Kemantha Jayawardhana, Thayer School of Engineering, Dartmouth College, Hanover, NH; Eric D. Larson and Fuat Celik, Princeton University, Princeton, NJ; and Bruce E. Dale, Department of Chemical Engineering, Michigan State University, Lansing, MI

8:40 am Oral Presentation 2-02. **Technical and Economic Considerations of Biorefineries,** *Bill Dean,* Tim Dodge, Fernando Valle, and Gopal Chotani, Genencor International, Palo Alto, CA

9:10 am Oral Presentation 2-03. **The Development of an Integrated Biorefinery Concept for Production of Fuels and Chemicals Derived from Lignocellulosic Biomass,** *Birgitte K. Ahring,* Marie J. Mikkelsen, Slawek Dabrowski, Tania I. Georgieva, and Frank D. Haagensen, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark

9:40 am Break

10:10 am Oral Presentation 2-04. **Existing Biorefinery Operations that Can Benefit from Fractal Based Process Intensification,** *Vadim Kochergin* and Mike Kearney, Amalgamated Research Inc., Twin Falls, ID

10:40 am Oral Presentation 2-05. **High-Cetane Diesel Fuels from Biomass,** *Edwin S. Olson,* Ramesh K. Sharma, and Ted R. Aulich, Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND

11:10 am Oral Presentation 2-06. **The Importance of Utility Systems in Today's and Tomorrow's Biorefineries,** *Tim Eggeman* and Dan Verser, ZeaChem Inc., Lakewood, CO

11:40 am Closing Remarks—Session Chair/Co-Chair

Special Topic A:

International Energy Agency Task #39 - Liquid Biofuels

Chair: Jack Saddler, University of British Columbia

2:00 pm Opening Remarks—Session Chair

2:05 pm Oral Presentation Special Topic A-01. **Technical Progress In Bioconversion: Steps Towards Commercialization,** W.E. Mabee, D.J. Gregg, N. Gilkes, and *J.N. Saddler,* University of British Columbia, Vancouver, BC, Canada

2:10 pm Oral Presentation Special Topic A-02. **Fuel Ethanol R&D in Sweden,** *Barbel Hahn-Hägerdal,* Applied Microbiology, LTH/Lund University, Lund, Sweden

2:20 pm Oral Presentation Special Topic A-03. **Making a Business with Biofuels,** *Manfred Wörgetter,* Bundesanstalt fur LandTechnik, Wieselburg, Austria

2:30 pm Oral Presentation Special Topic A-04. **Present Situation and Prospects on Bioethanol in Asian Countries,** *Shiro Saka,* Graduate School of Energy Science, Kyoto University, Kyoto, Japan

2:40 pm Oral Presentation Special Topic A-05. **Liquid Biofuels in South Africa,** *Bernard A. Prior,* Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa

2:50 pm Oral Presentation Special Topic A-06. **Global Biofuel Potential – Sugarcane Contribution,** *José Roberto Moreira,* CENBIO (National Centre of Biomass), São Paulo, Brazil

3:00 pm Oral Presentation Special Topic A-07. **Key Barriers for Commercializing Biofuels from Lignocellulosic Biomass,** *Birgitte K. Ahring,* BioCentrum-DTU, Lyngby, Denmark

3:10 pm Oral Presentation Special Topic A-08. **Perspectives on Biofuels Market Development in Canada,** *Don O'Connor,* $(S\&T)^2$ Consultants Inc., Delta, BC, Canada

3:20 pm Oral Presentation Special Topic A-09. **Potential Barriers to Biomass Ethanol Commercialization,** *Quang Nguyen*, Abengoa Bioenergy R&D, Inc., Chesterfield, MO

3:30 pm Oral Presentation Special Topic A-10. **Genencor's Perspective on the Key Barriers to Commercializing Biofuels,** *Colin Mitchinson*, Genencor International, Inc., Palo Alto, CA

3:40 pm Oral Presentation Special Topic A-11. **Progress on Enzymes for Biomass Utilization and Prospects for the Future,** *Joel R. Cherry*, Novozymes Inc., Davis, CA

3:50 pm Oral Presentation Special Topic A-12. **The Key Barriers to Commercialisation of Transport Biofuels,** *Tony Sidwell*, British Sugar, Norfolk, UK

4:00 pm General Discussion

4:50 pm Closing Remarks—Session Chair

Monday, May 2, 2005

Special Topic B:

"Outside of a Small Circle of Friends:" Changing Attitudes About Biomass as a Sustainable Energy Supply

Chair: John Sheehan, National Renewable Energy Laboratory

2:00 pm Opening Remarks—Session Chair

2:05 pm Oral Presentation Special Topic B-01. **Growing Energy: How Biofuels Can Help End America's Oil Dependence,** *Jeff Fiedler,* Natural Resources Defense Council, Washington, DC

2:20 pm Oral Presentation Special Topic B-02. **Biomass as a Strategy in Winning the Oil Endgame,** *Joel N. Swisher*, Rocky Mountain Institute, Snowmass, CO

2:35 pm Oral Presentation Special Topic B-03. **Expanding the Use of Biomass for Fuels – A View From Today's Bioethanol Industry,** *Rick Tolman,* National Corn Growers Association, Chesterfield, MO

2:50 pm Oral Presentation Special Topic B-04. **"Twenty-Five by Twenty-Five," Agriculture's Role in Ensuring U.S. Energy Security-A Blueprint for Action,** *Michael Bowman,* Bowman Family Farms, Wray, CO

3:05 pm Oral Presentation Special Topic B-05. **Fear and Loathing on the Energy Trail: Confusion, Convergence, and Divergence in the Public Dialogue about the Future of America's Energy Supply,** *John Sheehan*, National Renewable Energy Laboratory, Golden, CO

3:20 pm General Discussion

3:55 pm Closing Remarks—Session Chair

6:45-9:30 pm Poster Session B (3A, 3B, 4, 5)

Tuesday, May 3, 2005

Session 3A: Plant Biotechnology and Feedstock Genomics

Chair: Sean Simpson, AgriGenesis Biosciences, Ltd.
Co-Chair: Wilfred Vermerris, Purdue University

8:00 am Opening Remarks—Session Chair/Co-Chair

8:10 am Oral Presentation 3A-01. **Application of Functional Genomic Tools for Exploring Switchgrass Feedstocks Improved through Divergent Selection,** *G. Sarath,* Kenneth P. Vogel, and Rob Mitchell, USDA-ARS, Lincoln, NE; Paul Twigg, University of Nebraska-Kearney, Kearney, NE; Christian Tobias, USDA-ARS, Albany, CA; and Lisa M. Baird, University of San Diego, San Diego, CA

8:35 am Oral Presentation 3A-02. **Genetic Improvement of Shrub Willow (*Salix*) as a Dedicated Crop for Bioenergy, Biofuels, and Bioproducts,** *Lawrence B. Smart,* Juan Lin, Richard F. Kopp, Ingrid S. Phillips, and Kimberly D. Cameron, Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, Syracuse, NY and Timothy A. Volk, Edwin H. White, and Lawrence P. Abrahamson, Forest and Natural Resources Management, SUNY College of Environmental Science and Forestry, Syracuse, NY

9:00 am Oral Presentation 3A-03. **Functional Genomics in Forestry: Solutions for the Biomass Economy,** *Robert J. Kodrzycki,* Katrina C. Gause, Heather M. Holley, Donald J. Kaczmarek, Kimberly F. McManus, Samantha A. Miller, H. Dayton Wilde, and Maud A. W. Hinchee, ArborGen, Summerville, SC; and Mark F. Davis, National Renewable Energy Laboratory, Golden, CO

9:25 am Oral Presentation 3A-04. **Development of Biologically Confined Transgenic Crops for Renewable Energy,** *Mariam Sticklen,* C. Ransom, H. Oraby, H. Salehi, R. Ahmad, and Z. Seddighi, Department of Crop & Soil Science, Michigan State University, East Lansing, MI

9:50 am Break

10:20 am Oral Presentation 3A-05. **Manipulating the Phenolic Acid Content and Digestibility of Forage Grasses by Targeted Expression of Fungal Cell Wall Degrading Enzymes,** *M.M.de O. Buanafina,* T. Langdon, S.J. Dalton, B. Hauck, N. Dunn-Coleman, and P. Morris, Plant Animal and Microbial Science Department, Institute of Grassland & Environmental Research, Aberystwyth, UK

10:45 am Oral Presentation 3A-06. **Direct Imaging of Pretreatment and Enzymatic Digestion Effects on Corn Stover Ultrastructure,** *Stephen R. Decker,* Michael Selig, Michael E. Himmel, Shi-You Ding, and Todd B. Vinzant, National Renewable Energy Laboratory, Golden, CO

11:10 am Oral Presentation 3A-07. **Enhanced Processing Characteristics via Simultaneous Optimization of Stover Composition and Pretreatment Conditions,** *Javier Campos,* Ana Saballos, Yulin Lu, Alma Armenta Medina, Gebisa Ejeta, Nathan S. Mosier, and Wilfred Vermerris, Department of Agronomy, Department of Agricultural & Biological Engineering and Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN

11:35 am Closing Remarks—Session Chair/Co-Chair

Tuesday, May 3, 2005

Session 3B: Biomass Pretreatment and Hydrolysis

Chair: Rick Elander, National Renewable Energy Laboratory
Co-Chair: Mohammed Moniruzzaman, Genencor International

8:00 am Opening Remarks—Session Chair/Co-Chair

8:10 am Oral Presentation 3B-01. **Biomass Recalcitrance: The Major Hurdle for the Lignocellulosic Biorefinery Industry,** *Michael Himmel,* Stan Bower, Mark Ruth, and Tom Foust, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO and Amy Miranda, Office of the Biomass Program, U.S. Department of Energy, Washington, DC

8:35 am Oral Presentation 3B-02. **Fundamental Factors Affecting Enzymatic Reactivity,** *Jonathan O'Dwyer,* Li Zhu, and Mark Holtzapple, Department of Chemical Engineering, Texas A&M University, College Station, TX

9:00 am Oral Presentation 3B-03. **Optimal Combination of Cellulase and Xylanase for Enzymatic Hydrolysis of AFEX Treated Corn Stover,** *Farzaneh Teymouri,* Darold MaCalla, and Mark Stowers, MBI International, Lansing, MI; Bruce E. Dale, Michigan State University, East Lansing, MI; and Stephen Decker, National Renewable Energy Laboratory, Golden, CO

9:25 am Oral Presentation 3B-04. **Hydrolysis and Fermentation of Lime-Pretreated Biomass,** *Robert R. Bakker,* Ronald H.W. Maas, Ed de Jong, and Ruud A. Weusthuis, Agrotechnology & Food Innovations, Wageningen, the Netherlands and Mirjam A. Kabel and Henk A. Schols, Laboratory of Food Chemistry, Wageningen University, The Netherlands

9:50 am Break

10:20 am Oral Presentation 3B-05. **Preliminary Results on Optimising Hydrothermal Treatment Used in Co-Production of Biofuels,** *Mette Hedegaard Thomsen* and Anne Belinda Thomsen, Plant Research Department, Risoe National Laboratory, Roskilde, Denmark; Henning Jørgensen, Danish Center for Forest, Landscape and Planning, KVL, The Royal Veterinary and Agricultural University, Taastrup, Denmark; and Børge Holm Christensen, Sicco K/S, Aalsgaarde, Denmark

10:45 am Oral Presentation 3B-06. **Bioconversion of Lignocellulose to Ethanol: a Robust Pretreatment Method for Processing Mixed Feedstocks,** *Renata Bura,* Alex Berlin, Neil Gilkes, Douglas Kilburn, and Jack Saddler, Biotechnology and Biomaterials Group, Faculty of Forestry, University of British Columbia, Vancouver, BC, Canada

11:10 am Oral Presentation 3B-07. **Cellulose to Glucose Conversion via Swelling-Decrystallization and Subsequent Low Severity Acid Hydrolysis,** *K. Bélanger,* E. Capek, H. Gauvin, and R. D'Amour, Université de Sherbrooke, Dépat. Génie chimique, Sherbrooke, QC, Canada and E. Chornet, Enerkem Technologies Inc., Montréal, QC, Canada

11:35 am Closing Remarks—Session Chair/Co-Chair

Session 4: Industrial Biobased Products

Chair: Ray Miller, E.I. DuPont de Nemours and Company, Inc.
Co-Chair: Matt Tobin, Codexis

7:00 pm Opening Remarks—Session Chair/Co-Chair

7:05 pm Oral Presentation 4-01. **Gasification of Ethanol-Derived Switchgrass Lignin,** *Chris J. Zygarlicke,* Michael L. Swanson, Ann K. Henderson, and Mark A. Musich, Energy & Environmental Research Center, University of North Dakota Grand Forks, ND; and Millicent R. Moore, Tennessee Valley Authority, Muscle Shoals, AL

7:30 pm Oral Presentation 4-02. **Fed-Batch Production of Succinic Acid from Glucose: Xylose Feedstocks Using *E. coli,*** *Christian Andersson,* Ulrika Rova, and Kris A. Berglund, Division of Biochemical and Chemical Process Engineering, Luleå University of Technology, Luleå, Sweden

7:55 pm Oral Presentation 4-03. **Continuous Butanol Production from Glucose and Butyrate by *Clostridium acetobutylicum* in a Fibrous Bed Bioreactor: Effects of Butyrate and Long-Term Stability,** David E. Ramey, Environmental Energy Inc., Blacklick, OH; and Wei-Cho Huang and *Shang-Tian Yang*, Department of Chemical and Biomolecular Engineering, Ohio State University, Columbus, OH

8:20 pm Oral Presentation 4-04. **The Development of Cement and Concrete Additive Based on Xylonic Acid Derived via Bioconversion of Xylose,** *Byong-wa (Zen) Chun,* Ara Jeknavorian, and Charlotte Porteneuve, Grace Performance Chemicals, W. R. Grace & Co, Cambridge, MA

8:45 pm Oral Presentation 4-05. **Commercialization of PHA Bioplastics: Current and Future Developments,** Oliver P. Peoples and *Johan van Walsem*, Metabolix Inc., Cambridge, MA

9:10 pm Oral Presentation 4-06. **Top Value Added Chemicals from Biomass (Volume 1),** T. A. Werpy, *J. E. Holladay*, and J. F. White, Pacific Northwest National Laboratory, Richland, WA; G. Peterson, J. Bozell, and A. Aden, National Renewable Energy Laboratory, Golden, CO; and Amy Manheim, U.S. Department of Energy, Washington, DC

9:35 pm Closing Remarks—Session Chair/Co-Chair

Wednesday, May 4, 2005

Session 5: Microbial Catalysis and Metabolic Engineering

Chair: Lisbeth Olsson, BioCentrum-DTU, Technical University of Denmark
Co-Chair: Aristos Aristidou, NatureWorks LLC

8:00 am Opening remarks—Session Chair/Co-Chair

8:10 am Oral Presentation 5-01. **Alcoholic Fermentation of Lignocellulose Hydrolysates Has Become Feasible,** *Eleonora Bellissimi,* Marko Kuyper, Maurice Toirkens, Hans van Dijken, and Jack Pronk, Department of Biotechnology, Delft University of Technology, Delft, The Netherlands and Ron Winkler and Hans van Dijken, Bird Engineering B.V., Rotterdam, The Netherlands

8:35 am Oral Presentation 5-02. **Genetic Engineering of *S. cerevisiae* for Pentose Utilization,** *Peter Richard,* Ritva Verho, John Londesborough, and Merja Penttilä, VTT Biotechnology, Finland

9:00 am Oral Presentation 5-03. **Development of Recombinant Xylose- and Arabinose-Utilising *Saccharomyces cerevisiae* Strains,** Kaisa Karhumaa, Valeria Wallace Salinas, Bärbel Hahn-Hägerdal, and *Marie F. Gorwa-Grauslund*, Department of Applied Microbiology, Lund University, Lund, Sweden; and Beate Wiedemann and Eckhard Boles, Institut für Mikrobiologie, Goethe-Universitaet Frankfurt, Frankfurt am Main, Germany

9:25 am Oral Presentation 5-04. **A "First-Look" at the Genome of the Xylose-Fermenting Yeast, *Pichia stipitis*,** *Thomas W. Jeffries* and José M. Laplaza, USDA-Forest Service, Forest Products Laboratory, Madison, WI; Igor Grigoriev and Paul Richardson, DOE Joint Genome Institute, Walnut Creek, CA; Yong-Su Jin, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA; and Volkmar Passoth, Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden

9:50 am Break

10:20 am Oral Presentation 5-05. **Evaluation of Cellulase System Components Expressed in *Saccharomyces cerevisiae* and Implications for Consolidated Bioprocessing,** *John McBride* and Lee R. Lynd, Thayer School of Engineering, Dartmouth College, Hanover, NH and Riaan den Haan, J. J. (Ancha) Zietsman, Ronel van Rooyen, Shaunita H. Rose, Danie La Grange, and W. H. (Emile) van Zyl, Department of Microbiology, University of Stellenbosch, Stellenbosch, South Africa

10:45 am Oral Presentation 5-06. **Metabolic Engineering of *Actinobacillus succinogenes* to Enhance Succinic Acid Production,** *Jian Yi,* Michael V. Guettler, Susanne Kleff, and Mark D. Stowers, MBI International, Lansing, MI

11:10 am Oral Presentation 5-07. **High-Purity Lactic Acid Fermentation Broth,** C.-L. Liu and *J. C. Lievense,* Tate & Lyle, Decatur, IL

11:35 am Closing Remarks—Session Chair/Co-Chair

Session 6: Bioprocess R&D

Chair: Michael Ladisch, Purdue University
Co-Chair: Peter Yu, Hong Kong Polytechnic University

1:15 pm Opening Remarks—Session Chair/Co-Chair

1:25 pm Oral Presentation 6-01. **Demonstration of Sustained Hydrogen Photoproduction by Immobilized, Sulfur-Deprived *Chlamydomonas reinhardtii* Cells,** *Alexander S. Fedorov,* Tatyana V. Laurinavichene, and Anatoly A. Tsygankov, Institute of Basic Biological Problems RAS, Pushchino, Russia; and Maria L. Ghirardi and Michael Seibert, Basic Sciences Center, National Renewable Energy Laboratory, Golden, CO

1:50 pm Oral Presentation 6-02. **Fermentation of Synthesis Gas to Fuel Ethanol,** Asma Ahmed and *Randy S. Lewis,* School of Chemical Engineering, Oklahoma State University, Stillwater, OK; Bruno G. Cateni, Danielle D. Bellmer, and Raymond L. Huhnke, School of Biosystems and Agricultural Engineering, Oklahoma State University, Stillwater OK; and Ralph S. Tanner, Department of Botany and Microbiology, University of Oklahoma, Norman, OK

2:15 pm Oral Presentation 6-03. **Enzymatic Conversion of Waste Cooking Oils into Alternative Fuel—Biodiesel,** *Guanyi Chen,* Xiangmei Meng, Weizun Li, and Liran Chen, Section of Bioenergy and Energy-Efficient Buildings, Faculty of Environmental Science and Engineering, Tianjin University, Tianjin, China

2:40 pm Oral Presentation 6-04. **Production of Statins from *Pleurotus* Mushrooms,** *Gerardo Daniel López,* INGAR (CONICET) – Universidad Tecnológica Nacional, Santa Fe, Argentina; Susana G. Gervasio, INTEC (CONICET), Paraje el Pozo, Santa Fe, Argentina; and Mary Lopretti, CIN - Facultad de Ciencias, Laboratorio de Bioquímica y Biotecnología, Universidad de la República-Uruguay, Montevideo, Uruguay

3:05 pm Break

3:35 pm Oral Presentation 6-05. **Simultaneous Saccharification and Mixed Sugar Fermentation (SSMSF) of Acid Pretreated Rice Straw by Carbon Catabolite De-Repressed (CCR^-) *Lactobacillus pentosus* JH5XP5 and *Lactobacillus brevi*,** *Jae-Han Kim*, Department of Food Science and Technology, University of California, Davis; David E. Block and David A. Mills, Department of Viticulture and Enology, University of California, Davis; and Sharon P. Shoemaker, California Institute of Food and Agricultural Research, University of California, Davis, CA

4:00 pm Oral Presentation 6-06. **Bio-Ethanol from MSW Derived Paper,** *M. Clark Dale,* Bio-Process Innovation, Inc., Lafayette, IN and Daniel Musgrove, Universal Entech, Phoenix, AZ

4:25 pm Oral Presentation 6-07. **Oligosaccharide Hydrolysis in Plug Flow Reactor Using Strong Acid Catalyst,** *Youngmi Kim,* Rick Hendrickson, Nathan Mosier, and Michael R. Ladisch, Laboratory of Renewable Resources Engineering, Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN

4:50 pm Closing Remarks—Session Chair/Co-Chair

List of Poster Presentations

Sunday Evening, May 1, 2005 6:45 - 9:30 pm

Chair: Nancy Dowe
National Renewable Energy Laboratory

All posters should be up by Sunday afternoon, May 1, 2005. Presenters should be near their respective posters as below:

Sessions: 1A, 1B, 2, 6 Sunday 6:45 pm - 9:30 pm

Feedstock Supply and Logistics

Poster 1A-08. **Assessing the Value of a Targeted Corn Stover Harvest by Understanding the Distribution of Inorganic Nutrients.** *Tom Schechinger,* IronHorse Farms, Harlan, IA; Steven Thomas, National Renewable Energy Laboratory, Golden, CO

Poster 1A-09. **Development of a Multi-Criteria Model for Ranking Biomass Feedstock Collection and Logistics.** *Amit Kumar,* University of British Columbia, Vancouver, British Columbia, Canada; Shahab Sokhansanj, Oak Ridge National Laboratory, Oak Ridge, TN; Peter C. Flynn, University of Alberta, Edmonton, Alberta, Canada

Poster 1A-10. **Tools and Logistics for Selective Feedstock Harvesting.** *Reed Hoskinson,* Kevin Kenney, Idaho National Laboratory, Idaho Falls, ID; K. Blair Duguid, University of Kentucky, Lexington, KY

Poster 1A-11. **Assessment of Crop Residual in North Carolina as a Sustainable Feedstock for Bioethanol Production.** *Abolghasem Shahbazi,* Saed Sayyar, Shiva Gorugantu, Yebo Li, North Carolina A&T State University, Greensboro, NC

Poster 1A-12. **Feedstock: What is the Value? A Regional Study.** Joseph E. Atchison, Atchison Consultants, Charlotte, NC; *James R. Hettenhaus,* cea Inc., Charlotte, NC

Poster 1A-13. **Upgrading Corn Stover to Slurried Feedstock for Optimized Transport and Conversion.** *Klein E. Ileleji,* Bo Zhou, Purdue University, West Lafayette, IN

Poster 1A-14. **Combustion Profile of Biodiesel Manufactured from Rapeseed Oil in Diesel Engine.** *Gwi-Taek Jeong,* Don-Hee Park, Eui-Yeon Yu, Choon-Hyoung Kang, Chonnam National University, Gwangju, Korea; Si-Wouk Kim, Chosun University, Gwangju, Korea

Poster 1A-15. **Characterization and Thermogravimetric Analysis of Corn Stover SSF Residues.** Seung-Soo Kim, *Foster A. Agblevor,* Virginia Polytechnic Institute and State University, Blacksburg, VA; Daniel J. Schell, Bonnie R. Hames, National Renewable Energy Laboratory, Golden, CO

Poster 1A-16. **Economic Feasibility Analysis of Municipal Solid Waste to Ethanol Conversion.** *Satish Joshi,* Osamu Sakamoto, Michigan State University, East Lansing, MI; Heather L. MacLean, University of Toronto, Ontario, Canada

Poster 1A-17. **New HPLC Methods for Analysis of Biomass Pretreatment Processes and Hydrolysates.** *Foster A. Agblevor,* Aubrey Murden, Virginia Polytechnic Institute and State University, Blacksburg, VA; Bonnie R. Hames, Daniel J. Schell, Helena L. Chum, National Renewable Energy Laboratory, Golden, CO

Poster 1A-18. **Biomass Availability for the Biorefining Strategy in Canada: Suggestions for Policy Reform.** *Warren Mabee,* Paul McFarlane, Jack Saddler, University of British Columbia, Vancouver, British Columbia, Canada; Evan Fraser, University of Leeds, Leeds, United Kingdom.

Poster 1A-19. **Experimental and Numerical Analysis of the Biomechanical Characteristics of Agricultural Crop Residues.** *Christopher T. Wright,* Peter A. Pryfogle, Richard L. Williamson, Nathan A. Stevens, David Gallup, J. Richard Hess, Eric D. Steffler, Idaho National Laboratory, Idaho Falls, ID

Poster 1A-20. **Biofiltration Methods for Biological Removal of Phenolic Residues.** *Luiz Carlos Martins das Neves,* Tábata Miyamura, Dante Augusto Moraes, Thereza Christina Vessoni Penna, University of São Paulo, São Paulo-SP, Brazil; Attilio Converti, Universitá degli Studi di Genova, Genova, Italy

Poster 1A-21. **Rail Transport of Biomass in Canada.** *Hamed Mahmudi*, Peter C. Flynn, University of Alberta, Edmonton, Alberta, Canada

Poster 1A-22. **Year One: Agronomic Experience in Growing No Tillage Switchgrass Focusing on Seeding Rate, Nutrient Needs, and Landscape.** *Don Tyler,* Burton C. English, Marie Walsh, Roland Roberts, University of Tennessee, Knoxville, TN

Poster 1A-23. **Comparison of Physical, Chemical, and Anatomical Characteristics of Corn Anatomical Fractions.** *Stephanie Porter,* Cheryl Jurich, David Templeton, Claudia Ishizawa, Mark Davis, Michael Himmel, Steven Thomas, National Renewable Energy Laboratory, Golden, CO

Poster 1A-24. **Estimated Economic Impacts on the Agricultural Sector and the Nation's Economy of Supplying Feedstock to an Energy Sector.** *Burton C. English,* R. Jamey Menard, Daniel De La Torre Ugarte, Chad Hellwinkel, Marie E. Walsh, University of Tennessee, Knoxville, TN

Poster 1A-25. **Potential Perennial Biomass Feedstocks for the Southern United States.** *William F. Anderson,* Crop Genetics and Breeding Research Unit, USDA-Agricultural Research Service, Tifton, GA; Danny E. Akin, David S. Himmelsbach, W. Herbert Morrison, Russell Research Center, USDA-Agricultural Research Service, Athens, GA; David Bransby, Auburn University, Auburn, AL; Robert Cobill, Sugarcane Research Unit, USDA-Agricultural Research Service, Houma, LA

Poster 1A-26. **The Use of Organic Dyes in Biomass Storage.** *D. Brad Blackwelder,* LaKenya McNear, William Bond, Corey W. Radtke, J. Richard Hess, Idaho National Laboratory, Idaho Falls, ID

Poster 1A-27. **Effect of Fertility Management on Composition of Plant Fractions from Wheat.** K. Blair Duguid, *Michael D. Montross*, Scott A. Shearer, University of Kentucky, Lexington, KY; Reed L. Hoskinson, Idaho National Laboratory, Idaho Falls, ID

Poster 1A-28. **Corn Stover Quantity and Composition as Influenced by Agronomic Practices.** *Michael D. Montross,* Czarena L. Crofcheck, Scott A. Shearer, Dennis W. Hancock, University of Kentucky, Lexington, KY; Bonnie Hames, National Renewable Energy Laboratory, Golden, CO

Enzyme Catalysis and Engineering

Poster 1B-08. **Expression of Nisin by *Lactococcus lactis* in a Fermenter and Detection by Two Nisin-Sensitive Bacteria.** *Angela Faustino Jozala,* Thomas Rodolfo Gentille, Adalberto Pessoa Jr., Ângelo Samir Melin Miguel, Thereza Christina Vessoni Penna, University of São Paulo, São Paulo-SP, Brazil; Olivia Cholewa, Molecular Probes, Inc., Eugene, OR

Poster 1B-09. **Stability of Green Fluorescent Protein (GFPuv) in Chlorine Solutions of Varying pH.** *Marina Ishii,* Elaine Chau, Thereza Christina Vessoni Penna, University of São Paulo, São Paulo-SP, Brazil; Olivia Cholewa, Molecular Probes, Inc., Eugene, OR

Poster 1B-10. **Thermal Stability of Recombinant Green Fluorescent Protein (GFPuv) in Glucose Solutions at Various pH Conditions.** Marina Ishii, Juliana Sayuri Kunimura, *Thereza Christina Vessoni Penna,* University of São Paulo, São Paulo-SP, Brazil; Olivia Cholewa, Molecular Probes, Inc., Eugene, OR

Poster 1B-11. **Influence of Feeding Rate Type on Fed-Batch Cultivation to Produce Glucose-6-Phosphate Dehydrogenase by *Saccharomyces cerevisiae* W303-181.** *Ângelo S.M. Miguel,* Adalberto Pessoa Jr., University of São Paulo, São Paulo-SP, Brazil

Poster 1B-12. **Sub-Cellular Distribution of Alcohol Oxidase (Ao) Activity and Its Role in Aliphatic Hydrocarbon Biodegradation Pathway in Yr-1 Strain of *Mucor circinelloides*, a Potential Bioremediator.** Hortencia Silva-Jiménez, Yadira del Carmen G. Bárcenas-Contreras, Arelí Durón-Castellanos, Vanesa Zazueta-Sandoval, *Roberto Zazueta-Sandoval,* University de Guanajuato, Noria Alta, Mexico

Poster 1B-13. **Application of Lipases by Entrapment in Hydrophobic Sol-Gel Materials to Concentrate Polyunsaturated Fatty Acids.** Cleide M.F. Soares, Juliana E. Itako, State University of Maringá, Maringá-PR, Brazil; Heizir F. de Castro, Faculdade de Engenharia Quimica de Lorena, Lorena-SP, Brazil; Flávio F. de Moraes, *Gisella M. Zanin,* State University of Maringá, Maringá-PR, Brazil

Poster 1B-14. **Preparation of Cyclodextrins from Starch Using Immobilized CGTase from *Thermoanaerobacter* on Glyoxyl-Agarose.** Paulo W. Tardioli, *Gisella M. Zanin,* Flávio F. de Moraes, State University of Maringá, Maringá-PR, Brazil

Poster 1B-15. **Effect of Inhibitors Released During Steam-Explosion Treatment of Barley Straw on Enzymatic Hydrolysis.** Mª Prado García-Aparicio, Ignacio Ballesteros, Alberto González, Mercedes Ballesteros, *Mª Jose Negro,* CIEMAT, Madrid, Spain

Poster 1B-16. **Assessment of Effects of Process Variables on Enzymatic Activity of Three Lipases in Compressed Fluids.** *Denise M.G. Freire,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Andresa C. Feihrmann, Cláudio Dariva, Marco Di Luccio, Débora de Oliveira, J. Vladimir Oliveira, URI-Campus de Erechim, Erechim-RS Brazil

Poster 1B-17. **Computational Modeling of the Interaction of the Binding Domain of *T. reesei* Cel7A with Cellulose.** *Mark R. Nimlos,* Stan Bower, Michael E. Himmel, National Renewable Energy Laboratory, Golden, CO; Michael F. Crowley, Scripps Research Institute, La Jolla, CA; Giridhar Chukkapalli, Michael Cleary, San Diego Supercomputer Center, La Jolla, CA; John Brady, Cornell University, Ithaca, NY

Poster 1B-18. **RSM Analysis of the Effects of the Oxygen Transfer Coefficient and Inoculum Concentration on the Production of Xylitol by *C. guilliermondii*.** Mariana Peñuela Vásquez, Nei Pereira Jr., *Mauricio B. de Souza Jr.,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-19. **Renaturing Foam-Fractionated Cellulase with Artificial Chaperones.** Vorakan Burapatana, Ales Prokop, *Robert D. Tanner,* Vanderbilt University, Nashville, TN

Poster 1B-20. **Comparative Performance of *Candida rugosa* Lipase Immobilized on Non-Commercial Matrixes to Yield Alkyl Esters in a Solvent Free System.** *Daniele Urioste,* Michele Miranda, Heizir F. de Castro, Faculdade de Engenharia Química de Lorena, Lorena-SP, Brazil

Poster 1B-21. **Development of Lipase Immobilization on Silica Gel Using Cross-Linking Method for the Production of Biodiesel.** Cheol Hee Park, Jong Mo Yeo, Dong Hwan Lee, *Seung Wook Kim,* Korea University, Seoul, Korea; Kyeong Keun Oh, Dankook University, Choongnam, Korea

Poster 1B-22. **Withdrawn.**

Poster 1B-23. **Small-Scale Cellulose Conversion Assay for the Evaluation of Cellulase Mixtures.** *Bradley Kelemen,* Bob Caldwell, Bill Cuevas, David Elgart, Vicky Huynh, Edmundo Larenas, Colin Mitchinson, Genencor International, Palo Alto, CA

Poster 1B-24. **Production of Enzyme Feed Additive from *Aspergillus oryzae*.** *Liu Cheng-Geng,* Yang Jan-Jun, Zhang Hong-Yan, Chen Wei-Feng, Enzyme Engineering Institute of Shaanxi Academy of Science, Xi'an, China

Poster 1B-25. **Energetics and Conformations of the Hydrated Cel7A Linker Peptide.** *Tauna Rignall,* Colorado School of Mines, Golden, CO; Clare McCabe, Vanderbilt University, Nashville, TN; Michael E. Himmel, National Renewable Energy Laboratory, Golden, CO

Poster 1B-26. **Characterization of the N-Linked Glycosylation Sites of Native and Recombinant *Penicillium funiculosum* Cel7A Cellobiohydrolase.** *William Michener,* Tina Jeoh, Michael E. Himmel, William S. Adney, National Renewable Energy Laboratory, Golden, CO

Poster 1B-27. **Enzyme Immobilization onto Polydimethylsiloxane Using Layer-by-Layer Self-Assembly Techniques.** Jie Wen, James Palmer, Bill Elmore, Louisiana Tech University, Ruston, LA; *Frank Jones,* University of Tennessee at Chattanooga, Chattanooga, TN

Poster 1B-28. **Evaluation of the Transferability of Mutational Effects Between Homologous Glycosyl Hydrolase Family One ß-D-Glucosidases.** *Mursheda Ali,* John O. Baker, Tina Jeoh, Eric Knoshaug, Michael E. Himmel, William S. Adney, National Renewable Energy Laboratory, Golden, CO

Poster 1B-29. **Raw Starch Hydrolyzing α–Amylase from the Newly Isolated *Geobacillus thermodenitrificans* HR010: Production Optimization Using 2^4 and 3^2 Factorial Designs.** *Thaddeus C. Ezeji,* University of Illinois, Urbana, IL; Hubert Bahl, University of Rostock, Rostock, Germany

Poster 1B-30. **Catalytic Performance of Invertase Adsorbed on Anionic Exchange Resin.** Ester Junko Tomotani, *Michele Vitolo,* University of São Paulo, São Paulo-SP, Brazil

Poster 1B-31. **Enzymatic Synthesis of Sorbitan Methacrylate According to Acyl Donors and Its Application.** *Hye-Jin Lee,* Don-Hee Park, Gwi-Taek Jeong, Kyoung-Min Lee, Woo-Tai Lee, Changshin Sunwoo, Chonnam National University, Gwangju, Korea; In-Heung Kim, Daehan Vaccum Co., Seoul, Korea; Hae-Sung Kim, Myongji University, Yongin, Korea

Poster 1B-32. **The Evaluation of Individual Cellulase Performance on Complex Biomass.** *Edmundo Larenas,* Bob Caldwell, Bill Cuevas, David Elgart, Vicky Huynh, Bradley Kelemen, Colin Mitchinson, Genencor International, Palo Alto, CA

Poster 1B-33. **Effect of Surfactants and Polyethylene Glycol on Enzymatic Hydrolysis of Wheat Straw.** *Henning Jørgensen,* Maria Helene Bruun, Royal Veterinary and Agricultural University, Taastrup, Denmark; Johan Börjesson, Folke Tjerneld, Lund University, Lund, Sweden

Poster 1B-34. **Production and Characterization of a New Cyclodextrin Glycosyltransferase of Alkalophylic Bacilli Isolated from Brazilian Soil.** Cristiane Moriwaki, Glauciane de Lara Costa, Rubia Pazzetto, Flávio F. de Moraes, *Gisella M. Zanin,* Márcia Portilho, Graciette Matioli, State University of Maringá, Maringá-PR, Brazil

Poster 1B-35. **Beyond the Barriers: Bioconversion Studies of Transgenic Trees to Ethanol.** *Lori A. Henderson,* Hou-Min Chang, Laigeng Li, Ying-Hsuan Sun, Vincent Chiang, North Carolina State University, Raleigh, NC; Kevin Wenger, Novozymes, Raleigh, NC

Poster 1B-36. **Enzymatic Saccharification of Pretreated Corn Fiber for Production of Sugars.** *Bruce S. Dien,* Xin-Liang Li, Michael A. Cotta, National Center for Agricultural Utilization Research, USDA-Agricultural Research Service, Peoria, IL

Poster 1B-37. **Inhibition of Cellulase, Xylanase and ß-Glucosidase Activities by Lignin Fractions Derived from Softwood.** *Alex Berlin,* Neil Gilkes, John Kadla, Vera Maximenko, Douglas Kilburn, Jack Saddler, University of British Columbia, Vancouver, British Columbia, Canada; Mikhail Balakshin, North Carolina State University, Raleigh, NC; Satoshi Kubo, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan

Poster 1B-38. **Probing the Role of N-linked Glycosylation in Stability and Activity of Cellobiohydrolases by Mutational Analysis.** *Tina Jeoh,* Yat-Chen Chou, John O. Baker, William Michener, Michael E. Himmel, William S. Adney, National Renewable Energy Laboratory, Golden, CO

Poster 1B-39. **Withdrawn.**

Poster 1B-40. **Production of LiP and MnP and Use of Cell-Free Culture Broth of *Phanerochaete chrysosporium* for Biodegradation of Phenolic Compounds.** Sue Hyung Choi, Hwa Young Lee, Seung-Hyeon Moon, *Man Bock Gu*, National Research Laboratory on Environmental Biotechnology, Gwangju Institute of Science and Technology Gwangju, Korea

Poster 1B-41. **Cloning and Characterization of Biomass Degrading Enzymes.** *Charles C. Lee,* Rena E. Accinelli, Sarah B. Batt, Tina G. Williams, Dominic W.S. Wong, Kurt Wagschal, George H. Robertson, WRRC, USDA-Agricultural Research Service, Albany, CA

Poster 1B-42. **Simultaneous Liquefaction and Saccharification of Grain Mash: A Modified Process for More Effective Enzyme Utilization.** *Bradley A. Saville,* Chunbei Huang, University of Toronto, Toronto, Ontario, Canada

Poster 1B-43. **Synthesis of the Enzyme Cyclodextringlucosyltransferase by *Bacillus firmus* for the Production of ß-CD in Presence of Different Starch Concentration and Sources**. Monique B.A. Marques, Daniel T. Vareschini, José E. Olivo, Flávio F. de Moraes, *Gisella M. Zanin,* State University of Maringá, Maringá-PR, Brazil

Poster 1B-44. **Enzymatic Hydrolysis of Water-Soluble and Insoluble Wheat Arabinoxylan.** *Hanne R. Sørensen,* Anne S. Meyer, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark; Sven Pedersen, Novozymes A/S, Bagsvaerd, Denmark

Poster 1B-45. **Cloning and Characterization of Thermostable Esterase from *Archaeoglobus fulgidus* DSM4304.** *Sang-Young Yoon,* Seung-Bum Kim, Yun-Jung Kim, Yong-Sung Kim, Yeon-Woo Ryu, Ajou University, Suwon, South Korea

Poster 1B-46. **Enzymatic Digestibility of Lignin-Blocked Substrates.** *Bin Yang*, Deidre M. Willies, Alvin O. Converse, Charles E. Wyman, Dartmouth College, Hanover, NH

Poster 1B-47. **Lactose Hydrolysis and Formation of Galactooligosaccharides by a Novel Intracellular ß-Galactosidase from *Talaromyces thermophilus*.** *Phimchanok Nakkharat,* Silpakorn University, Nakorn Pathom, Thailand; Dietmar Haltrich, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Poster 1B-48. **Evaluation of Cell Recycle of *Pichia pastoris* GS115 on Fed-Batch Xylanase Production.** Verônica Ferreira, Patricia Cláudio Nolasco, Aline Machado de Castro, Mônica Caramez Triches Damaso, *Nei Pereira Jr.,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-49. **Cellulase and Lignin-Blocking Protein Adsorption on Cellulosic Substrates.** *Deidre M. Willies,* Bin Yang, Charles E. Wyman, Dartmouth College, Hanover, NH

Poster 1B-50. **Production of Cellulases and Hemicellulases from Agricultural Wastes by *Aureobasidium pullulans* on Solid State Fermentation.** Rodrigo Simoes Ribeiro Leite, Eleni Gomes, *Roberto da Silva,* Universidade Estadual Paulista, São José do Rio Preto-SP, Brazil

Poster 1B-51. **Activities Determination and Stability of Peroxidase and Polyphenol Oxidase Obtained from Maté Tea Leaves (*Ilex paraguariensis*).** *Giovana C. Ceni,* Eliana M. Baldissera, Cláudio Dariva, Débora de Oliveira, URI-Campus de Erechim, Erechim-RS, Brazil; E. Guilhermo O. Arbazua, Octávio A. C. Antunes, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-52. **Obtaining Chelating Agents through Enzymatic Oxidation of Lignin by the Polyphenoloxidase.** *Adilson R. Gonçalves,* Gabriela M.M. Calabria, DEBIQ - FAENQUIL, Lorena-SP, Brazil

Poster 1B-53. **Reuse of the Enzyme Xylanase in the Biobleaching Process of Sugarcane Bagasse Acetosolv Pulp.** *Luís R.M. Oliveira,* Regina Y. Moriya, Adilson R. Gonçalves, DEBIQ - FAENQUIL, Lorena-SP, Brazil

Poster 1B-54. **Characterization of a Thermophilic Lipase Produced by *Penicillium simplicissimum* in Solid-State Fermentation.** Melissa L. E. Gutarra, *Denise M. G. Freire,* Leda R. Castilho, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Francisco Maugeri Filho, Maria Isabel Rodrigues, Campinas State University, Campinas-SP, Brazil

Poster 1B-55. ***Ab initio* Study of the Interaction Mechanisms Between Aromatic Amino Acids and Cellulose.** *Xianghong Qian*, Rx-Innovation, Inc., Fort Collins, CO; Mark R. Nimlos, Michael E. Himmel, National Renewable Energy Laboratory, Golden, CO

Poster 1B-56. **Adaptation of a Foam Fractionation Column for ß-Glucosidase Purification Using a Crude Cellulase Solution.** *Oscar García-Kirchner,* Patricia Rodríguez-Pascual, Rodrigo Martinez-Zuñiga, Carlos Orozco-Álvarez, UPIBI/IPN, Mexico, Distrito Federal, Mexico

Poster 1B-57. **Purification and Characterization of Two Thermostable Xylanases From Alcalophilic *Bacillus licheniformis* 77-2.** Valquiria B. Damiano, Richard Ward, Eleni Gomes, *Roberto da Silva,* Universidade Estadual Paulista, São José do Rio Preto-SP, Brazil

Poster 1B-58. **Conversion of Di- and Tri-Methoxybenzyl Alcohols by Laccases and Peroxidases.** *Feng Hong,* Donghua University, Shanghai, China; Leif J. Jönsson, Karlstad University, Karlstad, Sweden; Knut Lundquist, Yijun Wei, Chalmers University, Göteborg, Sweden

Poster 1B-59. **Heterologous Expression of *Trametes versicolor* Laccase in *Pichia pastoris* and *Aspergillus niger*.** *Christina Bohlin,* Leif J. Jönsson, Karlstad University, Karlstad, Sweden; Robyn Roth, W. H. (Emile) van Zyl, University of Stellenbosch, Matieland, South Africa

Poster 1B-60. **Kinetic Investigation of Cellulase Enzyme Using Non-Crystalline Cellulose and Cello-Oligosaccharides.** *Suma Peri,* Y. Y. Lee, Auburn University, Auburn, AL

Poster 1B-61. **Rapid Measurement of Cellulase Activity Using Non-Crystalline Cellulose.** *Hatem Harraz,* Y. Y. Lee, Auburn University, Auburn, AL

Poster 1B-62. **Peroxidase Production and Application in the Isosafrole Biotransformation into Piperonal.** Joyce L. Andrade, Mariana S. Lemos, Alexandre S. Santos, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Alberdan S. Santos, Federal University of Para, Belem, Para, Brazil; Octavio A.C. Antunes, *Nei Pereira Jr.,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-63. **Production and Characterization of Thermo- and pH-Stable Cellulase from *Streptomyces sp* M23.** Karin Willquist, University of Lund; Lund, Sweden; Ayla Sant'Ana, Gilberto Domont, Rosalie R. R. Coelho, *Elba P.S. Bon,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-64. **Immobilization of *Yarrowia lipolytica* Lipase by Adsorption on Hydrophobic Support.** Aline Gomes Cunha, Cintia da Silva Lima, Lucia M. Campos Paiva, *Denise M.G. Freire,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Juliana Vaz Bevilaqua, Lidia M. de Melo Santa Anna, Petrobras Reserch and Development Center, Brazil; Jaqueline Destain, Universite de Liege, Liege, Belgium

Poster 1B-65. **Characterization of Hemicellulose Degrading Enzymes Using Natural Substrates.** *Kurt Wagschal,* Diana Franqui-Espiet, Charles C. Lee, George H. Robertson, Dominic W.S. Wong, WRRC, USDA-Agricultural Research Service, Albany, CA

Poster 1B-66. **Docking Studies of Lipase Hydrolysis Applied to a New Prototype Anti-Asthma Drug.** *Rodrigo Volcan Almeida,* Juliana Vaz Bevilaqua, Emanuel G. Amarante, Lidia M. Lima, Eliezer J. Barreiro, Aline G. Cunha, Lucia M.C. Paiva, Denise M.G. Freire, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 1B-67. **Thermoacidophilic Cellulases and Hemicellulases from *Alicyclobacillus acidocaldarius*.** *Vicki Thompson,* David Thompson, Kastli Schaller, William Apel, Idaho National Laboratory, Idaho Falls, ID

Poster 1B-68. **Evaluation of Solid and Submerged Fermentations for the Production of Cyclodextrin Glycosyltransferase by Thermophilic Bacterium H69-3: Charactization of Crude Enzyme.** *Heloiza F. Alves-Prado*, Roberto da Silva, Eleni Gomes, Universidade Estadual Paulista, São José do Rio Preto-SP, Brazil

Poster 1B-69. **Cellulose Hydrolysis by *Penicillium echinulatum* Cellulases.** Leonardo F. Martins, *Luiz P. Ramos,* Federal University of Paraná, Curitiba, Parana, Brazil; Marli Camassola, Aldo José P. Dillon, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil

Poster 1B-70. **Properties and Performance of Glucoamylases for Fuel Ethanol Production.** *Bradley A. Saville,* Chunbei Huang, University of Toronto, Toronto, Ontario, Canada

Poster 1B-71. **Purification and Characterization of a Novel Cyclodextrin Glycosyltransferase from Thermophilic Bacterium H69-3.** *Heloiza F. Alves-Prado,* Roberto da Silva, Eleni Gomes, Universidade Estadual Paulista, São José do Rio Preto-SP, Brazil

Today's Biorefineries

Poster 2-07. **Optimization of Biodiesel Production from Castor Oil.** N. Silva Lima, *Maria R. Wolf Maciel,* Cesar B. Batistella, State University of Campinas, Campinas-SP, Brazil

Poster 2-08. **Liquefaction of Agricultural Residues to Biopolyols at Atmospheric Pressure and Low Temperature.** Lingyun Liang, Zhihuai Mao, China Agricultural University, Beijing, China; *Yebo Li,* North Carolina A&T State University, Greensboro, NC

Poster 2-09. **Fermentative Hydrogen Production from Animal Manure.** *Zhiyou Wen,* Shulin Chen, Washington State University, Pullman, WA

Poster 2-10. **Production of Acetone Butanol from Corn Fiber Xylan Using *Clostridium beijerinckii* P260.** *N. Qureshi,* X.L. Li, B.C. Saha, Michael A. Cotta, National Center for Agricultural Utilization Research, USDA-Agricultural Research Service, Peoria, IL

Poster 2-11. **Process Modeling and Economic Analysis of MBI Biorefinery.** *Srinivasan Rajagopalan,* Darold McCalla, Mark D. Stowers, MBI International, Lansing, MI

Poster 2-12. **Techno-Economic Assessment of Hemicellulose Extraction of Wood Chips for Ethanol Production.** William S. Adney, Richard T. Elander, *John L. Jechura,* National Renewable Energy Laboratory, Golden, CO

Poster 2-13. **Extraction of Hyperoside and Quercitrin from Mimosa (*Albizia juribrissin*) Foliage.** A.K. Ekenseair, L. Duan, D.J. Carrier, *E.C. Clausen,* University of Arkansas, Fayetteville, AR

Poster 2-14. **Evaluation of Soybean Hulls as a Source of Ethanol.** *Jonathan R. Mielenz,* John S. Bardsley, Charles E. Wyman, Dartmouth College, Hanover, NH

Poster 2-15. **Development of New Antifoam Technologies for Fermentation Processes.** *Ariane Etoc,* Dow Corning, Seneffe, Belgium; Frank Delvigne, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium; Jean-Paul Lecomte, Dow Corning, Seneffe, Belgium; Philippe Thonart, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium

Bioprocess R&D

Poster 6-08. **Grape Pomace as a Source of Fuels and Chemicals.** G.J. Johnson, T.A. House, W.R. Cook, D.J. Carrier, *E.C. Clausen,* University of Arkansas, Fayetteville, AR

Poster 6-09. **Concentrating Egg Albumin Solution to Solid Using Foam Fractionation.** *Thomas P. Niedringhaus,* Vorakan Burapatana, Robert D. Tanner, Vanderbilt University, Nashville, TN

Poster 6-10. **Simultaneous Saccharification and Fermentation of Steam-Pretreated Spruce Using Yeast Cultivated on the Pretreatment Liquid.** *Andreas Rudolf,* Bärbel Hahn-Hägerdal, Mats Galbe, Gunnar Lidén, Lund University, Lund, Sweden.

Poster 6-11. **Supercritical Extraction Process for Pro-Vitamin A Recovery: Simulation and Optimization.** *E.B. Moraes,* M.E.Torres Alvarez, M.R.Wolf Maciel, State University of Campinas, Campinas-SP, Brazil

Poster 6-12. **An Evolutionary Training Method for Hybrid Models of Fermentation Processes.** *Elmer Ccopa Rivera,* Aline C. da Costa, Rubens Maciel Filho, State University of Campinas, Campinas-SP, Brazil

Poster 6-13. **Simultaneous Saccharification and Fermentation of Steam Pretreated *Salix* at High Consistency in Order to Increase the Ethanol Concentration.** *Per Sassner,* Mats Galbe, Guido Zacchi, Lund University, Lund, Sweden

Poster 6-14. **Optimization of Lactic Acid Production from Cheese Whey Using *Lactobacillus helveticus* under Batch Conditions.** *Marcelo T. Leite,* Raquel M. Santos, Eloízio J. Ribeiro, Marcos A.S. Barrozo, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

Poster 6-15. **Operation of a Percolation Column Apparatus for Oxidative Lime Pretreatment and Solid State Conversion of Chipped Yard Waste to Carboxylic Acids.** *Reddy Adapala,* G. Peter van Walsum, Baylor University, Waco, TX

Poster 6-16. **On-Site Lime Pretreatment and Conversion of Dairy Manure to Mixed Acids for the Production of Chemical Feedstocks via the Mix-Alco Process.** *Michael Flatt,* G. Peter van Walsum, Baylor University, Waco, TX

Poster 6-17. **Closure of the Phosphate Balance in the Conversion of Lime-Pretreated Dairy Manure to Mixed Acids.** *Erin Doyle,* G. Peter van Walsum, Baylor University, Waco, TX

Poster 6-18. **Direct Capture Immobilized Separator: A Novel Approach to Separations.** *Paula Moon,* Carl Landahl, Steven McConnell, Argonne National Laboratory, Argonne, IL; Henry Kolesinski, Robert Cooley, Prime Separations, Inc., Lowell, MA

Poster 6-19. **Lactic Acid Recovery from Cheese Whey Fermentation Broth Using Membrane System.** *Yebo Li,* Abolghasem Shahbazi, Seku Coulibaly, North Carolina A&T State University, Greensboro, NC

Poster 6-20. **Inulin Containing Biomass for Bioethanol Production: Fructose Extraction Methods and Fermentation Assays.** M. José Negro, Ignacio Ballesteros, Paloma Manzanares, J. Miguel Oliva, Felicia Sáez, *Mercedes Ballesteros,* CIEMAT, Madrid, Spain

Poster 6-21. **Butanol Extraction from Fermentation Broth: Mathematical Equations.** *Patrick M. Karcher,* Thaddeus C. Ezeji, Hans P. Blaschek, University of Illinois, Urbana, IL; Nasib Qureshi, National Center for Agricultural Utilization Research, USDA-Agricultural Research Service, Peoria, IL

Poster 6-22. **Development of Functional Link Hybrid Neural Model for an Alcoholic Fermentation Process.** Ivana C.C. Mantovaneli, Aline C. da Costa, *Rubens Maciel Filho,* State University of Campinas, Campinas-SP, Brazil

Poster 6-23. **Kinetic Analysis of Growth and Xanthan Production with *Xanthomonas campestris* TISTR 1100 in Coconut Water.** *Sasithorn Kongruang,* Sumontip Kontun, King Mongkut's Institute of Technology, Bangkok, Thailand

Poster 6-24. **Influence of the Concentration of Sucrose and Temperature in Alcoholic Fermentation.** *Eloízio Júlio Ribeiro, Gustavo P. Ribeiro,* Kelcilene Cristina Lucas, Flávia Silvério Amaral, Carlos Henrique Barbosa Beraldo, Valéria Viana Murata, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

Poster 6-25. **Integrated Process for Microbial Formation of Poly (ß-Hydroxyalkanoates) from Activated Sludges with Enhancement of Wastewater Treatment.** Lam Wai, H. Chua, W. H. Lo, *Peter H. Yu,* Hong Kong Polytechnic University, Hong Kong, China

Poster 6-26. **Cloning and Expressing the PHA Synthase Gene *phaC1* and *phaC1AB* into *Bacillus subtilis*.** Yujie Wang, Lifang Ruan, Qun Yan, H. Chua, W. H. Lo, *Peter H. Yu,* Hong Kong Polytechnic University, Hong Kong, China

Poster 6-27. **Effect of Ca^{2+}, Mg^{2+} and Trace Heavy Metal Ions on PHB-Producing Bacteria.** K. W. Lo, H. Chua, W. H. Lo, *Peter H. Yu*, Hong Kong Polytechnic University, Hong Kong, China

Poster 6-28. **Enhanced Production of mcl-Polyhydroxyalkanoates by *Pseudomonas aeruginosa*.** P. L. Chan, H. Chua, W. H. Lo, *Peter H. Yu*, Hong Kong Polytechnic University, Hong Kong, China

Poster 6-29. **Evaluation of Experimental Systems for Xylitol Separation.** Tihany Morita Antero dos Santos, Francisco Maugeri Filho, State University of Campinas, Campinas-SP, Brazil; *Silvio Silvério da Silva,* School of Chemical Engineering of Lorena, Lorena-SP, Brazil

Poster 6-30. **Scale-Up of Two-Stage Biofilter System for Removal of Odorous Compounds.** Gwang Yeon Lee, Gwi-Taek Jeong, *Don-Hee Park,* Chonnam National University, Gwangju, Korea; Young Seon Jang, Jin Myeong Cha, Sung Rock Joung, B&E Tech. Co. Ltd., Gwangju, Korea

Poster Presentations

Poster 6-31. **Simultaneous Removal of SO_2 and NO_x Using Aqueous Homogeneous Catalyst**. Si Eun An, Kyung Hun Jung, In Hwa Lee, Chosun University, Gwangju, Korea; Si Hyung Kang, Young Seon Jang, *Jin Myeong Cha,* B&E Tech. Co Ltd., Gwangju, Korea

Poster 6-32. **Macroscopic Mass and Energy Balance of a Pilot Plant Anaerobic Bioreactor Operated Under Thermophilic Conditions.** *Teodoro Espinosa-Solares,* Autonomous University of Chapingo, Chapingo, Mexico; John Bombardiere, Mark Chatfield, Max Domaschko, Michael Easter, David A. Stafford, West Virginia State University, Institute, WV; Nehemias Castellanos-Hernandez, Saul Castillo-Angeles, Autonomous University of Chapingo, Chapingo, Mexico

Poster 6-33. **Use of Granular Starch Hydrolyzing Enzymes for Low Energy Grain Ethanol Production.** Nigel Dunn-Coleman, Suzanne Lantz, Michael Pepsin, Tim Dodge, Jay K. Shetty, Genencor International, Inc., Palo Alto, CA; O.J. Lantero, James Miers, *Craig Pilgrim,* Genencor International Inc., Beloit, WI

Poster 6-34. **Ethyl Alcohol Production Optimization by Coupling Genetic Algorithm and Multilayer Perceptron Neural Network.** *Elmer Ccopa Rivera,* Aline C. da Costa, Rubens Maciel Filho, State University of Campinas, Campinas-SP, Brazil

Poster 6-35. **Studies on Glucose and Fructose Adsorption by Activated Carbon.** *Renata M. R. G. Almeida,* Pollyanna S. Oliveira, Gustavo T. S. Gonzaga, Mirelle M. S. Cabral, Federal University of Alagoas, Maceió, Alagoas, Brazil

Poster 6-36. **Application of RAPD Technique for Microorganism Screening in the Bioconversion of Limonene.** Geciane Toniazzo, Federal University of de Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Lindomar Lerin, *Francine Padilha,* Cláudio Dariva, Rogerio Cansian, Débora de Oliveira, URI-Campus de Erechim, Erechim-RS, Brazil; Enrique G.O. Abarzua, Octavio A.C. Antunes, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 6-37. **Assessment of the Influence of Process Variables on the Biotransformation of (+)-Limonene by *Penicillium digitatum* ATCC 26821.** Geciane Toniazzo, Federal University of de Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Elisa F.B. de Camargo, Josiane Brock, Cláudio Dariva, *Débora de Oliveira,* URI-Campus de Erechim, Erechim-RS, Brazil; Enrique G.O. Abarzua, Octavio A.C. Antunes, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 6-38. **Production and Rheological Characterization of Biopolymer of *Sphingomonas capsulata* ATCC 14666, Using Conventional and Industrial Media.** Ana Luisa da Silva Berwanger, Natalia Molossi Domingues, Larissa Tonial Vanzo, URI—Campus de Erechim, Erechim-RS, Brazil; Adilma Regina, Pippa Scamparini, State University of Campinas, Campinas-SP, Brazil; Helen Treichel, *Francine Ferreira Padilha,* URI—Campus de Erechim, Erechim-RS, Brazil

Poster 6-39. **Biosurfactant Production by a Strain of *Pseudomonas aeruginosa* in a Bioreactor Coupled to a Membrane Oxygenator.** *Frederico A. Kronemberger,* Cristiano Piaseck Borges, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil; Lidia M. Santa Anna, Petrobas, Brazil; Valeria F. Soares, Reginaldo Ramos Menezes, Denise M.G. Freire, Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 6-40. **Influence of the Concentration of Sacarosis and Temperature in Alcoholic Fermentation.** *Eloízio Júlio Ribeiro, Gustavo Paiva Ribeiro,* Kelcilene Cristina Lucas, Flávia Silvério Amaral, Carlos Henrique Barbosa Beraldo, Valéria Viana Murata, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

Poster 6-41. **Anaerobic Degradation of Horticultural Wastes in Batch Process.** *Fábian Robles-Martinez,* Claudia Sanchez-Valle, Enrique Durán-Páramo, Unidad Profesional Interdisciplinaria de Biotecnología del IPN, Mexico, Distrito Federal, Mexico

Poster 6-42. **Affinity Foam Fractionation for Selective Separation of *Trichoderma* Cellulase.** *Qin Zhang,* Chi-Ming Lo, Lu-Kwang Ju, University of Akron, Akron, OH

Poster 6-43. **Coupling Foam Fractionation with Fermentation for *Trichoderma* Cellulase Production.** *Lu-Kwang Ju,* Chi-Ming Lo, Qin Zhang, University of Akron, Akron, OH

Poster 6-44. **Syngas Fermentation as a Route to Hydrogen and PHA from Biomass.** *Alan DiSpirito, Robert C. Brown,* Theodore J. Heindel, Basil Nikolau, Iowa State University, Ames, IA

Poster 6-45. **Industrial Biomaterials and Fuels from Lignocellulosic Biomass: Development Initiatives in Australia and New Zealand.** *Sean D. Simpson,* Richard Forster, Agri/Genesis, Auckland, New Zealand; Les A. Eyde, Bill Doherty, Sugar Research Institute, Auckland, New Zealand

Poster 6-46. **Acetate Removal from the Hydrolyzate of Lignocelluloses by Methanogens: The Feasibility Study.** *Chuanbin Liu,* Bo Hu, Shulin Chen, Washington State University, Pullman, WA

Poster 6-47. **Inulinase Production by *Kluyveromyces marxianus* NRRL Y-7571 Using Solid State Fermentation.** João Paulo Bender, Márcio Antônio Mazutti, Débora de Oliveira, Helen Treichel, *Marco Di Luccio,* URI-Campus de Erechim, Erechim-RS, Brazil

Poster 6-48. ***In situ* Batch Extractive Fermentation Using *Clostridium beijerinckii* BA101: Scale-Up and Mixing.** *Patrick M. Karcher,* Hans P. Blaschek, University of Illinois, Urbana, IL

Poster 6-49. **Experimental Development, Instrumentation and Control of an Extractive Fermentation Process to Ethanol Production.** Daniel I.P. Atala, *Francisco Maugeri Filho,* UNICAMP, Campinas-SP, Brazil

Poster 6-50. **The Effect of Varying Structured Catalyst Packing on Conversion in Microchannel-Based Bioreactors.** *Frank Jones,* Chris Martin, Robert Bailey, James Hiestand, University of Tennessee at Chattanooga, Chattanooga, TN

Poster 6-51. **Reactions Involved in Raw Sewage Sludge Combustion.** *Hong Cui,* Yan Cao, Wei-Ping Pan, Western Kentucky University, Bowling Green, KY; Y. Ninomiya, Chubu University, Aichi, Japan

Poster 6-52. **Process Improvements for Corn Ethanol Production.** *M. Clark Dale,* Bio-Process Innovations, West Lafayette, IN

Poster 6-53. **Biomass Gasification and Progress Towards Commercial Application.** *Darren D. Schmidt,* University of North Dakota, Grand Forks, ND

Poster 6-54. **Hybrid Neural Modeling of Batch Alcoholic Fermentation.** Elver Radke, *Aline C. Costa,* Rubens Maciel Filho, University of Campinas, Campinas-SP, Brazil

Poster 6-55. **Spreadsheet Tools for Determination of Derivatives and Microbial Kinetics**. *K. Thomas Klasson,* Southern Regional Research Center, USDA-Agricultural Research Service, New Orleans, LA

Poster 6-56. **Biotechnological Production of Xylitol from Sugar Cane Bagasse: Effect of Glucose on Xylose Reductase and Xylitol Dehydrogenase Activities.** Débora Danielle V. Silva, Priscila V. Arruda, Rita de Cássia L.B. Rodrigues, *Maria das Graças de A. Felipe,* Silvio Silvério da Silva, Faculty of Chemical Engineering of Lorena, Lorena-SP, Brazil

Poster 6-57. **Butanol Extraction from Fermentation Broth: Mathematical Equations.** *Patrick M. Karcher,* Thaddeus C. Ezeji, Hans P. Blaschek, University of Illinois, Urbana, IL; Nasib Qureshi, National Center for Agricultural Utilization Research, USDA-Agricultural Research Service, Peoria, IL

Poster 6-58. **Production of Lactate Ester by Extractive Fermentation and Enzymatic Esterification.** Hanjing Huang, *Shang-Tian Yang,* Ohio State University, Columbus, OH

Poster 6-59. **Mathematical Modeling of Cell-Immobilized Bioreactors for Alcohol Production.** Márcio de A. Batista, Gustavo Ribeiro, Eloízio J. Ribeiro, *Luís C. Oliveira-Lopes,* Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

Poster 6-60. **Polysaccharide Production by *Haemophilus influenzae* Type B**. Maria do Carmo Medeiros Gonçalves, Oak Ridge National Laboratory, Oak Ridge, TN; Ellen Jessouron, Argonne National Laboratory, Argonne, IL; *Eliana Flavia Camporese Sérvulo,* Pacific Northwest National Laboratory, Richland, WA

Poster 6-61. **Combined Effects of Acetic Acid, Formic Acid and Hydroquinone on *Debaryomyces hansenii* Physiology**. Luís C. Duarte, *Florbela Carvalheiro,* Joana Tadeu, Francisco M. Gírio, INETI, Lisboa, Portugal

Poster 6-62. **Dynamic Modeling of a Fermentation Process for Ethanol Production by Deterministic and Hybrid Models.** *Vera Lúcia Reis de Gouvêia,* Rubens Maciel Filho, State University of Campinas, Campinas-SP, Brazil

Poster 6-63. **Use of Different Adsorbents for Sorption and *Bacillus polymixa* Protease Immobilization.** Irem Kirkkopru, Cenk Alpaslan, *Didem Omay,* Yüksel Güvenilir, Istanbul Technical University, Istanbul, Turkey

Poster 6-64. **Effect of Different Parameters on the Enrichment of DHA by Enzymatic Hydrolysis from Cod Liver Oil.** Ozlem Sirin, Tuba Yasar, *Didem Omay,* Yüksel Güvenilir, Istanbul Technical University, Istanbul, Turkey

Poster 6-65. **Hydrogen Generation from Sugars via Aqueous-Phase Reforming**. *Randy D. Cortright*, Virent Energy Systems, Madison, WI

Poster 6-66. **Countercurrent Fermentation and Continuum Particle Distribution Modeling of Rice Straw in the MixAlco Process.** *Frank K. Agbogbo,* Mark T. Holtzapple, Texas A&M University, College Station, TX

Poster 6-67. **Molecular Distillation: A Powerful Technology for Obtaining Tocopherols from Soya Sludge.** E. B. Moraes, P. F. Martins, C. B. Batistella, M. E. Torres Alvarez, *M. R. Wolf Maciel,* State University of Campinas, Campinas-SP, Brazil

Poster 6-68. **Evaluation of the Chromatographic Column Method for Xylitol Recovery Obtained by Fermentative Pathway.** Walter C. Ricardo Filho, Solange I. Mussatto, Júlio C. Santos, *Silvio S. Silva,* Faculdade de Engenharia Química de Lorena, Lorena-SP, Brazil

Monday Evening, May 2, 2005 6:45 - 9:30 pm

Chair: Mildred Zuccarello
National Renewable Energy Laboratory

All posters should be up by Sunday afternoon, May 1, 2005. Presenters should be near their respective posters as below:

Sessions: 3A, 3B, 4, 5 Monday 6:45 pm - 9:30 pm

Plant Biotechnology and Feedstock Genomics

Poster 3A-08. **Variation of S/G Ratio and Lignin Content in a Populus Family Influences the Release of Fermentable Sugars by Dilute Acid Hydrolysis.** *Brian H. Davison,* Sadie R. Drescher, Gerald A. Tuskan, Oak Ridge National Laboratory, Oak Ridge, TN; Mark F. Davis, National Renewable Energy Laboratory, Golden, CO; Nhuan P. Nghiem, Martek Biosciences Corporation, Winchester, KY

Poster 3A-09. **Enhanced Secondary Metabolite Production by Elicitation in Transformed Plant Root System**. *Gwi-Taek Jeong,* Don-Hee Park, Hwa-Won Ryu, Baik Hwang, Chonnam National University, Gwangju, Korea; Je-Chang Woo, Mokpo National University, Chonnam, Korea

Poster 3A-10. **Identification and Characterization of Maize Mutants with Potential Use as Green Chemical Feedstocks.** *Javier Campos,* Angela Valadez, Nick Carpita, Purdue University, West Lafayette, IN; Mark F. Davis, Steven Thomas, National Renewable Energy Laboratory, Golden, CO; Wilfred Vermerris, Purdue University, West Lafayette, IN

Poster 3A-11. **Recombinant Cellulase Expression in the Chloroplast of Tobacco Plants.** *Ginette Turcotte,* Ryerson University, Toronto, Ontario, Canada; Hyeun-Jong Bae, Chonnam National University, Gwangju, Korea; Serge Laberge, Agriculture and Agri-Food Canada, Ste-Foy, Canada

Poster 3A-12. **Direct Observation of the Maize Primary Cell Wall Using Atomic Force Microscopy.** *Shi-You Ding,* Michael E. Himmel, National Renewable Energy Laboratory, Golden, CO

Biomass Pretreatment and Hydrolysis

Poster 3B-08. **Simultaneous Saccharification and Fermentation of Steam-Pretreated Corn Stover at High Dry-Matter Concentration for Fuel Ethanol Production.** *Karin Öhgren,* Mats Galbe, Guido Zacchi, Lund University, Lund, Sweden

Poster 3B-09. **AFEX Pretreatment of Corn Fiber-Ethanol Fermentation and Animal Feed Analysis of Residue.** Alaina Hoffman, Michael V. Guettler, Tonya Tiedje, *Srinivasan Rajagopalan,* Darold McCalla, Mark Stowers, MBI International, Lansing, MI

Poster 3B-10. **The Dilute Acid Pretreatment of Corn Stover Anatomical Fractions.** *David K. Johnson,* Claudia Ishizawa, David Templeton, Steven Thomas, National Renewable Energy Laboratory, Golden, CO

Poster 3B-11. **Porosity Measurements on Dilute Acid Pretreated Corn Stover.** *Claudia Ishizawa,* Mark Davis, David K. Johnson, National Renewable Energy Laboratory, Golden, CO

Poster 3B-12. **Pretreatment of Waste Papers for Citric Acid Fermentation by *Aspergillus niger.*** *Ramida Watanapokasin,* Sirinun Nilwarangkoon, Srinakharinwirot University, Bangkok, Thailand; Nitisak Sawasjirakij, North Bangkok College, Bangkok, Thailand

Poster 3B-13. **Kinetic Modeling of Xylo-Oligosaccharides Production from Brewery's Spent Grains Autohydrolysis**. *Florbela Carvalheiro,* Luís C. Duarte, Francisco M. Gírio, INETI, Lisboa, Portugal

Poster 3B-14. **High-Solids Enzymatic Saccharification of Cellulose.** *David B. Hodge,* Colorado State University, Fort Collins, CO; M. Nazmul Karim, Texas Tech University, Lubbock, TX; Jody Farmer, Daniel J. Schell, James D. McMillan, National Renewable Energy Laboratory, Golden, CO

Poster 3B-15. **Factors Affecting Scale-Up of High Solids Saccharification from Shake Flasks to Stirred Tank Reactors.** *David B. Hodge,* Colorado State University, Fort Collins, CO; Ali Mohagheghi, John O. Baker, Daniel J. Schell, James D. McMillan, National Renewable Energy Laboratory, Golden, CO

Poster 3B-16. **Bioethanol Production from Ammonia Pretreated Waste Oak Wood by SSF.** *Nam Yee Kim,* Hyunjoon Kim, Jun Seok Kim, Kyonggi University, Suwon, Korea; Jin Suk Lee, Korea Institute of Energy Research, Daejeon, Korea

Poster 3B-17. **Two-Step Pretreatment of Corn Stover by White Rot Fungi and Dilute Sulfuric Acid for Ethanol Production.** Su Donghai, *Sun Junshe,* China Agriculture University, Beijing, China

Poster 3B-18. **Genus Reciprocity of White Rot Fungi on the Pretreatment of Corn Stover in Mixed Culture.** Su Donghai, *Sun Junshe,* China Agriculture University, Beijing, China

Poster 3B-19. **Bioethanol from Cellulose with Supercritical Water Treatment Followed by Enzymatic Hydrolysis.** *Toshiki Nakata,* Shiro Saka, Hisashi Miyafuji, Kyoto University, Kyoto, Japan

Poster 3B-20. **Effect of Pressure on Organic Acid Production from Japanese Beech Treated in Supercritical Water.** *Kei Yoshida,* Katsunobu Ehara, Shiro Saka, Kyoto University, Kyoto, Japan

Poster 3B-21. **Effects of Sulfuric Acid Loading and Residence Time on the Composition of Sugarcane Bagasse Hydrolysate Obtained in a 250-L Batch Reactor and Its Relation with the Xylose-to-Xylitol Bioconversion.** *Walter de Carvalho,* Zuzel R. Matos, Julio C. Santos, Silvio S. Silva, Chemical Engineering College of Lorena, Lorena-SP, Brazil

Poster 3B-22. **Development of a Multi-Stage Fermentation System for Maximizing Solids Concentration and Optimizing Enzyme Loading for Continuous SSF of Pretreated Biomass.** John S. Bardsley, Jonathan R. Mielenz, *Charles E. Wyman,* Dartmouth College, Hanover, NH

Poster 3B-23. **Catalytic Mechanisms of Dilute Acid Depolymerization Hydrolysis of Xylan.** Todd A. Lloyd, *Charles E. Wyman,* Dartmouth College, Hanover, NH

Poster 3B-24. **A Simple and Effective Pretreatment for Biomass Materials.** Jill Burdett, Paresma Patel, *Brian Vande Berg,* Brian Carr, Nicholas Duck, Gregory Lewis, Michael Koziel, Athenix Corporation, Research Triangle Park, NC; James R. Hettenhaus, Chief Executive Assistance, Inc. Charlotte, NC

Poster 3B-25. **Enzymatic Hydrolysis of Corn Stover Pretreated with Dilute Acid: Effect of Pretreatment Conditions on Enzyme-Substrate Interactions.** *Rajeev Kumar,* Charles E. Wyman, Dartmouth College, Hanover, NH

Poster 3B-26. **Enhancement of the Enzymatic Digestibility of Waste Newspaper Using Tween.** *Sung Bae Kim,* Hyun Joo Kim, Chang Joon Kim, Gyeongsang National University, Jinju, Korea.

Poster 3B-27. **Dilute Acid Hydrolysis Pretreatment of Agro-Food Wastes for Bioethanol Production.** Irantzu Alegría, Inés del Campo, Maite Zazpe, Mikel Echeverría, Ibai Funcia, *Inés Echeverría,* CENER, Navarra, Spain

Poster 3B-28. **Steam Stripping as an Unwashed PCS Detoxification Method Proof of Concept.** *Guillermo Coward-Kelly,* Mads Torry Smith, Novozymes North America, Franklinton, NC

Poster 3B-29. **Xylose Fermentation with Non-GMO *Saccharomyces cerevisiae* Utilizing an Extra Cellular Xylose Isomerase.** *Mads Torry Smith,* Guillermo Coward-Kelly, Novozymes North America, Franklinton, NC

Poster 3B-30. **Enzymatic Hydrolysis of Manure Fiber Pretreated by Metal Ions and Hydrogen Peroxide.** *Wei Liao,* Yan Liu, Zhiyou Wen, Shulin Chen, Washington State University, Pullman, WA

Poster 3B-31. **Identifying Relations between Pretreatment Parameters, Enzymatic Hydrolysis, and Inhibition of Thermophilic Xylose Fermentation Using Alkaline Wet Oxidation of Rice Straw.** *Frank D. Haagensen,* Birgitte K. Ahring, University of Denmark, Lyngby, Denmark; Enikö Varga, Anne B. Thomsen, Risoe National Laboratory, Roskilde, Denmark

Poster 3B-32. **Development of a Technology Combining Thermal Hydrolysis, Wet Oxidation, and Steam Explosion for Pretreatment of Lignocellulosic Biomass.** *Frank D. Haagensen,* Thomas Andersen, Birgitte K. Ahring, University of Denmark, Lyngby, Denmark; Jens Munch, Cambi Bioethanol, Copenhagen, Denmark

Poster 3B-33. **Test of Steam Explosion after Dilute Acid Percolation for the Maximum Recovery of Sugars from Agricultural Waste Feedstocks.** *Lee Jun-Pyo,* Lee Jin-Suk, Park Soon-Chul, Korea Institute of Energy Research, Daejeon, Korea; Oh Kyeong-Keun, Dankook University, Cheonan, Korea

Poster 3B-34. **Ethanol Production from Steam-Explosion Pretreated Wheat Straw.** Ignacio Ballesteros, M. José Negro, J. Miguel Oliva, Araceli Cabanñas, *Paloma Manzanares,* Mercedes Ballesteros, CIEMAT, Madrid, Spain

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Poster 5-46. **Improvement of Ethanol Production by *Pichia stipitis* in High-Xylose-Content Liquor Obtained for Sugarcane Bagasse Hydrolyses.** Gabriel B. Vargas, Alexandre S. Santos, *Nei Pereira Jr.,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 5-47. **Evidence for Enzyme-Microbe Synergy in Cellulose Utilization by *Clostridium thermocellum*.** *Yanpin Lu,* Y.-H. Percival Zhang, Lee R. Lynd, Dartmouth College, Hanover, NH

Poster 5-48. **Whole Genome Transcription Analysis of Yeast Stress Response During Very High Gravity Ethanol Fermentation.** *Rasmus Devantier,* Sven Pedersen, Novozymes A/S, Bagsvaerd, Denmark; Lisbeth Olsson, University of Denmark, Lyngby, Denmark

Poster 5-49. **Biosurfactant Production by *Rhodococcus erythropolis* Growing on Glycerol as Carbon Source.** Elisa M. P. Ciapina, Walber C. Melo, Alexandre S. Santos, Denise M. G. Freire, *Nei Pereira Jr.,* Federal University of Rio de Janeiro, Rio de Janeiro-RJ, Brazil

Poster 5-50. **Developing Viable Cereal-Based Biorefineries.** A. A. Koutinas, R. Wang, G. M. Campbell, *C. Webb,* University of Manchester, Manchester, United Kingdom

Poster 5-51. **Metabolic Engineering to Improve Ethanol Production in the Xylose Utilizing Thermophile *Thermoanaerobacterium saccharolyticum* JW/SL-YS485.** *A. Joe Shaw,* Sunil G. Desai, Lee R. Lynd, Dartmouth College, Hanover, NH

Poster 5-52. **Induction of Pleiotropic Drug Resistance (PDR) Gene Expression Indicates Important Roles of PDR to Cope with Furfural and 5-Hydroxymethylfurfural Stress in Ethanologenic Yeast.** *Z. Lewis Liu,* Patricia J. Slininger, National Center for Agricultural Utilization Research, USDA-Agricultural Research Service, Peoria, IL; Brad Andersh, Bradley University, Peoria, IL

Poster 5-53. **Effect of Mixing in Anaerobic Digestor on Conversion of Farm Waste to Methane in 100L Upflow Bioreactor.** *Abhijeet P. Borole,* Oak Ridge National Laboratory, Oak Ridge, TN; K. Thomas Klasson, Southern Regional Research Center, USDA-Agricultural Research Service, New Orleans, LA; Whitney Ridenour, Justin Holland, Oak Ridge Institute for Science and Engineering, Oak Ridge, TN

Poster 5-54. **Removal of Mercury from Coal via Microbial Bioleaching.** *Abhijeet P. Borole,* Catherine K. McKeown, Oak Ridge National Laboratory, Oak Ridge, TN; K. Thomas Klasson, Southern Regional Research Center, USDA-Agricultural Research Service, New Orleans, LA; Choo Y. Hamilton, University of Tennessee, Knoxville, TN

Poster 5-55. **Optimization of Pentose Fermentation in *Zymomonas mobilis* through Kinetic Modeling and Experimental Analysis.** Mete M. Altintas, *Dhinakar S. Kompala,* University of Colorado, Boulder, CO; Chris Eddy, James D. McMillan, Min Zhang, National Renewable Energy Laboratory, Golden, CO

Abstracts for Oral Presentations

ORAL PRESENTATION 1A-01

Agricultural Residue Availability in the United States

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The National Energy Modeling System (NEMS) is used by the Energy Information Administration to forecast U.S. energy production, consumption, and price trends for a 25 year time horizon. Biomass is one of the technologies within NEMS which plays a key role in several scenarios. An endogenously determined biomass supply schedule is used to derive the price-quantity relationship of biomass. There are four components to the NEMS biomass supply schedule including: agricultural residues, energy crops, forestry residues, and urban wood waste/mill residues. The Energy Information Administration's *Annual Energy Outlook 2005* includes updated estimates of the agricultural residue portion of the biomass supply schedule. The changes from previous agricultural residue supply estimates include: revised assumptions concerning corn stover and wheat straw residue availabilities, inclusion of non-corn and non-wheat agricultural residues (such as barley, rice straw, and sugarcane bagasse), and the implementation of assumptions concerning increases in no-till farming. This paper will discuss the impact of these changes on the supply schedule and on forecasts of the growth of biomass for power generation. The biomass forecast under different scenarios will also be discussed.

ORAL PRESENTATION 1A-02

New Technology for Reed Canary Grass Production

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In Finland the target is to increase the use of renewable energy at least by 50 % (34,9 TWh) by the year 2010. In the attempt to reach the targets, forest residue and agrobiomass fuels play a leading role. The target is to increase the use of forest residues up to 10 TWh and the use of agro-biomass up to 0,6 TWh. In Finland the interest to use reed canary grass (RCG) for fuel is increased during the last few years. At the moment the cultivation area of RCG is about 5 000 hectares (0,15 TWh) and it is continuously increasing. RCG is a perennial crop with life cycle of 8 -10 years. Annual yield is about 30 MWh/ha. RCG is cultivated on fallow land but also on cutover peatlands. VTT has developed harvesting and combustion technologies together with RCG producers and users. Both loose and bale harvesting methods have been developed and demonstrated. Bale method is the dominating method at the moment. The main problem with this method is to find effective crushing or chopping equipment. RCG is used to produce heat and electricity at plants planned to use wood and peat as a fuel. The main problems of RCG use is the low energy density (0,3 MWh/m^3) and high alkal metal content which causes corrosion in the boiler. Therefore RCG is used as a mix fuel together with wood and peat. The share of RCG in the fuel is 10 – 15 % of the energy content. At the moment the RCG fuel costs at the power plant are 15 euros per MWh. The harvesting of RCG is subsidised which improves competitiveness for instance compared with peat (11 euros/MWh).

ORAL PRESENTATION 1A-03

Corn Stover Fractions Related to Bioenergy: Chemical Composition and Structure

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Corn stover is a potential lignocellulosic feedstock to expand the production of bioethanol. Information is needed on its structure and composition to optimize pretreatments and cost efficiency, since the raw substrate is recalcitrant to conversion. Samples of corn stover were evaluated for % of plant fractions, aromatics in different fractions, and response to cellulase. Leaf portions were about 60% of standing plants and about 48% of baled stover. Levels of aromatic compounds were greater in the stem fraction, with ester-linked *p*-coumaric acid substantially higher in the stem tissues than other fractions. Leaf blade laminae had the lowest amounts of both *p*-coumaric and ferulic acids. Leaves were about 3 X more susceptible to breakdown by cellulase than stems. Commercial ferulic acid esterase, as a pretreatment before cellulase, produced filtrates with substantially more available sugars and freed phenolic acids as potential byproducts. The information should help develop and optimize strategies for use of corn stover in biofuels, particularly related to the most appropriate plant fractions for bioconversion while leaving the most suitable residues for soil erosion control.

ORAL PRESENTATION 1A-04

The BTL2 Process of Biomass Utilisation Entrained Flow Gasification of Pyrolysed Biomass Slurries

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A high amount of organic residues consists of thin-walled lignocellulosic substances like straw and hay. These have a low energy density due to a bulk density <150 kg/m^3, and the scale of a conversion plant for straw is limited by the costs for transport. The "BiomassToLiquid2" (BTL2) concept of Forschungszentrum Karlsruhe is introduced to overcome this logistic problem: Dry biomass with a high ash content is flash-pyrolysed in a regional plant (first step) and gasified in a central plant (second step). In the regional plant, a slurry of pyrolysis products of wheat straw is mixed, which is more suitable for transport and storage than original straw. Some aspects of this concept, including the feeding of the gasifier, are described by the present paper. The mechanical characteristics of the Process Demonstration Unit (PDU) (15 kg/h) for straw pyrolysis are examined and the particle sizes of the char powder reported. For gasification in the 3 MW pressurised entrained flow gasifier of Future Energy, Freiberg, Germany, 30 t of wood pyrolysis products were mixed and successfully converted into synthesis gas, which is tar-free and can be used for Fischer-Tropsch synthesis after gas cleaning. Products of straw pyrolysis are more difficult to handle due to the higher amount of char and its higher porosity. The influence of temperature, porosity, milling, and mixing on the slurries is shown as is the possibility of producing stable and pumpable feeds for gasification. Considerations of sustainability, economy and efficiency of BTL2 are presented.

ORAL PRESENTATION 1A-05

Ethanol Production from Wet-Oxidized Wheat Straw by Different Recombinant *Saccharomyces cerevisiae* Strains

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Agricultural waste materials are an attractive renewable source for bioconversion to ethanol. Current research and development is being directed towards the substitution of the higher-cost sugar feedstocks with the low-cost lignocellulosic biomass. In this perspective wheat straw represents an abundant energy source for bioconversion processes. The efficient bioconversion of lignocellulosic biomass to ethanol calls for some form of pretreatment to break down the lignocellulose into the three major polymeric constituents. Furthermore, this step can introduce a major problem since the produced hydrolysate contains, not only the fermentable sugars, but also a broad range of coumpounds which can be toxic to the microorganism and can affect the enzymes in the hydrolysis step.

In our study we investigated the effect of toxic compounds on the cellulase and hemicellulase components during the hydrolysis of wet oxidized wheat straw and on the ethanolic fermentation by different recombinant *S. cerevisiae* strains. The body of the experimental data indicates that syringaldehyde and, mainly, the acid-inhibiting components of the wet oxidized wheat straw slurry, require some selective removal prior to biological conversion or else a major dilution to ensure a high process productivity.

ORAL PRESENTATION 1A-06

Under What Conditions is Ethanol from Corn Stover Economically Viable?

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This research examines how many ethanol plants would be economically feasible in the Midwest when ethanol is priced at $1.15, $1.25, or $1.35 per gallon, and what size plant (1,000 or 2,000 metric tons per day) should be constructed. Ethanol production process engineering design and economic costs of conversion were obtained from the National Renewable Energy Lab. The Policy Analysis System (POLYSYS) model was used to obtain estimates on available corn stover residues, which were then adjusted to account for environmental concerns. The Oak Ridge Integrated Bioenergy Analysis System (ORIBAS) model was used to obtain estimates on feedstock and transportation costs, which were then used to determine the potential of the industry.

The results of the study indicate that in every state analyzed the construction and operation of an ethanol plant provides substantial estimated economic impacts for total industry output, employment, and value-added. For example, the number of new jobs generated in the construction phase ranges from 1,712 to 2,117 in a plant processing 1,000 MT of corn stover a day. The corresponding number of jobs generated in the annual operation phase ranges from 576 to 910. In the case of an ethanol plant processing 2,000 MT/day, the number of jobs created ranges from 2,820 to 3,489 and from 1,104 to 2,107, respectively.

The study also showed that the number of feasible ethanol plants in each state could vary substantially based on the prices of ethanol and corn stover and plant size. The smaller plant size, 1,000 MT/day of corn stover, is much more sensitive to the prices of ethanol than to the price of the corn stover. While 17 plants are feasible if the ethanol price is at $1.15/gallon and the corn stover is at the breakeven price, 136 plants are feasible if the price of ethanol is $1.35/gallon at a breakeven stover price. With corn stover priced 30% above the breakeven price, the number of feasible plants changes from 0 to 118 as the price of ethanol increases from $1.15 to $1.35/gallon. The economies of size present in the larger plant, 2,000 MT/day, make this plant less sensitive to the changes in feedstock prices as the number of plants ranges from 52 to 72, and from 28 to 67 in the corresponding two price scenarios outlined above for the 1,000 MT/day plant.

ORAL PRESENTATION 1A-07

Modeling Cellulosic Ethanol Production and Distribution in the United States

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Alternative fuels and infrastructure are likely to be important in the future as security and environmental concerns come forward in national priorities. We consider the economic costs of various ethanol fuel blends for transportation scenarios in the United States as a substitute for petroleum-based fuel. The current infrastructure in the US for shipping and refining petroleum-based fuels has been highly optimized over time and contributes a relatively small portion of costs (about 3 cents/gal). Our estimates for various ethanol replacement scenarios yield higher cost (2.5 to 9 cents per gallon of ethanol blend for downstream transportation costs only) but remain a relatively small fraction of total fuel cost. If ethanol is to be a competitive option in the long run, more efficient shipment infrastructure will need to be developed, such as pipelines. Unfortunately when using ethanol in low level blends (e.g. E10 (10% ethanol/90% gasoline)) existing petroleum and product would still be needed for gasoline based portion of the fuel. Building new pipelines to deliver ethanol would be cost-effective in the short run but if ethanol were to replace gasoline in the long run then pipeline overcapacity would exist.

ORAL PRESENTATION 1B-01

Chemical Complementation: A Potentially General Method of Engineering Enzymes Through Genetic Selection

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We have developed a yeast-based three-component system called "chemical complementation" that links the survival of cells to the presence of a specific small molecule. In chemical complementation the small molecule enables yeast to grow by binding a nuclear receptor, triggering expression of an essential gene. The interaction between the small molecule ligand and the receptor provides an effective, potentially general system for genetic selection. We use chemical complementation to engineer new receptors that activate transcription in response to chosen small molecules. Libraries of receptors are created based on structure-guided codon randomization with 10^5 to 10^6 members. Chemical complementation evaluates all the library members in parallel- only yeast harboring functional receptors grow. The engineered receptors should find applications for conditional gene expression in gene therapy and as research tools for target validation. This method also promises a generally applicable method for engineering enzymes through genetic selection and for screening otherwise intractable enzymes. Progress on engineering receptors toward arbitrary small molecules will be presented.

ORAL PRESENTATION 1B-02

Effect of Cellulase Supplementation on Cookline Operation in A Dry Mill Ethanol Plant

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Trials were conducted to assess the benefits of cellulase supplementation on cookline operation. Brix, viscosity, and process measurements were obtained following cellulase addition to the slurry tank and liquefaction tank at 1.5 and 0.1 kg/tonne of substrate, respectively, and compared to measurements obtained during normal operation.

Cellulase supplementation to the slurry tank caused the Brix to increase by approximately 0.5 units in the slurry tank, but there was no statistical difference in Brix for vessels beyond the jet cooker. Cellulase supplementation to the liquefaction tank caused the Brix to increase by approximately 0.7 units. Theoretically, cellulose hydrolysis could produce an additional 0.1 gallons of ethanol per bushel of corn.

Cellulase supplementation produced a statistically significant reduction in mash viscosity. The viscosity in the slurry tank was typically reduced by 10-15%, and by 20 to 30%in the liquefaction tank. Other qualitative process measurements (pump and valve settings) confirmed the reduction in viscosity. Significant savings in steam and energy costs may be realized from this reduction in viscosity. An economics assessment suggests that, even if only half of theoretical ethanol yield increase is obtained, a net financial benefit of ~$350,000 USD/y could be obtained from cellulase supplementation to the liquefaction system of a 20 MM USPGY dry mill operation.

ORAL PRESENTATION 1B-03

Improving Cellulose Hydrolysis with New Cellulase Compositions

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Novozymes has been actively working to reduce the cost of cellulases for the conversion of lignocellulose to fermentable sugars since the 2001 award of a research subcontract from NREL with funds from the U.S. Department of Energy. One of the approaches pursued was to improve the hydrolysis of the target substrate, dilute-acid pretreated corn stover (PCS), by *Trichoderma reesei* cellulases. This presentation will describe our efforts in elucidating the substrate and enzyme characteristics that limit complete saccharification of lignocellulose in PCS. Several methods aimed at overcoming these limitations have been tested and resulted in substantial decrease in the enzyme loading requirement. This talk will summarize the results to date and suggest future research paths towards improving the economy of cellulase-mediated bioethanol production.

ORAL PRESENTATION 1B-04

Production of Granular Starch Hydrolyzing Enzymes for Low Energy Grain Ethanol Production

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Production of fuel ethanol from grain continues to expand at double-digit rates. Use of energy in the process is a major cost with cooking of the grain to aid in enzymatic digestion a significant expense. Recently, novel enzyme systems (granular starch hydrolyzing enzymes, or GSHE) have been developed that are capable of converting granular (i.e., uncooked) starch to fermentable sugars in a simultaneous saccharification fermentation. This presentation will focus on the successful discovery, expression, and properties of these enzymes.

ORAL PRESENTATION 1B-05

A High Throughput Micro-Assay to Evaluate Enzymatic Hydrolysis of Lignocellulosic Substrates from Agricultural and Forest Residues

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Agricultural residues such as corn stover and cereal straw, and softwood or hardwood residues from the forest industry are viewed as potential feedstocks for bioconversion to fuel-grade ethanol and other commodity chemicals. Typically, bioconversion processes use a pretreatment step to disrupt the feedstock, followed by enzymatic hydrolysis of the cellulose and hemicellulose components to provide fermentable sugars. Process development is now focused on the selection and optimization of feedstock pretreatment technologies and more cost-effective enzyme production. Both of these activities involve large-scale screening to optimize pretreatment process parameters and enzyme compositions. However, current assay procedures are time-consuming, labor-intensive, and require substantial quantities of reagents.

Attempts to scale down and automate standard cellulase assay procedures (e.g., the filter-paper assay) have been reported previously. However, it is now widely recognized that such assays do not provide a reliable prediction of enzyme performance on lignocellulose because cellulose hydrolysis is profoundly affected by residual hemicellulose and lignin, factors which are neglected when using model cellulosic substrates. Consequently, we have developed a high thru-put micro-assay using realistic substrates such as pretreated corn stover and hardwood. Good correlation between data obtained with this method and a standard flask-based assay is demonstrated for a wide range of pretreated lignocellulosic materials.

ORAL PRESENTATION 1B-06

Development of Enzyme Cocktails for Feedstock Conversion to Fermentable Sugars

Lishan Zhao, Kelvin Wong, Myoung Kim, Lori Preston, Flash Bartnek, Yoko Philips, Chris Lyon, Uvini Gunawardena, Charles Tweedy, Olen Yoder, Mike Lafferty, and Kevin Gray

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Efficient, economic saccharification of feedstocks is critical to the successful commercialization of biomass conversion to ethanol and/or other products. Funded by DOE and in collaboration with DuPont and National Renewable Energy Laboratory, Diversa is developing cellulase and hemicellulase enzymes capable of effectively converting complex biomass feedstocks to fermentable monomeric sugars. By utilizing our proprietary technologies to discover novel genes from diverse sources, we have discovered several hundred unique enzymes that are involved in biomass degradation. These enzymes belong to many of the glycosyl hydrolase families and have broad pH and temperature optima. Many of these enzymes have potential utility in a biorefinery process. This presentation will cover the discovery of these enzymes, the development of specialized automation technologies to evaluate enzyme activity on insoluble substrates and the performance of various enzyme cocktails on feedstock substrates.

ORAL PRESENTATION 1B-07

Poly(Ethylene Glycol) as Additive for Increased Conversion of Lignocellulose

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Lignocellulose is an attractive substrate for ethanol production. However, high cellulose conversion requires high enzyme loading. We have earlier shown that addition of surfactants increases the conversion of cellulose into soluble sugars by reducing enzyme binding to lignin. Experiments were designed to explore mechanisms of surfactant effects. A number of surfactants were screened for their ability to improve enzymatic hydrolysis of steam pretreated spruce using the cellulase system of *Trichoderma reesei*. Non-ionic surfactants with increasing length of hydrophilic ethylene oxide (EO) chain showed an increase in conversion with EO length. We have shown that with additions of polyethylene oxide polymers (PEG) we could reach similar or even higher effects on lignocellulose conversion as with surfactants. Rate of cellulose hydrolysis was increased and higher degree of conversion was achieved with additions of PEG 4000, e.g. addition of 2.5 g/l of PEG 4000 could increase cellulose conversion from 50 to 78% in 16 h hydrolysis. With PEG addition, hydrolysis of spruce lignocellulose could be performed at temperature of 50 °C due to increased hydrolysis rate. Conversions of 80 % was reached after only 48 h compared to 72 h at 40 °C. The mechanism for the PEG effect is suggested to be due to hydrogen bonding between ethylene oxide units on PEG and phenolic hydroxyls in lignin. The adsorption of polyethylene oxide on lignin surface hinders enzyme binding to lignin resulting in more enzymes available for cellulose conversion.

ORAL PRESENTATION 2-01

Tomorrow's Biomass Refineries

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Today's biorefineries provide vitally important proofs of concept and points of departure. Analysis of tomorrow's biorefineries featuring mature processing technology is also important in order to consider what could potentially be achieved and to motivate and perhaps guide efforts to realize this potential. Since the major sustainability and security challenges we face arise primarily from energy use, biomass processing must make a significant impact on energy production and utilization if it is to play more than a minor role in responding to these challenges. This presentation examines this possibility, drawing from the results of a recently-completed multi-institutional project entitled "The Role of Biomass in America's Energy Future".

Mature processing technology scenarios for cellulosic biomass will be presented based on detailed computer (ASPEN) models. These include stand-alone production of power, Fischer-Tropsch (FT) fuels, and hydrogen, as well as coproduction scenarios involving ethanol-power, ethanol-power-FT fuels, ethanol-hydrogen, ethanol-FT fuels-natural gas, and several of these product combinations in conjunction with feed protein. Observations and insights will be presented regarding product combinations that are particularly promising from the point of view of economic, thermodynamic, and environmental efficacy. Our results suggest that both the overall attractiveness of biomass processing as well as the attractiveness of several specific product combinations increase markedly when viewed in the context of mature technology as compared to current technology. In particular, we project that some mature processing technology scenarios will have overall efficiency (heating value of products/heating value of biomass) in excess of 70% and be economically competitive with conventional processes based on fossil resources at prices seen over recent years. The cost-competitive and efficient energy production processes we project would provide the "energy backbone" and related economies of scale conducive to production of many industrial chemicals. In addition to detailed consideration of mature biorefining scenarios, perspectives will be offered on the adequacy of biomass resources in the context of large scale provision of energy services.

ORAL PRESENTATION 2-02

Technical and Economic Considerations of Biorefineries

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Genencor is a leader in developing technologies that are critical to the development of biorefineries. We are delivering innovation through cost effective enzymes for low temperature conversion of corn to bioethanol, advanced cellulases utilizing cellulosic biomass as the carbon source of the future, and pathway engineered microbes providing efficient conversion of glucose to industrial products. The carbon sources feeding the biorefinery will likely evolve from a solely starch based operation to one with slip streams of carbon derived from cellulosic biomass, and perhaps ultimately to cellulosic biomass becoming a major portion of the feedstock processed into fermentable carbon.

The biorefinery will emerge as a bioprocessing factory that will separate biomass (the entire plant) into component streams, and convert carbon streams into a wide variety of products and co-products. Enzymatic modifications and biotransformations will play a prominent role in these processes. In this paper, we'll present examples of biorefinery elements such as feedstock processing by enzymes to convert starch (and cellulose) to products.

ORAL PRESENTATION 2-03

The Development of an Integrated Biorefinery Concept for Production of Fuels and Chemicals Derived from Lignocellulosic Biomass

Birgitte K. Ahring, Marie J. Mikkelsen, Slawek Dabrowski, Tania I. Georgieva, and Frank D. Haagensen

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The technology of producing renewable energy sources such as ethanol, methane and hydrogen from biomass holds the potential of creating in-house energy resources while lowering the emission of greenhouse gasses as demanded by the Kyoto protocol. Recently, goals were defined for the European Union determining that 5.75% of the transportation fuel has to come from biofuels in year 2010. A large-scale implementation of biofuels into the transportation sector will demand that lignocellulosic biomass, which is found in a surplus throughout the World is used as the raw material for the production process.

The presentation will include a comprehensive description of the special bio-refinery concept developed in Denmark for production of biofuels and other valuable products from straw. The concept includes several innovative steps such as a pre-treatment method using wet oxidation, on-site production of enzymes and a continuous fermentation process using a genetic modified thermophilic bacterium. By co-producing several biofuels in the plant optimal use of the biomass has been assured and the price of for instance of bioethanol is getting close to conventional oil-based fuels. Optimizing each step in the bio-refinery, while having the full integration in mind, will be the way to make an economical viable biofuel production. In the presentation we will present our road map for achieving this goal in the nearest future.

ORAL PRESENTATION 2-04

Existing Biorefinery Operations that Can Benefit from Fractal Based Process Intensification

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Ion exchange, adsorption and chromatography are examples of separation processes used in present biorefineries. The particular tasks for which these technologies can be successfully applied are highly influenced by capital cost and efficiency. There exists a potential for significantly increasing the efficiency of these processes while simultaneously decreasing their size and capital cost. This potential for process intensification can be realized with the use of engineered fractal equipment. The cost savings potential and the possibilities for broadening the use of fractal based separation technologies in future biorefinery concepts will be illustrated by examples of full-scale implementation in the sugar and sweetener industries.

ORAL PRESENTATION 2-05

High-Cetane Diesel Fuels from Biomass

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In previous symposia, we have described the production of gasoline and diesel oxygenates, starting with ethanol and methanol and converting these to higher alcohols via one-stage Guerbet condensations. Although both the higher alcohols and ethanol are good additives for gasoline, these alcohols have a detrimental effect on cetane number when blended with diesel fuel. However, Guerbet products can be easily converted to various ether and acetal derivatives for use as diesel additives. Several ether and acetal derivatives evaluated have very high cetane values (BCN up to 200). Further extensions of this concept of condensation and etherification using a variety of feedstocks derived from biomass will be reported for the production of high-cetane fuels.

ORAL PRESENTATION 2-06

The Importance of Utility Systems in Today's and Tomorrow's Biorefineries

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Energy, in the form of steam or electric power, is a major operating cost in today's biorefineries. Utility system design and operation also affect the environmental benefits of bio-based products. Heat and power systems commonly found in today's corn dry mill, corn wet mill, pulp mills, and sugar cane mills will be reviewed. Their impact on the overall economics and environmental benefits of the corresponding bio-based products will be discussed.

Concepts for advanced biorefineries often include biomass gasification/syngas production, bioprocessing, or combinations of the two. Integration of utility systems with these advanced concepts is especially important since it leads to improvements in overall system efficiency. These technical advances, along with nascent markets for green power, will be key to the development of future biorefineries.

An example, based on an indirect process for producing ethanol, will be used illustrate the importance of utility systems in future biorefineries. The process combines fermentation, gasification/syngas production, and hydrogenolysis steps to produce ethanol at high chemical energy efficiency. We will shown that the incremental capital required for a gas turbine topping cycle is justified because of improvements in overall system efficiency derived by the integration of process and utility system design.

ORAL PRESENTATION 3A-01

Application of Functional Genomic Tools for Exploring Switchgrass Feedstocks Improved through Divergent Selection

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Switchgrass (*Panicum virgatum* L.) is an important native perennial forage and biomass crop. Success in developing elite lines of switchgrass as a future feedstock will require traditional breeding methods coupled with biotechnology. USDA-ARS scientists have generated a large assemblage of switchgrass germplasm bred for a variety of uses. These collections provide an outstanding resource for dissecting traits that impact a wide range of plant processes. We needed to develop appropriate tools in functional genomics to fully exploit these populations for future breeding of elite germplasm, and to provide insights into switchgrass biology.

To this end, we have generated cDNA libraries from different plant parts and have end sequenced over 5,000 individual cDNA clones. We have begun to identify and characterize transcripts that encode for proteins involved in cell-wall metabolism. One of our goals is to build microarrays with a limited set of genes that can be used to screen switchgrass populations. In addition, proteomic and microscopic methods are being used to document the biochemical and anatomical changes that take place during tiller development. These protocols are being used to query existing germplasm. We are also studying changes in the levels of several enzymes from select switchgrass plants utilizing protein-chip arrays. Data from these and related experiments will be discussed.

ORAL PRESENTATION 3A-02

Genetic Improvement of Shrub Willow (*Salix*) as a Dedicated Crop for Bioenergy, Biofuels, and Bioproducts

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The domestication and development of fast-growing shrub willow crops to serve as a dedicated and custom-tailored feedstock for the production of bioenergy, biofuels, and bioproducts can provide a long-term, sustainable replacement for fossil fuels and stimulate rural development. This project will reduce the cost of willow biomass by developing varieties with consistently greater yields and with optimal wood chemistry for low-input pretreatment, fractionation, and conversion in the biorefinery. We have established and maintain the largest willow breeding program in North America. This includes assembling a nursery of diverse clones collected from natural sites across the U. S. and provided by international collaborators. We are using AFLP markers and microsatellites to fingerprint these clones and to characterize the diversity of populations of *Salix purpurea* and *S. eriocephala* in New York. Gains have been achieved through controlled pollinations producing >200 families since 1998. Crosses involving *S. miyabeana* with *S. sacchalinensis*, *S. purpurea*, and *S. viminalis* have generated progeny with growth that is 20-40% greater than that of a standard cultivar, *S. dasyclados* 'SV1', in replicated selection trials. Increased, reliable yields demonstrated in regional trials over the long term will encourage widespread adoption of willow crops in the United States.

ORAL PRESENTATION 3A-04

Development of Biologically Confined Transgenic Crops for Renewable Energy

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Hydrolysis enzymes produced in fermentation tanks are expensive. A method of reducing costs is to produce these enzymes in biomass crops, extract as soluble proteins and add to pretreated matter for enzymatic hydrolysis. The U.S. National Academies recently suggested the use of biologically confined transgenic crops to avoid risks (Kirk et al, 2004). We have created transgenic crops characterized by (1) delay in flowering to increase the biomass and to avoid transgene flow through pollen transfer (Salehi et al., 2005) and (2) the production of microbial endoglucanase to aid post-harvest hydrolysis of crop biomass.

Footnotes

Kirk T., Carlson J., Ellstrand N., Kapuscinski, Lumpkin T., Magnus D., Nester E., Peloquin J, Snow A., Sticklen M., and Turner P. (2004). NRC Report. The Natl. Acad. Press. Washington, DC. 255p.

Salehi H., Ransom C., Oraby H., and Sticklen M. (2005). J. Plant Physiol. In press.

ORAL PRESENTATION 3A-05

Manipulating the Phenolic Acid Content and Digestibility of Forage Grasses by Targeted Expression of Fungal Cell Wall Degrading Enzymes

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We have shown previously the expression of a ferulic acid esterase gene from *Aspergillus niger* in *Festuca arundinacea* and the potential of the expressed FAE to break phenolic cross-links and to release monomeric and dimeric ferulic acids on cell death in vacuole targeted FAE plants. This was enhanced several fold by the addition of exogenous recombinant xylanase (Buanafina et al., 2002).

Here we show that targeting FAE to the apoplast and ER/golgi system resulted in a significant reduction in the levels of monomeric and dimeric cell wall phenolics in the leaves of some plants when expressed constitutively. Apoplast targeting might be expected to directly affect cell wall composition by removing ferulic acids, whereas ER/golgi targeting may affect cell wall composition, indirectly, by reducing feruoylation of the arabinoxylans in the ER/golgi destined to the cell wall. We also show that the potential of expressed FAE to break phenolic cross-links in vacuole targeted FAE plants leads to increased initial rates of fermentation.

We have now produced *F. arundinacea* plants expressing endo-ß-1,4-xylanase from *Trichoderma reesi* and we report whether expression of xylanase will increase release of ferulates further.

Footnotes

Buanafina, M.M de O., Langdon, T., Hicks, H.C., Hauck, B., Dalton, S.J. and Morris, P. Targeted expression of a ferulic acid esterase from *Aspergillus niger* in leaves of forage grasses. In: Proc. 19th European Grassland Federation – General Meeting La Rochelle (France) 27030 May 2002. Pg. 66-67.

ORAL PRESENTATION 3A-06

Direct Imaging of Pretreatment and Enzymatic Digestion Effects on Corn Stover Ultrastructure

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Thermochemical and mechanical pretreatment has long been known to result in biochemical changes to biomass in terms of both chemical composition and susceptibility to enzymatic digestion. The wide variety of pretreatment chemistries currently under investigation in various laboratories has shown that though linked in some instances, changes in chemical composition do not always result in predictable digestibility patterns. Although indirect evidence has suggested physical changes in the arrangement of plant cell wall structural components is at least in part responsible for these biochemical changes, direct observation of these changes has been limited. The new Biomass Surface Characterization Laboratory at the NREL has recently enabled direct imaging of ultrastructural changes in biomass at scales ranging from millimeters to nanometers through Environmental Scanning Electron, Atomic Force, Confocal, and Transmission Electron Microscopy. Visualization of cell wall components, including intracellular pits, fiber and vessel structural components, cellulose macro- and microfibrils, hemicellulose matrices, and other structural features has been correlated to pretreatment chemistries and changes in enzymatic digestibility, giving us new insight into the mechanisms of biomass recalcitrance and raising new questions in our endeavor towards economical biomass conversion.

ORAL PRESENTATION 3A-07

Enhanced Processing Characteristics via Simultaneous Optimization of Stover Composition and Pretreatment Conditions

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Considerable improvements are necessary before it is economically feasible to use maize and sorghum stover as a source of fermentable sugars for the production of ethanol and green chemical feedstocks. We are investigating the impact of cell wall composition on bio-processing efficiency, and have shown that changes in the composition of the cell wall polymer lignin can improve the yield of glucose by as much as 50% during lab-scale saccharification experiments. In order to make further progress toward the development of efficient biomass crops, we are cloning several sorghum genes involved in lignin biosynthesis with the aim of facilitating plant breeding, and we are investigating the potential of sweet sorghum with enhanced cell wall composition as a dual-purpose biomass crop. The processing of stover can be further enhanced by liquid hot water pretreatment, particularly after addition of sulfuric or maleic acid in low concentrations. Changes in cell wall composition appear to have an impact on the optimum pretreatment conditions, and we are evaluating which chemical changes in the cell wall will result in high yields of fermentable sugars under mild pretreatment conditions.

ORAL PRESENTATION 3B-01

Biomass Recalcitrance: The Major Hurdle for the Lignocellulosic Biorefinery Industry

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Plants use evolutionarily honed strategies for defending structural sugars, stored in the form of cell wall polysaccharides, from attack by microbes and men. Apart from obvious chemical barriers to invasion, such as lignin and the microcrystallinity of cellulose, the complexity of plant ultrastructure also affords protection. This situation offers both challenges and promises for the new biorefinery industry planning to utilize fermentable sugars from lignocellulosic biomass. The promises lie in the wealth of non starchy biomass available for conversion (which exist largely without competitive markets) and the challenges lie in overcoming the powerful natural mechanisms protecting non starchy polysaccharides in plants. Lignocellulosic feedstocks, estimated to cost from $35 to $55/ton delivered to the biorefinery, must be optimally utilized by the respective bioconversion process to ensure the lowest feedstock costs. We now understand that the highest feedstock sugar yields and the lowest biorefinery processing costs can only come from targeted research carefully designed to overcome key knowledge barriers regarding the natural resistance of biomass to deconstruction. New strategies for addressing biomass recalcitrance in the context of the DOE Office of the Biomass Program will be discussed.

ORAL PRESENTATION 3B-02

Fundamental Factors Affecting Enzymatic Reactivity

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The literature indicates that the following factors affect the enzymatic reactivity of biomass: (1) lignin content, (2) crystallinity, (3) acetyl content, (4) degree of polymerization, (5) accessible surface area, (6) pore volume, and (7) particle size. This study focuses on the first three structural features.

A total of 147 model biomass substrates were prepared with widely different lignin contents, crystallinities, and acetyl contents. The enzymatic conversion to glucose and xylose is correlated with enzyme loading using the following empirical equation:

$$x = B \ln (E_o) + A$$

where x is the conversion, E_o is the enzyme loading (FPU/g biomass), and A and B are the intercept and slope, respectively. The slope and intercept – which characterize the enzymatic reactivity of the biomass – are correlated with the three structural features. To test the validity of the correlations, the model predictions are compared to data collected for a variety of biomass feedstocks (e.g., corn stover, sugarcane bagasse) treated with a variety of industrially relevant pretreatments (e.g., AFEX, lime, dilute acid).

The significance of this work is that it allows the design of optimal pretreatment and saccharification systems that trade the costs of more extensive pretreatments against the savings of lower enzyme consumption.

ORAL PRESENTATION 3B-03

Optimal Combination of Cellulase and Xylanase for Enzymatic Hydrolysis of AFEX Treated Corn Stover

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The cost of many important fermentation products strongly depends on the cost of carbohydrate raw material. Lignocellulosic biomass conversion offers the potential for less expensive fermentable sugars. Two important research objectives for this potential are: 1) development of effective and economical biomass pretreatment to increase the yield of the fermentable sugar from biomass hydrolysis and 2) maximal utilization of the various polymeric sugars available in the heterogeneous lignocellulosic material. Complete and balanced cellulolytic and xylanolytic systems are required to achieve maximum hydrolysis of plant cell wall polymers at minimal cost. It has been shown that the hydrolysis of glucan and xylan are intimately linked and whatever enhances glucan conversion also tends to increase xylan conversion (and vice versa). In this study we attempt to take advantage of this fact by trading off cellulase and xylanase activities. It is important to provide not only adequate total hemicellulase activity but also the correct distribution of enzyme activities between cellulase and hemicellulase mixture. Enzymatic hydrolysis of optimally AFEX treated corn stover with different levels of xylanase in combination with different cellulase loadings has been performed to identify the best combinations of enzymes.

ORAL PRESENTATION 3B-04

Hydrolysis and Fermentation of Lime-Pretreated Biomass

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A consortium comprised of 3 R&D institutes, 3 companies and one University recently launched a 4-year project to develop lignocellulosic biomass-to-ethanol technology in the Netherlands. As part of this project, we investigated mild-temperature, lime ($Ca(OH)_2$) pretreatment of lignocellulosic biomass for enzymatic hydrolysis purposes. Lime pretreatment has regained interest because of perceived advantages compared to other pretreatment techniques, including low formation of fermentation inhibitors. This presentation will include results of pretreatment and hydrolysis work at both lab-, bench- (2L) and pilot-scale (30L), as well as fermentation results to evaluate fermentability of produced substrates to ethanol. Key parameters that impact on the industrial feasibility of the process, including chemical recovery, will also be discussed.

Wheat straw was submitted to impregnation with $Ca(OH)_2$ at varying water-biomass ratios and alkali loading rates, followed by enzymatic hydrolysis. Solid and liquid analysis indicate that lime pretreatment at mild temperatures does not lead to significant dissolving of xylan or lignin. Enzymatic hydrolysis trials show considerable glucan-to-glucose (73-88%) and xylan-to-xylose (55-68%) conversion, depending on enzyme selection and -loading rate. Fermentation tests of unwashed hydrolysates showed comparable conversion to ethanol as standard glucose fermentation, although ethanol production rate was affected by acetic acid concentration in the substrate. Removal of acetic acid from the substrate led to improvements in fermentability.

ORAL PRESENTATION 3B-05

Preliminary Results on Optimising Hydrothermal Treatment Used in Co-Production of Biofuels

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In December 2002, an EU-project for co-production of biofuels was started. The overall objective is to develop cost and energy effective production systems for co-production of bio ethanol and electricity based on integrated biomass utilization. During the first 12 months period of the project, a pilot plan reactor for hydrothermal treatment (and other pre-treatments including wet oxidation) with a capacity of 100 kg/hour was constructed and tested for pre-treatment of wheat straw to be used for ethanol and/or electricity production. Pre-treatment by hydrothermal treatment (or wet oxidation) is performed to solubilize the hemicellulose fraction in straw, open the cellulose structure to increase accessibility of enzymes, and to remove the alkaline salts (*e.g.* potassium chloride). Alkaline salts cause corrosion problems in conventional boilers during incineration of straw for electricity production. The solubilised hemicellulose is in a second step converted by either enzymes or weak acid hydrolyses to monomeric sugar compounds for ethanol production. The cellulose fraction containing the lignin will be burned for electricity or part of it may be used for ethanol production by means of SSF. By-products from the pre-treatment and fermentation processes will be concentrated and used for animal feed.

Several trials were made with varying parameters of water level, chemical addition and flow in the reactor. All experiments were performed at 190°C, except for a single experiment performed at 200°C. The results illustrates that it is possible to extract more than 95% of the alkaline salts (at 200°C) leaving a solid cellulose rich biofuel for combustion or for further treatment in the ethanol process. In the experiments performed at 190°C, the best total glucose yield after pre-treatment and following enzymatic hydrolysis was found in the experiment with Na_2CO_3 addition (61% glucose yield), but improved conversion was found in the experiment at 200°C (97% glucose yield). Very good glucose recovery (80-100%) was found in all of these trials. The highest total yield of hemicellulose (67%) was found in the experiment with high flow. In this experiment the hemicellulose recovery was 87%. The highest hemicellulose recovery (91%) was found in the experiment with Na_2CO_3 addition.

ORAL PRESENTATION 3B-06

Bioconversion of Lignocellulose to Ethanol: a Robust Pretreatment Method for Processing Mixed Feedstocks

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One of the economic barriers impeding widespread commercialization of a lignocellulose-to-ethanol bioconversion process is feedstock availability. An ideal pretreatment method would allow processing of a diverse range of feedstocks, thereby reducing fluctuations in supply due to seasonal variation and other factors. In an effort to develop a versatile and robust bioconversion process, we have explored the possibility of mixing various feedstocks in a single pretreatment reactor to provide material for enzymatic hydrolysis.

In the current study, we pretreated mixed agricultural residues (corn fibre and corn stover) using SO_2-catalysed steam explosion (190°C, 5 min and 3% SO_2) and recovered carbohydrates in high yield for hydrolysis and subsequent fermentation. Using the same conditions, we demonstrated that is feasible to combine mixed agricultural residues with a hardwood feedstock (hybrid poplar) at various ratios for pretreatment. The study incorporates a novel, high thru-put micro-assay for optimization of pretreatment and hydrolysis conditions.

ORAL PRESENTATION 3B-07

Cellulose to Glucose Conversion Via Swelling-Decrystallization and Subsequent Low Severity Acid Hydrolysis

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Sequential steam-aqueous fractionation of biomass leads to the production of a hemicellulosic-rich liquor, a lignin-rich stream and a cellulosic-rich residue. The latter can be separated, if desired, into fines and fibers. This paper discusses an approach to decrystallize the cellulosic matrix (as fines or fibers) via swelling in a concentrated acid solution leading to the formation of a viscous hydrogel. A mediator is then used to change the ionic concentration of the viscous hydrogel which is converted into glucose at moderate severities (100 C and 30 min being typical). High yields of glucose are consistently obtained (> 80 % of theoretical). Recovery of acid and mediator permits to envisage their reuse in the process. The mediator facilitates the production of hydrolyzates with 10 – 12 wt% glucose concentration.

ORAL PRESENTATION 4-01

Gasification of Ethanol-Derived Switchgrass Lignin

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The viability of gasifying switchgrass lignin residue for power production was studied. The lignin was derived from the conversion of Midwestern U.S. switchgrass to ethanol using a concentrated acid hydrolysis process for converting cellulose and hemicellulose to sugar. A continuous (4 lb/hr) pilot-scale fluidized-bed gasifier housed at the Energy & Environmental Research Center was employed. Lignin (100%) and lignin–coal blends (20/80%) were reacted with steam and oxygen in a reducing or oxygen-starved atmosphere at temperatures between 700°–923°C, under 15 psig pressure, 1.7 scfm flow rates, and1.5–3.23 lb/hr feed rates. Product gas for the 100% lignin runs averaged 8.5% H_2, 7.4% CO, 4.0% CH_4, 16.7% CO_2, and 63.4% N_2 with a higher heating value of 92 Btu/scf. The higher heating value of the lignin/coal (20/80%) blend was 152 Btu/scf.

These tests show that lignin-derived fuel gas can be combusted directly in boilers or gas turbines without the impurities and solid contaminants formed during the direct combustion of the same feedstock, and the synthesis gas can also be utilized in the production of other fuels or chemicals. The air-dried switchgrass lignin gasified well without bed agglomeration, ash fouling potential, or hot gas filter plugging, even at higher operating temperatures. Ash problems were reduced mainly because of the high melting point refractory affect of the 73% silicon in the lignin and the low alkali content. Sulfur levels were somewhat elevated because of remnant calcium sulfate in the lignin from a neutralization stage of the acid in the hydrolysis process. Although the 100% lignin was not as reactive as the western U.S. subbituminous coal, the methane yields were twice as high, and the tar and condensable organics were very low (< 0.01 wt% for all fuels).

ORAL PRESENTATION 4-02

Fed-Batch Production of Succinic Acid from Glucose:Xylose Feedstocks Using *E. coli*

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Succinic acid production from glucose based feedstocks is possible in high yields. In order to reduce feedstock costs, carbon sources from a wide range of industrial sources should be considered. While starch hydrolysates contain only glucose, a wider range of potential sources for sugar from cellulosics and lignocelluloics result in mixtures of sugars, where the two most abundant sugars are glucose and xylose. A metabolically engineered *E. coli* strain, the AFP184, which is able to produce succinic acid by fermentation from both glucose and xylose feedstocks, was used for succinic acid production. The performance of the succinic acid fermentation including yield and volumetric productivity from these experiments will be discussed.

ORAL PRESENTATION 4-03

Continuous Butanol Production from Glucose and Butyrate by *Clostridium acetobutylicum* in a Fibrous Bed Bioreactor: Effects of Butyrate and Long-Term Stability

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Conventional ABE fermentation involves two phases (acidogenesis and solventogenesis) that are difficult to control, and suffers from low butanol productivity and yield. A stable continuous fermentation for butanol production from glucose was developed by co-feeding the bioreactor with butyrate, the precursor for butanol formation, so that the fermentation would shift to and stay in the solventogenesis phase, thus producing more butanol from glucose and reducing the byproducts (e.g., ethanol and acetone). Continuous fermentation of glucose and butyrate by *C. acetobutylicum* in a fibrous bed bioreactor was studied at 35 °C and pH 4.3 for over two months. The effects of butyric acid concentration on butyrate uptake rate and butanol production were studied. In general, increasing the butyrate concentration (up to 7.4 g/L) also increased butyrate uptake rate (to 2.4 g/L/h) and butanol production. High butanol productivity of ~5.6 g l^{-1} h^{-1} and yield of ~0.28 g g^{-1} (~0.43 g g^{-1} for total solvents) were obtained. The fermentation was operated continuously without degeneration for the entire period studied. The enhanced butyrate uptake rate and sustained culture stability with high butanol productivity and yield by using the fibrous bed bioreactor should provide an economic and energy-efficient process for butanol production from sugars.

ORAL PRESENTATION 4-04

The Development of Cement and Concrete Additive Based on Xylonic Acid Derived via Bioconversion of Xylose

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Aldonic acids, in general, are known metal chelating agent and inorganic dispersant. Xylonic acid is a pentoaldonic acid and is an inorganic dispersant. The present work focused on utilizing xylose by converting it to an aldonic acid. The obtained xylonic acid was tested in cement & concrete as a dispersing agent.

Xylose was converted to xylonic acid *via* enzyme or microbial fermentation as well as chemical oxidation. The enzyme conversion was successfully done using a commercial glucose oxidase although the conversion rate was slower than in the case of glucose oxidation. The microbial oxidation of xylose in accordance with Buchert *et al.* was also successfully done. The microbial conversion proceeded even with the presence of large amount of lignosulfonate.

The xylonic acid was tested in cement paste, mortar and concrete as a cement dispersant. It was found that xylonic acid was approximately twice as effective as lignosulfonate whereas it showed more cement hydration retardation. Overall, xylonic acid can be effectively utilized in cement and concrete admixture application.

ORAL PRESENTATION 4-05

Commercialization of PHA Bioplastics: Current and Future Developments

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Metabolix has applied advanced metabolic pathway engineering at the genome level to solve the manufacturing challenges for polyhydroxyalknoate (PHA) copolymers. Utilizing the same basic manufacturing platform, PHA copolymers can now be produced economically from renewable resources with pre-specified polymer properties. Projected manufacturing costs of PHA resins have been reduced to levels competitive with existing materials. Major advances have also been made in PHA resin processing using existing industrial thermoplastic processing equipment, delivering competitive processing economics for a range of initial target applications including injection molding, cast film and extrusion coating. This technology is now being commercialized in a strategic alliance with Archer Daniel Midland Co.

Metabolix's PHAs are a broad and versatile family of plastics, ranging in properties from rigid, strong and stiff to tough and highly elastomeric. They can be made as resins or aqueous dispersions with excellent film forming characteristics. Robust in use, yet biodegradable, PHAs offer a renewable and environmentally friendly alternative in many applications now served by synthetic plastics, including fiber, film, molded goods, extruded products, adhesives, and coatings.

ORAL PRESENTATION 4-06

Top Value Added Chemicals from Biomass (Volume 1)

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This report identifies a Top list of chemical building blocks that provide the best opportunity to produce chemicals and materials from biomass derived sugars and syngas as well as secondary chemicals from these building blocks. Both the building blocks and the secondary chemicals can serve as economic drivers for a biorefinery and most importantly as models for identifying technical challenges needing R&D. The list is comprised of twelve sugar-based building blocks.

Biomass derived syngas also represents a potentially valuable building block but that analysis has been considered and is being published elsewhere.

An initial list of over 300 candidates was reduced to a list of the top 30 potential candidates by using an iterative review process based on the petrochemical model of defining building blocks, chemical data, known market data, properties and performance of the potential candidates and most importantly the experience of the team. From this top 30 list, additional criteria were employed to select a Top Ten list such as examining the potential markets for the building blocks and their derivatives and the technical complexity of the synthesis pathways. A second set of building blocks was also identified as viable candidates but with lower potential. These include gluconic acid, lactic acid, malonic acid, propionic acid, the triacids - citric and aconitic; xylonic acid, acetoin, furfural, levoglucosan, lysine, serine and threonine.

The synthesis of the top building blocks and their derivatives is comprised of two pathways. One was the transformation of sugars to the building blocks. The other was the conversion of the building blocks to the secondary chemicals or derivatives. It is observed that biological transformations comprise the largest number of pathways to the building blocks, but chemical transformations predominate in the conversion of building blocks to derivatives. The challenges and complexity of these pathways, as they relate to the use of biomass derived sugars and chemicals, were briefly examined in order to highlight R&D needs that could help improve the economics of producing these building blocks and derivatives. Not surprisingly, many of the transformations and barriers revealed in this analysis are common to the bioprocessing of lignocellulosics and starch to fuels such as ethanol. Lastly, recommendations for follow-on efforts will be discussed.

ORAL PRESENTATION 5-01

Alcoholic Fermentation of Lignocellulose Hydrolysates has Become Feasible

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Due to the instability and increasing prices in the petroleum market the demand for a biobased fuel replacement, such as ethanol produced by fermentation continues to escalate. To meet these demands alternative agricultural substrates have been examined. Currently much attention is being focused on the use of lignocellulosic biomass as it is an ideal renewable resource for the production of fuel ethanol via fermentation. *Saccharomyces cerevisiae* has been the most exploited organism for the production of various products including the industrial production of ethanol. It is known that wild type *S. cerevisiae* is not capable of fermenting many of the pentose sugars (xylose for example) released during lignocellulosic hydrolysis. After extensive genetic modification and strain selection via evolutionary engineering, a *S. cerevisiae* strain has been obtained that rapidly and efficiently co-ferments glucose and xylose. Fermentation of xylose is as efficient as that of glucose. Under anaerobic conditions (pH 4 and 30°C), a mineral medium containing a mixture of glucose (100 g/L) and xylose (50 g/L), growth factors such as nicotinic acid, sterols and an unsaturated fatty acid, inoculated with 6 g of biomass/L, the sugar mixture is converted within 24 hours into alcohol with only glycerol and minimal biomass as by-products.

ORAL PRESENTATION 5-02

Genetic Engineering of *S. cerevisiae* for Pentose Utilization

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The two most widespread pentose sugars in our biosphere are D-xylose and L-arabinose. The pentose catabolic pathways are relevant for microorganisms living on decaying plant material and also in biotechnology when cheap raw materials such as plant hydrolysates are fermented to ethanol. In fungi, i.e. in yeast and mold, L-arabinose is sequentially converted to L-arabinitol, L-xylulose, xylitol and D-xylulose and enters the pentose phosphate pathway as D-xylulose 5-phosphate. In molds the reductions are NADPH-linked and the oxidations are NAD^+-linked. We recently identified the two missing genes in this pathway. The functional overexpression of all the genes of the pathway in *S. cerevisiae* led to growth on L-arabinose and ethanol production under anaerobic conditions however at very low rates. In this communication we show that in a yeast species the L-arabinose pathway is similar, i.e. it has the same two reduction and two oxidation reactions, but the reduction by L-xylulose reductase is not performed by a strictly NADPH-dependent enzyme as in molds but by a strictly NADH-dependent enzyme. To our knowledge this is the first report of an NADH-linked L-xylulose reductase. D-xylose fermentation to ethanol with recombinant *S. cerevisiae* is often slow and has a low yield. One reason is that the catabolism of these pentoses through the corresponding fungal pathways creates an imbalance of redox cofactors. The process, although redox neutral, requires NADPH which must be regenerated in a separate process. To facilitate the NADPH regeneration, the recently discovered gene *GDP1* coding for a fungal NADP GAPDH was expressed in a *S. cerevisiae* strain with the D-xylose pathway. Glucose 6-phosphate dehydrogenase is the main path for NADPH regeneration, however it causes futile CO_2 production and creates a redox imbalance on the pathway for anaerobic fermentation to ethanol. The deletion of the corresponding gene, *zwf1*, in combination with overexpression of *GDP1* could stimulate D-xylose fermentation with respect to rate and yield; i.e. less CO_2 and xylitol were produced. Through redox engineering a yeast strain, which was mainly producing xylitol and CO_2 from D-xylose, was converted to a strain producing mainly ethanol.

ORAL PRESENTATION 5-03

Development of Recombinant Xylose- and Arabinose-Utilising *Saccharomyces cerevisiae* Strains

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Baker's yeast, *Saccharomyces cerevisiae,* is currently used in biofuel ethanol production due to its robustness, ease of production and its ability to tolerate high ethanol concentrations and inhibitory compounds. However, the cost of production of biofuel ethanol from various raw materials (wood, corn stover, sugar cane bagasse etc) would be significantly reduced with the development of strains able to ferment, in addition to hexoses, the pentose sugars xylose and arabinose. Recombinant xylose- and arabinose-utilising *S. cerevisiae* have previously been independently developed. In this study, both xylose and arabinose pathways together are being introduced in laboratory- and industrial strains that are further tested for mixed sugar fermentations.

ORAL PRESENTATION 5-04

A "First-Look" at the Genome of the Xylose-Fermenting Yeast, *Pichia stipitis*

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The entire 15.9 Mbp of the *Pichia stipitis* genome was sequenced and annotated. *P. stipitis* Pignal (1967) is a predominantly haploid, heterothallic yeast related to *Candida shehatae* and other pentose metabolizing ascomycetous yeast species.[1],[2] It has the highest native capacity for xylose fermentation of any known microbe.[3] Strains of *P. stipitis* are among the best xylose-fermenting yeasts in type culture collections.[4] Fed batch cultures of *P. stipitis* produce up to 47 g/L of ethanol from xylose at 30°C under low aeration conditions.[5]

P. stipitis has six chromosomes ranging from 1 to 3 mbp. It belongs to a group of yeasts that uses an alternative nuclear genetic code in which CUG codes for serine rather than leucine. Approximately 20% of the *P. stipitis* genes contain introns. Surprisingly, the sequenced genome (CBS 6054) does not contain a xylanse, but it does include seven Family 3 beta-glucosidases and more than 40 putative sugar transporters. The completion of this genome will enable large-scale expression analysis and it will greatly facilitate the engineering of *P. stipitis* for fermentation of hemicellulose hydrolysates.

Footnotes

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4 van Dijken, J. P., E. van den Bosch, J. J. Hermans, L. R. de Miranda, and W. A. Scheffers. 1986. Alcoholic fermentation by 'non-fermentative' yeasts. Yeast 2:123-127.

5 du Preez, J. C., B. van Driessel, and B. A. Prior. 1989. Ethanol tolerance of *Pichia stipitis* and *Candida shehatae* strains in fed-batch cultures at controlled low dissolved-oxygen levels. Appl Microbiol Biotechnol 30:53-58.

ORAL PRESENTATION 5-05

Evaluation of Cellulase System Components Expressed in *Saccharomyces cerevisiae* and Implications for Consolidated Bioprocessing

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For biomass conversion to commodity products, many studies have anticipated process configurations in which production of saccharolytic enzymes is accomplished in a step separate from production of the desired product via anaerobic fermentation. We are exploring an alternative, microbially-oriented, approach involving production of saccharolytic enzymes and fermentation products in a single step, termed "Consolidated Bioprocessing" (CBP). Expression and co-expression of genes encoding cellulases in *Saccharomyces cerevisiae* will be addressed, as well as evaluation of recombinant yeast strains in batch fermentations on non-native glucan substrates. We report functional expression in *S. cerevisiae* Y294 of several cellobiohydrolase (CBH) enzymes, including CBH1 from *Trichoderma reesei*, CBH1-4 from *Phanerochaete chrysosporium*, CBH2 from *T. reesei*, and CBHB from *Aspergillus niger*, as well as the specific activity of these recombinant enzymes. Activity of the recombinant enzymes was measured via a cellulase assay adapted for low concentrations of enzyme using an affinity purification technique. Mass concentrations of CBH1 and CBH1-4 were measured by an ELISA method, while a general protein assay was used for the other recombinant enzymes. Soluble glucans resulting from the action of CBH enzymes and endoglucanases are converted to glucose via ß-glucosidase and so we have also evaluated the expression of the *BGL1* gene of *Saccharomycopsis fibuligera* in *S. cerevisiae*. Strains expressing *BGL1* grew on cellobiose (0.18 h^{-1} compared to 0.25 h^{-1} on glucose) with bioethanol production (0.41 g of ethanol /g of cellobiose) under anaerobic conditions. Co-expression of different endoglucanase and cellobiohydrolase encoding genes together with the *BGL1* gene in *S. cerevisiae* strains to enable degradation of longer glucans will also be reported. These results will be considered in the context of a quantitative analysis of prospects for obtaining growth on insoluble cellulosic substrates. Paths for future organism development will also be discussed.

ORAL PRESENTATION 5-06

Metabolic Engineering of *Actinobacillus succinogenes* to Enhance Succinic Acid Production

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Actinobacillus succinogenes is a facultatively anaerobic bacterium that ferments simultaneously a broad range of sugars to succinate as the major product and pyruvate, acetate and ethanol as minor products. Metabolic flux analysis was used to evaluate the effect of different carbon sources on succinic acid production in batch fermentations. The analyses established that the major route to succinic acid flows from phosphoenolpyruvate, oxaloacetate, malate, fumarate to succinate in *A. succinogenes*. Glyoxylate shunt and PTS (phosphotransferase system) appear not to be used in the organism. Glucose fermentations typically reach concentrations of 65g/L succinic acid at about 100% yield (wt SA/ wt Glc). Fermentations with more reduced carbon sources such as sorbitol or mannitol produced higher amounts of succinic acid with higher yields than glucose fermentations implicating a need for reducing power. In contrast, fermentations of pentose sugars (xylose and arabinose) produced lower amounts of succinate with lower yield, suggesting that reducing power limitation becomes even more severe in fermentations using pentose sugars. Mixed sugar fermentations, with a composition of sugars as released from treated corn biomass, showed similar confines. Routes to increase reducing power in the organism are under investigation, and their effect on succinic acid production will be discussed.

ORAL PRESENTATION 5-07

High-Purity Lactic Acid Fermentation Broth

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Established commercial lactic acid fermentations have several deficiencies: the acid must be neutralized *in situ* due to the production microbe's sensitivity to undissociated lactic acid; the fermentation medium often includes crude protein sources to meet the microbe's complex growth requirements; the microbe produces significant amounts of organic byproducts including acids, aldehydes and alcohols. The net effect is to make lactic acid difficult and costly to purify, particularly if it is to be used as a polymer feedstock, due to the amount and nature of impurities in the broth. An alternative fermentation method that overcomes all of these deficiencies has been developed. Acid tolerant yeasts have been genetically engineered to produce undissociated lactic acid from carbohydrates at near quantitive yields. The fermentation medium employs minimal amounts of simple inorganic salts. The yeast produces no ethanol and hardly any other organic byproducts. As a result, the complexity and cost of recovering (polymer-grade) lactic acid is reduced.

ORAL PRESENTATION 6-01

Demonstration of Sustained Hydrogen Photoproduction by Immobilized, Sulfur-Deprived *Chlamydomonas reinhardtii* Cells

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The team has demonstrated for the first time that immobilized, sulfur-deprived algal cultures can photoproduce H_2 gas for extended periods of time. After identifying the optimal materials and procedures for immobilization of Chlamydomonas reinhardtii at high cell density, we examined the effect of liquid mixing, sulfate content, acetate levels and light intensity on the H_2-production activity of the immobilized culture. Sulfur deprivation was used to induce H_2 production, as previously shown with suspended algal cultures. Our results indicate that (a) liquid mixing by argon bubbling is important to provide homogenous conditions for the immobilized culture; (b) the normal level of acetate required for suspension cultures is also optimal for immobilized cultures; and (c) high light intensity decreases H_2 production, possibly due to a simultaneous increase in photosynthetically-evolved O_2. Cell immobilization significantly increased the duration of the H_2-photoproduction phase compared to batch cell suspensions (from 4 days to ca. 4 weeks, respectively), maintained the specific rates of H_2 photoproduction and showed potential for large increases in H_2 production on a bioreactor volume basis. Possible factors affecting H_2 photoproduction in the immobilized algal system will be discussed. This work was supported in part by the US DOE Hydrogen, Fuel Cells and Infrastructure Technologies Program.

ORAL PRESENTATION 6-02

Fermentation of Synthesis Gas to Fuel Ethanol

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The fermentation of biomass-generated synthesis gas (syngas) was studied. Switchgrass and bermudagrass were gasified using three reactor operating conditions: air gasification, near pyrolysis, and steam. For switchgrass, the average CO concentration increased from 20 % with air to 47% with steam, while the H_2 concentration increased from 6% to 18%. For bermudagrass, the CO concentration increased from 16% with air to 34% with steam, while the H_2 concentration increased from 6% to 28%.

The syngas generated from air gasification was bubbled into a 3-liter bioreactor containing Strain P7. P7 was isolated from an agricultural lagoon and is a new species of *Clostridium* closely related to *Clostridium scatologenes*. Important characteristics of P7 include excellent culture stability, tolerance to oxygen, tolerance to high concentrations of ethanol, and an ability to grow in defined medium.

Syngas fermentation caused an increase in ethanol production compared to bottled gases of similar composition. However, exposure to the gas also resulted in cell dormancy and inhibition of hydrogen consumption. The cell dormancy was circumvented by additional cleaning of the gas using acetone scrubbing and a 0.025 µm filter. Gases known to cause hydrogenase inhibition, such as nitric oxide and acetylene, were evaluated with regards to hydrogenase activity.

ORAL PRESENTATION 6-03

Enzymatic Conversion of Waste Cooking Oils into Alternative Fuel—Biodiesel

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Environmental concern and energy security are the driving force for the development of alternative fuels from renewable resources. Biodiesel, an alkyl esters derived from vegetable oils by transesterification with methanol/ethanol, has been paid considerable attention during the past decade particularly for being a renewable, biodegradable, and nontoxic fuel. The transesterification can be carried out chemically or enzymatically. Recently, enzymatic transesterification using lipase has become more attractive for the production of biodiesel fuel from vegetable oils, since glycerol (the produced by-product) can easily be recovered and even re-transesterified. In addition, the purification of fatty methyl esters could be simple.

A significant barrier to the commercialization of this system is the cost of raw materials and lipase production. As a means of reducing the cost, waste cooking oils and immobilized lipases are considered in this paper. Therefore the paper is addressing the immobilized lipase conversion of waste cooking oils into biodiesel, focusing on process parameter study. Several factors which may influence on the quality of produced biodiesel were investigated, including the molar ratio of methanol to waste cooking oils, catalyst load, the way of adding catalyst, reaction temperature and pressure. The results indicate that methanol to oils ratio of 6, immobilized lipase to oils in weight ratio of 15-20%, temperature of 40°C are suitable for the production of biodiesel under 1atm. Two-step addition of lipase appears better than one-step addition. The biodiesel produced is good in quality and its productivity is satisfactory, up to 0.88-0.90kg/kg oils. The irreversible inactivation of the lipase by contact with insoluble methanol is confirmed here and a new route to reduce inactivation of commercial immobilized lipases is proposed. The results obtained should be very useful for the large-scale production of alternative fuel from waste cooking oils.

ORAL PRESENTATION 6-04

Production of Statins from *Pleurotus* mushrooms

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Statins have proved to be effective in reducing, at plasmatic level, total cholesterol (60-65%), as well as LDL (25- 40%) and triglycerides, while increasing HDL (5-20%). They act as inhibitor of the hepatic synthesis of cholesterol by blocking the betaHydroxi- beta-MethilGlutaril-Coenzime A (HMG-CoA) reductase. As cardiovascular diseases related to high levels of cholesterol are among the main causes of death in our societies, there is a high incentive for developing processes for the production of statins, an FDA approved drug. All natural statins have a common molecular structure, a hexahydro-naphthalene system and a -hydroxy-lactone, but they differ from each other due to side chains and a methyl group around the ring. Processes for the production and purification of statins from *Penicillium citrinum*, Monascum rubber, and other microorganisms have been described. Industrial processes for commercial production are mainly set up using *Aspergillus* strains. This fact creates an opportunity for designing alternative and viable technologies (which competitiveness should also be proved), employing alternative raw materials, in order to develop local production of high valued products, anchored on local know how. Furthermore, as lovastatin is present in high proportion in the edible kind of mushrooms known as Oyster Mushroom, Tree Oyster and Haritake (*Pleurotus ostreatus*), this fungi are an important food supplement for hypercholesterolemic patients and may also be a reasonable raw material for production of statins. The process we report in this work comprises different levels: culture of P. ostreatus at commercial level by local producers; characterization of these fungi; R&D on the relationship between nutrients and lovastatin levels; optimization of extraction procedures (ethanol, hexane, ethyl acetate) as basis for equipment design; process simulation and technical and economical feasibility evaluation.

ORAL PRESENTATION 6-05

Simultaneous Saccharification and Mixed Sugar Fermentation (SSMSF) of Acid Pretreated Rice Straw by Carbon Catabolite De-Repressed (CCR$^-$) *Lactobacillus pentosus* JH5XP5 and *Lactobacillus brevis*

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Lactic acid markets are growing rapidly worldwide, due to emerging applications as a platform chemical for food-grade organic solvents and biodegradable plastics. Despite the merits of lignocellulosic biomass serving as a feedstock for the biological production of lactic acid, commercialization has not been realized because of low yield and high processing costs. Higher yield at lower cost can be achieved with cost-efficient conversion of cellulose-to-glucose and complete utilization of all carbohydrates (pentoses and hexoses). Process design involving simultaneous enzymatic hydrolysis of cellulose coupled with concurrent fermentation of all carbohydrates to lactic acid has been found to reduce cost by increasing productivity and reducing the cost of enzyme. Lactobacillus brevis and Lactobacillus pentosus JH5XP5 were selected for this process due to a wide range of sugar utilization including lignocellulosic-derived carbohydrates. Without apparent carbon catabolite repression, both Lb. brevis and Lb. pentosus JH5XP5 were found to metabolize simultaneously and completely all fermentable carbohydrates present in a culture medium containing acid pre-processed rice straw hydrolysate. Lactic acid production was not affected by potential inhibitors in this mixture. Simultaneous saccharification and mixed sugar fermentations (SSMSF) were performed with 15 FPU (or CBU) / g-substrate of cellulase (Novozyme 188) and cellobiase (Spezyme cp) using acid preprocessed rice straw. Enzyme activities were maintained during the cell growth and lactic acid production of both strains, in the fed-batch operation of SSMSF. During the SSMSF, sugars are utilized concurrently and completely and enzyme activities were maintained maximum without feedback inhibition by glucose and/or cellobiose. As a result, it was shown that SSMSF required half the time and 6.6-fold less enzyme than a corresponding sequential hydrolysis and fermentation (SHF) process to produced same amount of lactic acid.

ORAL PRESENTATION 6-06

Bio-Ethanol from MSW Derived Paper

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BPI cooperated with UE to complete a pilot scale project to demonstrate bio-ethanol from garbage derived paper in 2004. The project consisted of: 1) separating paper from Municipal Solid Waste (MSW) using a 'pulverizer/air classifier being developed by UE- this classifier has the capability of separating paper (lights) from heavier components of MSW. 2) Taking the collected lights stream, and a) pulping the entire unsorted 'light fraction' from the classifier using a proprietary pulper and then collecting a purified paper pulp by running the stream over washing screens, 3) converting the collected pulp to ethanol in a 100 L pilot scaled Continuous Multi Stage Stirred Reactor Separator (CMSRS). We employed a 40 L semi-continuous cellulase 'hot saccharification' vessel followed by a 100 L 6 stage enzymatic Simultaneous Saccharification and Fermentation coupled with gas phase stripping of the ethanol.

In this paper we will describe our bench scale efforts to improve the process by developing a 'High Solids' hot saccharification to generate a feed stream of up to 200 g/L paper solids for the fermentation system and describe the performance of different cellulase enzymes.

ORAL PRESENTATION 6-07

Oligosaccharide Hydrolysis in Plug Flow Reactor Using Strong Acid Catalyst

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Liquid hot water pretreatment of plant biomass produces a liquid stream with dissolved oligosaccharides which are usually converted to fermentable sugars by enzyme hydrolysis. In previous work, strong cation exchanger, Amberlyst 35® W, has shown to hydrolyze cellobiose and oligosaccharides in liquid from corn fiber pretreatment at high conversion rates. This paper reports the effects of particle size, degree of cross-linking, and temperature on hydrolysis of oligosaccharides and degradation of monosaccharides. High temperature and short residence times were required to minimize formation of aldehydes and other fermentation inhibitors formation while achieving high glucose yield. The catalysts, SK104 (4% crosslinked gel type) and Amberlyst 35 (macroreticular sulfonic acid resin) were tested for hydrolysis of maltooligosaccharides at various reaction conditions. Maltooligosaccharides were used as a model oligosaccharides since their activation energy for bond breakage is similar to that of xylo- or cello-oligosaccharides, and since malto-oligosaccharides are more readily obtainable compared to the other types of oligosaccharides. Results show that low percentage cross-linked gel-type cation exchange resins give a higher glucose yield than macroreticular-type resins. The hydrolysis was diffusion limited in both resins. A mathematical model that quantifies diffusion and kinetic characteristics of this reaction is presented and potential application of plug flow reactors to hydrolysis of oligosaccharides obtained from pretreatment of cellulose is discussed.

Abstracts for Special Topics

ORAL PRESENTATION SPECIAL TOPIC A-01

Technical Progress in Bioconversion: Steps Towards Commercialization

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The world production levels of bio-based ethanol are rising rapidly in response to increased prices for oil, and a growing understanding of the need to improve the security of fuel supplies. Examples of policy decisions designed to support increased ethanol production and use are discussed in this paper, including the Brazilian experience of the 1980's and 1990's, as well as current Canadian initiatives for supporting commercial ethanol production. Lignocellulosics represent a new platform for the production of bio-based ethanol that may be adopted in many countries. Technology to support the lignocellulose-to-ethanol platform is beginning to be commercialized by industry. At least five major pilot facilities for lignocellulosic bioconversion currently exist or are under construction, including industry-led initiatives by logen and Abengoa. One of the remaining barriers to commercializing this process is the range of high costs associated with the enzymatic hydrolysis step. Research initiatives designed to address this problem are discussed. The move towards lignocellulosics will expand the range of areas suitable for the application of bioconversion technology. It will promote a higher level of transport fuel security by diversifying acceptable biomass sources.

ORAL PRESENTATION SPECIAL TOPIC A-02

Fuel Ethanol R&D in Sweden

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Sweden has built a unique R&D platform for the development of ethanol as a domestic renewable liquid fuel. A complete and integrated pilot plant for two-step dilute acid and enzymatic hydrolysis has been constructed. The pilot plant permits a large variation of process configurations and recirculation of various process streams. It has a capacity of 2 tons of dry substance equivalent to 500 l ethanol per 24 hours. A complete process development unit (PDU) with a scale of 10-100 liters in different unit operations, forms the link to fundamental research at universities and institutes. Work has focused on optimizing and maximizing the sugar yield in hydrolysis for a number of domestic and foreign raw materials including soft wood, hard wood and agricultural residues. For the fermentation of lignocellulose hydrolysates robust industrial yeast strains capable to ferment hexose and pentose sugars have been constructed using a number of metabolic engineering and fermentation strategies. Computer simulation permits technical and economical evaluation of the improvement of individual process steps, of variation in process configurations and of process integration in relation to the overall process economy.

ORAL PRESENTATION SPECIAL TOPIC A-03

Making a Business with Biofuels

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The energy crisis in 1973 gave the starting shot for the development of renewable transport fuels. Today security of supply, reduction of pollution and social development are worldwide acknowledged as the most important drivers for Biofuels. Besides technical barriers, which can be overcome by technological R&D, NTB´s - Non-Technical Barriers - are those, which impede the market development. NTB´s can be identified in the agriculture, the economic and financing sector, in the industry, on the market as well as in the legal framework but also in the awareness of the general public.

Sustainable transport systems based on renewable energy are must for the time after 2030. We should be aware that the change of energy systems is a long-lasting process. The challenge is the balance between short-term necessities and the long-term imperative. To be in time, strong efforts are needed today.

Success stories are based on wise political decisions. The bioethanol story in the US and in Brazil and the Biodiesel story in Europe show how to go forward. For the development reduction of production costs from the field to the road, efficient transport systems and an increased productivity of the agriculture as well as public awareness on the external benefits are needed. The future will not depend on the lack of energy - the availability of financial resources and missing political decisions are the real challenge.

ORAL PRESENTATION SPECIAL TOPIC A-04

Present Situation and Prospects on Bioethanol in Asian Countries

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Bioethanol as a substitute for fossil fuel is important in Asian countries, as in North America. An overview of the present situation for bioethanol production in Japan and other Asian countries will be presented, in terms of the current supply and demand conditions. The role of the Kyoto Protocol on how targets and policies are set in these countries will also be discussed. Based on these lines of information, future trends for bioethanol utilization will be forecast in Japan as well as in other Asian countries, allowing us to determine if the ethanol industry makes business sense for these countries. The possible opportunities for the Australian ethanol industry in Asia will be considered.

ORAL PRESENTATION SPECIAL TOPIC A-05

Liquid Biofuels in South Africa

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Bioethanol was produced from sugar cane in South Africa until the 1960s and then discontinued. Since the demise of apartheid, the country has ignored biomass as a potential source of liquid biofuels. Recently a white paper recently published by the South Africa government has addressed the national need to implement future renewable energy strategies and the government has recently attempted to stimulate biofuel production by reducing the fuel levy by 30%. This might assist in reaching the goal of renewable resources providing 4% of the total national energy requirements by 2013. Commercial production of biodiesel up to 80 000 tons per annum from soya will come on stream shortly although other crops are being currently considered. Considerable interest in bioethanol from sugar cane and maize has been expressed by industry although the process economics are still unfavourable without subsidies and price stability. The possibility of extensive job creation in depressed rural areas through new bioenergy industries is helping to stimulate further interest. No coordinated national bioenergy research program currently exists at present although various groups are working of the bioconversion of lignocellulosic biomass as feedstock for biofuels. South Africa recently joined IEA Bioenergy to interact with the liquid biofuel community.

ORAL PRESENTATION SPECIAL TOPIC A-06

Global Biofuel Potential – Sugarcane Contribution

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Biofuels can provide a significant share of the final energy used worldwide. Through identification of available sites for sugarcane plantation an assessment of future alcohol fuel supply and electricity cogenerated in sugar mill was performed. Result shows that all the new liquid fuel and electricity demand up to 2030 could be fully attended using presently available technologies with positive contribution to reduce poverty in rural areas. GHG emissions mitigation where quantified based in the full lifecycle emissions of both final energy forms – alcohol and electricity. The possibility of using carbon capture and storage technology during biofuel production and use yields negative GHG emissions. This complementary alternative should be considered either as a normal practice or as an emergency option in case abrupt climate charge risks and fast global actions to accelerate emissions mitigation are detected.

ORAL PRESENTATION SPECIAL TOPIC A-07

Key Barriers for Commercializing Biofuels from Lignocellulosic Biomass

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Biofuels and in particular bioethanol has gained increased interest during the last years mainly due to increased interest in decreasing dependency of oil, the interest in out-phasing MTBE and the interest for decreasing green-house gas emission from the transportation sector. Traditional bioethanol production from starch-rich crops such as corn, grain or sugar cane is well established and this industry is currently growing in size throughout the world partly driven by governmental subsidies for biofuels.

Bioethanol from lignocellulose such as agricultural residues, wood or energy crops is by far a more promising process and the over-all energy balance is far better compared to biofuels made from starch. Furthermore, lignocellulosic biomass will be able to deliver sugars for a price of less than half the price of corn or grain-based sugars. This will be necessary for competing with oil as a transportation fuel in the nearest future. The technology for lignocellulosic-based bioethanol is less mature and several processes steps are still lacking development. The technical status of lignocellulosic-based bioethanol is definitely one of the key barriers for large-scale investment in the area. During the last years promising steps have been taken both within pretreatment, enzymatic hydrolysis and fermentation, water reuse and by-product utilization. Pilot testing using more conventional technologies such as acid-hydrolysis as pretreatment is in progress in several countries today involving companies such as Abengoa and Shell. Furthermore, new concepts have been developed for instance in Denmark involving several new technical solutions such as the Danish Bioethanol Concept developed by DTU and Risø and the VEnzin Platform developed by Elsam. Both of these concepts are now going into pilot phase. Large-scale commercialization of lignocellulose-based bioethanol is awaiting results from all the different pilot tests, which now are in progress. If the outcome will be bioethanol to a price competitive with starch-based bioethanol we can foresee a bright future for bioethanol based on lignocellulosics just around the corner. However, if the same tests will not be able to deliver convincing results the development will await future technological jumps.

ORAL PRESENTATION SPECIAL TOPIC A-08

Perspectives on Biofuels Market Development in Canada

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The issue of creating markets for energy technologies has been the subject of considerable focus at the International Energy Agency over the past five years. The technological and market developments required to transform the energy system will be conceived and implemented largely in the private sector, while governments that value the wider benefits of cleaner and more efficient energy technologies will work in partnership with market actors to ensure there are real opportunities for technologies to make the difficult transition from laboratory to market. There are four primary and two secondary barriers to the development of an ethanol market in Canada. These include high ethanol price, inefficient market organization, finance risk and business risk (primary barriers), as well as price distortion and excessive/inefficient regulation (secondary barriers). In order for the ethanol industry to develop, there have to be solutions to address each of the primary market barriers. There are a variety of solutions to the issues that have been discussed along with the advantages and disadvantages of each solution.

ORAL PRESENTATION SPECIAL TOPIC A-09

Potential Barriers to Biomass Ethanol Commercialization

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Abengoa Bioenergy, a major fuel ethanol producer in the world, is currently operating starch ethanol production facilities in the U.S. and Spain. We are actively seeking opportunities to expand the ethanol production capacity worldwide. To ensure continuing long-term growth, we are developing biomass ethanol production technologies, and are in the process of designing and building a pilot plant facility in York, NE, and a commercial demonstration plant in Salamanca, Spain.

Biomass, because of its abundance and low cost, is an important feedstock for sustainable growth in the production of ethanol from renewable resources. However, there are a number of technical and economic hurdles that need to be resolved to make biomass ethanol commercially viable in the near future. Due to its unique chemical and physical characteristics, conversion of biomass to ethanol via enzymatic hydrolysis route, in comparison with cereal grain ethanol production, requires more processing steps which lead to higher capital and operating costs.

This presentation outlines several major technical and economic barriers of commercializing enzymatic biomass ethanol production and possible strategies for lowering these barriers.

ORAL PRESENTATION SPECIAL TOPIC A-10

Genencor's Perspective on the Key Barriers to Commercializing Biofuels

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Significant advances over the last 4 to 5 years within DOE-funded subcontracts have removed what clearly was the key technical barrier to commercializing biofuels, by developing cost-effective enzymes for the conversion of lignocellulosics. There are remaining technical challenges, including further improvements in enzyme specific performance, system integration, pretreatment, and the development of microbes for efficient conversion of biomass component sugars to ethanol and/or to other pathway products. In a traditional industrial biotechnology market, we would work with established processes and customers to address the remaining tehcnical steps at the level of optimization, integration and commercialization. However, the lignocellulosic conversion industry does not yet exist, and the further technical improvements are necessary but not sufficient for its creation. There is also a key commercial barrier: The cost of the technology and the process development for pioneer plants has to compete with low-cost incumbent technology and production systems. In these circumstances, public policy initiatives are crucial to successfully bridge the distance between basic discovery and commercial deployment. The presentation will review the current state of technology and describe Genencor's vision and role in the development of a biobased economy.

ORAL PRESENTATION SPECIAL TOPIC A-11

Progress on Enzymes for Biomass Utilization and Prospects for the Future

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Novozymes has worked for the past four years on reducing the cost of enzymes for the conversion of lignocellulosic biomass to fermentable sugars. During this time we have focused on decreasing the cost of producing enzymes on an industrial scale compatible with the biorefinery concept, as well as working to improve the efficiency of enzymes used in the saccharification of cellulose. This talk will summarize the state of the art in enzymes for biomass utilization, Novozymes' unique contributions to this area, and the cost impact of our work. In addition, prospects for further cost reductions and Novozymes role in the commercialization of biorefineries will be presented.

ORAL PRESENTATION SPECIAL TOPIC A-12

The Key Barriers to Commercialisation of Transport Biofuels

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At today's prices biofuels cost more to produce than the fossil fuels they replace. Unless consumers are willing to pay more, Governments have to give incentives to help to "make the market". Different countries have different drivers for encouraging biofuels for transport but the tools they can use are much the same: reducing taxes, implementing obligations, or giving capital grants. Even with incentives in place some countries do not see the growth in the use of transport biofuels that others do. This presentation discusses the possible reasons for this, and highlights the key barriers for the UK.

ORAL PRESENTATION SPECIAL TOPIC B-01

Growing Energy: How Biofuels Can Help End America's Oil Dependence

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A coalition of researchers analyzed the technical and economic potential of cellulosic biofuels production, and the R&D and policies required to achieve this potential. The potential is significant and can be achieved in a way that achieves environmental benefits (assuming sufficient protections for air quality and other issues), provides new markets for farmers, and is complementary to existing corn ethanol operations.

ORAL PRESENTATION SPECIAL TOPIC B-02

Biomass as a Strategy in Winning the Oil Endgame

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Liquid fuels made from farming and forestry wastes, or perhaps from energy crops, are normally considered to offer only a small potential at high cost. For example, classic ethanol production from corn, which now provides ethanol oxygenate equivalent to 2% of U.S. gasoline, could expand by only about half by 2025 if not subsidized. Modern plants of this type can yield net energy, but need favorable resale prices for their byproducts (mainly electricity and distiller's dried grains) to compete with gasoline.

However, that perspective is outdated. *State of the Art* technologies now permit biofuels in 2025 to provide 4.3 Mbbl/d of crude-oil equivalent at under $35/bbl ($0.75/gal gasoline-equivalent). This potential amounts to 15% of EIA's projected 2025 oil demand, or 31% of net demand after fully applying oil's end-use efficiency potential.

Of this potential, 99% is from ethanol, largely from lignocellulosic feedstocks. The new technologies whose roughly doubled yields, much lower energy inputs, and often lower capital costs make this potential so large and cost-effective include the Pearson Gasification Process, which is being demonstrated in pilot plants. The other 1% is biodiesel, an ester made by reacting an alcohol with vegetable oil.

Besides replacing transportation fuels with bio-based fuels, biomaterials offer a further 1 Mbbl/d of crude-oil displacement from petrochemical feedstocks, after petrochemical feedstock savings via plastics recycling, and a variety of biolubricants now emerging.

The main energy crops we examine are switchgrass and short-rotation woody crops such as hybrid willow and poplar, grown on the 35 million acres of arable land in the Conservation Reserve Program, which pays farmers to grow resource-conserving non-crop vegetation rather than traditional crops. Biofuels' domestic and largely rural production could benefit both the national economy and distressed rural economy, culture, and communities.

ORAL PRESENTATION SPECIAL TOPIC B-03

Expanding the Use of Biomass for Fuels – A View From Today's Bioethanol Industry

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The rapid expansion of the current bioethanol industry is being fueled and driven by the application of technology that starts with the production of corn. The U.S. has a comparative advantage in corn production and has expanded that advantage with the application of new technologies that are both cost effective and environmentally friendly.

The bioethanol industry is the first step in the vision of a transition for the U.S. from a hydrocarbon to a carbohydrate economy. The U.S. corn production industry has linked its future to this vision based on the twin benefits of expanded markets for their growing productivity and the opportunity to move up the value chain and take equity positions in these new industries. As this vision is fulfilled, the bioethanol industry is poised to supply the U.S. with sources of renewable energy, renewable materials, industrial chemicals and value-added food and feed products leading to an economic resurgence in rural America.

ORAL PRESENTATION SPECIAL TOPIC B-04

"Twenty-Five by Twenty-Five," Agriculture's Role in Ensuring U.S. Energy Security- A Blueprint for Action

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Project Overview

Through this initiative, grassroots agricultural leaders are working together to determine how large a role the ag sector can play in helping the nation move towards energy independence. The project has two primary goals: to help agriculture unite behind a common vision for energy production from America's farms and ranches, and to develop a comprehensive strategy to bring this vision to life.

Agriculture's Role

American Agriculture is well positioned to significantly expand its role in the development and implementation of new energy solutions. Long known and respected for its contributions to the production of food and fiber, an emerging opportunity exists for crop, livestock and grass producers, as well as tree farmers, to become major producers of another essential commodity—energy.

Consider these facts:

- The current carbon based energy paradigm is no longer sustainable
- Our nation and the world are looking for new energy solutions
- Energy, economic development, national security and environmental quality are inextricably linked

Corn and soybean producers have shown how the ag sector can become modern day energy providers—but it can play a larger role. America's farms and ranches can produce important fuels and feedstock needed to help our nation achieve energy independence.

Through emerging technology farmers and ranchers can dramatically increase the production of liquid transportation fuels; generate electricity by harnessing wind and solar energy and capturing and converting biogas emissions; and produce biomass and turn crop residues, ag byproducts and wastes into value added energy feedstocks.

Our Vision

Agriculture will provide 25 percent of the total energy consumed in the United States by 2025 while continuing to produce abundant, safe and affordable food and fiber.

Call to Action

The Ag Energy Work Group believes the time has come for the agricultural production sector to join together to develop and forcefully champion a bold vision that encompasses the production of clean, affordable and readily available feedstock for energy production.

We are inviting the leaders representing the nation's agricultural community to join us in an expanded dialogue about agriculture's role in ensuring U.S. energy independence.

For More Information:
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ORAL PRESENTATION SPECIAL TOPIC B-05

Fear and Loathing on the Energy Trail: Confusion, Convergence, and Divergence in the Public Dialogue about the Future of America's Energy Supply

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As a number of previous talks on this special session have pointed out, there is a real shift in attitudes—almost a sea change, if you will, regarding the view of bioethanol as part of a sustainable energy future. This is very exciting to see. In this talk, I will touch more broadly on the public debate about energy that frames these new points of view. On the one hand, there is a growing sense of concern about the immediate future of our oil supply. This growing concern has helped to catalyze the reassessment of biomass that we have witnessed in the past year.

But, the broader debate about energy is, in many ways, one that is so full of "noise" that there is a real risk that clear-sighted long term visions like the ones presented here could easily fall on deaf (if not confused) ears. Here is a case in point. Go to your local Barnes and Noble, and you might find these two books sitting on the shelf next to each other:

- Caltech professor David Goodstein's *Out of Gas: The End of the Age of Oil*
- Conservative think tank writer Peter Huber's" *Bottomless Well: The Twilight of Fuel, the Virtue of Waste, and Why We Will Never Run Out of Energy*

Such is the level of confusing and contradictory sound bites with which our public is constantly bombarded. Is it any wonder that the debate over energy in Congress is so badly broken?

Other examples abound, but my favorite is probably the long-standing stalemate of expert opinions regarding the "energy balance" of ethanol. The sense that ethanol made from corn has a "negative" energy balance has reached sufficiently mythical proportions that we can find very thoughtful and well-intentioned writers such David Goodstein declaring that ethanol's terrible energy balance "has long been known in the scientific community." Really? I find no such consensus in the literature. This is a great example of how sound bites can overwhelm the debate so much so that even the scientific community falls prey to its effects.

The point of these examples is not so much to bemoan the dreadful state of public debate, but to call even more strongly for responsible, informative, balanced and credible discussions about our energy future and the role that biomass can play in it. And let's start with some basic education about thermodynamics and clarity in terms. Let's avoid oversimplified, condescending discussions of energy, and really get down to the nitty gritty of the 2nd law of thermodynamics, which will help all of us to understand that all processes which convert one form of energy into a more useful form have a so-called "negative" energy balance. The likes of Sadi Carnot wouldn't have it any other way. Then let's move the discussion toward an understanding of renewable and sustainable energy sources versus fossil fuels. I am a firm believer in people's ability to "get it" if you give them a real chance. And if we can successfully inform the debate, we just might see things really happen for biomass over the next few years.

Poster Abstracts for Session 1A

Feedstock Supply and Logistics

POSTER PRESENTATION 1A-08

Assessing the Value of a Targeted Corn Stover Harvest by Understanding the Distribution of Inorganic Nutrients

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The overall objective of this project is to establish benchmarks for the distribution of inorganic nutrients within the stover portion of the corn plant after physiological maturity. An assessment of the effects of targeting specific sections of the plant for in-field separation during a stover harvest is of particular interest. This work will identify stover harvest methods that would minimize nutrient removal, and provide potential stover cost (savings) to biomass procurement, transportation and harvest, considering changes in density, dry matter, yield and typical retail value of various nutrients. This study was designed to address this issue over a period of four years. Results from the first two years are now available and some preliminary observations will be made.

Replicate rows of five popular hybrid corn varieties were planted at three locations that have different soil types. The variables explored in this experiment include the effect of location (3 locations; includes weather patterns, soil types and available nutrients), irrigation, year, planting date (early vs. late), tillage practice (till vs. no-till), population density (normal vs. high), and genetics (5 hybrids) on the amount of several nutrients sequestered in the stover. Stover was harvested at the same time as grain and partitioned into 3 segments along the vertical axis of the plants, simulating different cutting heights and stover removal percentages. The mass of dry stover represented by each fraction for each treatment was determined, as was the moisture content of each fraction at harvest. The amounts of P, K, S, Ca, Mg, Na, Fe, Mn, Cu and Zn sequestered in dried stover fractions were determined by standard analytical methods. The amount of free sugars remaining in stover fractions was also determined. The impact of stover removal on soil quality, farming practices, and biomass processing in the context of a continuous corn farming strategy will be discussed.

POSTER PRESENTATION 1A-09

Development of a Multi-Criteria Model for Ranking Biomass Feedstock Collection and Logistics

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This paper uses an innovative multi-criteria approach for ranking biomass feedstock collection and transportation options. Most of the studies done to evaluate these alternatives focus on the least cost option. For a long term sustainable biobased economy, social and environmental factors also need to be weighted. This study takes into account not only financial factors but also social and environmental factors to rank different options. Giving higher importance to one criterion can only be done at the expense of others. A multi-dimensional approach helps in solving this type of complex problem.

PROMETHEE (Preference Ranking Organization Method for Enrichment and Evaluations), is used to rank the different options based on selected criteria. PROMETHEE is a well-established decision support system and is used to rank *n* alternatives ($a_1, a_2, \ldots a_i, \ldots\ldots a_n$) based on *k* evaluation criteria ($f_1, f_2, \ldots f_i, \ldots f_k$). Alternatives are compared in pairs, based on each criterion, and the result is a preference of one over the other and is given as a real number, $P_i(a_1, a_2)$. $P_i(a_1, a_2)$ is estimated using a preference function. Six different preference functions are defined in the PROMETHEE, which covers almost all the possible criteria. Each criterion is allocated a particular weight, π_i which is a measure of the relative importance of the criterion. The multi-criteria preference index, $\Pi(a_1, a_2)$ for a particular alternative is the weighted average of the preference $P_i(a_1, a_2)$.

$$\Pi(a_1, a_2) = [\Sigma \pi_i P_i(a_1, a_2)] / \Sigma \pi_i$$

This preference index is used to estimate *leaving flow* $\Phi^+(a_1)$, *entering flow* $\Phi^-(a_1)$ and *net flow* $\Phi(a_1)$.

$\Phi^+(a_1) = \Sigma \Pi(a_1, a_i)$, where a_i varies from $a_1, a_2, \ldots a_n$.

$\Phi^-(a_1) = \Sigma \Pi(a_i, a_1)$, where a_i varies from $a_1, a_2, \ldots a_n$.

$\Phi(a_i) = \Phi^+(a_i) - \Phi^-(a_i)$, where a_i varies from $a_1, a_2, \ldots a_n$.

Leaving flow denotes the dominance of an alternative over other alternatives and is a measure of *outranking character*. *Entering flow* is the measure of *outranked character*. Alternatives with higher value of *leaving flow* and *net flow* are ranked higher. This method is used by *Decision Lab* software, developed by *Visual Decision Inc.*, to rank different alternatives.

In this study collection options for agricultural residues (corn stover and straw) include: (1) conventional baling three pass system (harvest grain, shred or rake crop residue, bale biomass, transport bales to the field edge and stack, load bales on truck and transport to biorefinery, unload and grind the biomass), (2) loafing two pass system (harvest grain, shred or rake crop residue, load a stacker (loafer) with biomass, transport the stacker (filled with biomass) to the side of the farm, unload the stacker, load a grinder, load the grind into truck, transport the filled truck to biorefinery), (3) chopping and ensiling two pass or single pass system (harvest grain, chop stover (forage harvester), load wagon, transport wagon to the silage, ensile, load silage truck, transport to biorefinery). The Integrated Biomass Supply Analysis and Logistics (IBSAL) model developed at ORNL is used to plan resources and the costs, energy use, and emissions for each of the collection options. Transportation options include: truck transport, pipeline transport, and rail transport. Criteria for ranking both the collection and transportation alternatives is based on financial, environmental, social and technical factors (for example, cost of biomass feedstock for each alternative, maturity of each technology, social impact, and environmental impact).

POSTER PRESENTATION 1A-10

Tools and Logistics for Selective Feedstock Harvesting

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The economics of bioenergy production will determine its success. Among the new technologies that must emerge to make bioenergy production feasible are technologies that will reduce the cost of the feedstocks delivered to the biorefinery.

The economics associated with delivered feedstock can be enhanced by improving the efficiency of the integrated feedstock production, harvest, and transport system, and by improving the quality of the delivered feedstock.

During wheat harvest in Idaho in 2003 and 2004, we field tested two selective harvest systems that used existing grain combines to selectively harvest subcomponents of the crop residue. In 2004 one of the systems was used to capture two separate discharge streams from the unmodified grain combine. Samples from these streams will be described for their physical and chemical makeup.

The two harvesting systems and the differences in the output streams will be discussed. This information will be used to suggest emerging technologies that should be explored to develop tools and logistics for selective harvest that will reduce the cost of the feedstock delivered to a biorefinery.

POSTER PRESENTATION 1A-11

Assessment of Crop Residual in North Carolina as a Sustainable Feedstock for Bioethanol Production

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The available agricultural residue for ethanol production in North Carolina was assessed based on the survey results. This presentation will include the available crop residue and the ethanol potential in North Carolina on county base. The economic feasibility of ethanol production in North Carolina will also be presented.

The amount of crops residue from corn, oats, sorghum, soybean and wheat that can be sustainable collected is estimated to be about 1.5 million dry tones/yr based on 2001 crop year. One third of these residues are corn stover, which has the highest potential for ethanol production. Currently about 45% of these residues are used for field cover, 25% for mulching, 16% for feeding and 5% for bedding. The majority of these feedstocks would be available for ethanol production in the near term while only a small portion is required to leave on the field. Only two counties in North Carolina have the potential to provide enough feedstock for an ethanol plant with a capacity of 10 million liters per year. All other counties need a cluster of two or more counties to provide enough feedstock for an ethanol plant of the same size.

POSTER PRESENTATION 1A-12

Feedstock: What Is The Value? A Regional Study

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In the next ten years biorefineries are expected to be processing biomass, each requiring One Million dry tons (dt) or more annually—initially crop residues like straw and stover for the production of fuels and chemicals. There remains a large amount of uncertainty in the feedstock supply, its cost, reliability and environmental impact of removal. Benefits for changing farming practices must be demonstrated to the farmer and the potential processor must be confident that the infrastructure is secure with stable pricing and a suitable Life Cycle Analysis.

A preliminary study initiated by the Imperial Young Farmers and Ranchers and the City of Imperial, NE estimated the surrounding area can supply *6 Million dt/yr at $30/dt delivered* to the biorefinery. The *net margin to the farmer will be $12/dt, $70/ha or more.* This feedstock is equivalent to *1.7 Billion liters ethanol annually*. Revised crop practices are estimated to sequester *1 Million tons soil C/yr.*

A 3 year, $3 million project is described to validate these economic and environmental benefits, with $2 million provided by the USDA NRCS. Will different cropping practices prove beneficial? Will the economics justify these investments? *Improved information is needed* to better enable the farmer and other stakeholders in the area to establish a better basis for decisions to be taken as biorefining opportunities emerge. Such information includes the following:

- Sustainable Removal
- Feedstock Value
- Innovative Collection, Storage and Transporting Systems
- Delivered Cost of the Feedstock
- Life Cycle Modeling of the System
- Feedstock Processing

The results will better identify where extensions are needed for insuring sustainable and economic supply.

POSTER PRESENTATION 1A-13

Upgrading Corn Stover to Slurried Feedstock for Optimized Transport and Conversion

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Transforming biomass on-farm to a slurry or liquid form that can be transported via pipelines to a biorefinery is a viable solution to cost-effective feedstock delivery. Slurry feedstock is compatible with the biomass-to-ethanol conversion platform and will reduce production cost by reducing the cost of feedstock handling, pretreatment/conditioning, and saccharification and fermentation. This paper will present a pathway for upgrading corn stovers into a higher value feedstock in slurry form. Preliminary studies of corn stover physical properties in relation to slurry transformation, slurry physical and flow properties, chemical composition, and mass and energy balances during slurrying will be presented. Issues regarding storage of slurry feedstocks, quality monitoring and ethanol conversion yields will also be presented. In addition, we will highlight the potential impacts of upgraded slurried feedstocks on the minimum ethanol selling price (MESP).

POSTER PRESENTATION 1A-14

Combustion Profile of Biodiesel Manufactured from Rapeseed Oil in Diesel Engine

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Fatty acid methyl esters show large potential applications as diesel substitutes, also known as biodiesel fuel. Biodiesel fuel as renewable energy is an alternative that can reduce energy dependence on petroleum and reduce air pollution. Several processes for the production of biodiesel fuel have been developed. Transesterification processes under alkali-catalyst with short-chain alcohols give high yields of methyl esters in short reaction times. The ratio of fatty acid methyl esters in mixed biodiesel fuel ranges between 5 and 30 wt%. Biodiesel is compatible with petro-diesel in compression-ignition engines, and mixtures of biodiesel and petro-diesel have been used without any need for engine modification. Also, biodiesel has many advantages compared to petro-diesel has a more favorable engine combustion emission profile, such as low emissions of carbon monoxide, particulate material, and unburned hydrocarbons in exhaust gases with proven carcinogenic and mutagenic effects is significantly lower. In this study, we performed the engine combustion properties of biodiesel manufactured from rapeseed oil.

POSTER PRESENTATION 1A-15

Characterization and Thermogravimetric Analysis of Corn Stover SSF Residues

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Simultaneous saccharification and fermentation (SSF) of pretreated biomass feedstocks produces residues constituting a significant fraction of the starting material. These residues are composed of lignin (50 - 60%), cellulose (10-12%), xylan (less than 2%), proteins (10-13%), and ash (10-16%). Several potential applications have been proposed for these residues including combustion and/or conversion to diesel fuel or adhesives. However, accurate characterization and analysis of this material is required to determine its value. We conducted elemental, calorific value, summative, and thermogravimetric analysis of corn stover SSF residues. The corn stover SSF residue, which was supplied by NREL, had a higher heating value (HHV) of 20.9 MJ/kg and a very complex thermal decomposition pattern.

The differential thermogram (DTG) in nitrogen atmosphere showed very sharp maxima at 328 °C and 408 °C at a heating rate of 5 °C/min. The DTG differ considerably from that of Aspen wood, which showed broad peaks at similar heating rates. Microcrystalline cellulose sample pyrolyzed at a similar heating rate had a sharp maximum at 400 °C, suggesting that the cellulose in the residue has microcrystalline characteristics. Since the residue is predominantly lignin, we attributed the first sharp peak at 328 °C to lignin, but this seems to contradict published literature, which shows a broad diffused peak for the lignin DTG. It appears that the pretreatment process has changed the residual lignin properties, imparting some unique decomposition characteristics.

POSTER PRESENTATION 1A-16

Economic Feasibility Analysis of Municipal Solid Waste to Ethanol Conversion

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Ligno-cellulosic portion of municipal solid waste (MSW) is considered a potential feed stock for fuel ethanol production. We review the trends in MSW generation, composition and disposal practices, and evaluate the aggregate and regional potential of MSW as a feedstock. We present an overview of the current technology of MSW to ethanol conversion. An attractive feature of MSW-ethanol conversion is that the feedstock is available at a negative cost; i.e. disposal facilities charge tipping fees ranging from $15-$100/ton to accept MSW. We assess the financial feasibility of a typical MSW-ethanol plant with a capacity of 500 tons per day under a number of scenarios with respect to tipping fees, ethanol prices, capital costs, byproduct prices and ethanol tax incentives. We find the profitability to be robust across scenarios. We then discuss technical, economic, environmental and social barriers that inhibit commercialization.

POSTER PRESENTATION 1A-17

New HPLC Methods for Analysis of Biomass Pretreatment Processes and Hydrolysates

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Pretreatment and chemical analyses are enabling steps in the bioconversion of biomass feedstocks to various products. For example, pretreatment processes can be used to increase surface area, reduce cellulose crystallinity, release hemicellulose from the biopolymer matrix and thereby increasing cellulose hydrolysis rates and yields

We have developed a new high performance liquid chromatography (HPLC) method for analyzing monomeric and dimeric sugars in biomass hydrolysates. The new method, which uses acetronitrile/water mobile phase, Prevail™ carbohydrate column, and an evaporative light scattering detector, does not require neutralization of the hydrolysate, and can achieve baseline resolution of most common sugars (arabinose, cellobiose, fructose, galactose, glucose, mannose, sucrose, and xylose) present in biomass hydrolysates. The method was used to monitor the influence of the pretreatment processes on the composition of corn stover hydrolysates. The xylose concentration ranged from 65 mg/ml to 28 mg/ml depending of the pretreatment condition, while the fructose and cellobiose varied inversely with the glucose concentration. The glucose concentration ranged from 44 mg/ml to 14 mg/ml, fructose ranged from 9 mg/ml to 1 mg/ml, and cellobiose ranged from 5 mg/ml to 0.4 mg/ml. The inverse relationship between the concentration of glucose and the other sugars was attributed to the decomposition of sucrose in the corn stover and the release of glucose from the hemicellulose or cellulose depending on the severity of the reaction conditions. Trace amounts of sucrose were detected in the hydrolysates.

POSTER PRESENTATION 1A-18

Biomass Availability for the Biorefining Strategy in Canada: Suggestions for Policy Reform

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A lignocellulosic-based biorefining strategy may be used to reduce North American dependence upon fossil oil, and to produce renewable energy, fuels and chemicals that can supply social needs. To keep the cost of these products low, a regular supply of inexpensive biomass must be made available, in essence diverting feedstock from higher-value agricultural or forest products. Significant amounts of biomass may be available through the use of innovative management techniques which reduce vulnerability of feedstock in the agricultural or forest products supply chain. Diversity, connectivity and wealth are variables that can describe increasing vulnerability in ecosystems as they progress through growth and harvest.

Energy plantations on marginal farmland may be used to increase the diversity of farming operations, which reduces the vulnerability of these systems to drought. Currently available marginal farmland might produce between 9 and 19.5 MMT of additional feedstock. Increased harvest residue removal, disturbance isolation, and pre-commercial thinnings might be combined to reduce the wealth and connectivity of forest operations, reducing the vulnerability of these systems to insect, disease, or fire disturbances. Conservative recovery of a fraction of this material might produce an additional 20 MMT of feedstock for the biorefinery. Specific policy reforms are suggested that are targeted to each of these variables.

POSTER PRESENTATION 1A-19

Experimental and Numerical Analysis of the Biomechanical Characteristics of Agricultural Crop Residues

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The lack of understanding of the biomechanical properties and structural characteristics of cellulosic feedstocks is a limiting factor in economically collecting and processing crop residues. To address this issue, a numerical analysis of the biomechanical and structural behavior of crop residues was performed using finite element analysis techniques and applying composite theory. Representative geometries of the specific structural components including the hypoderm, ground tissue and vascular bundles, were established using microscopy techniques. Material property data for the analysis was obtained from experimentally measured data. Numerical results from different mechanical loading models were compared with the respective experimental data. Results of this comparison help establish the validity of the models and provide a basis for applying their results to the mechanical operations of harvesting and preprocessing equipment. A thorough understanding of the biomechanical and structural behavior of crop residues can help optimizing plant varieties and develop more energy efficient harvesting practices.

POSTER PRESENTATION 1A-20

Biofiltration Methods for Biological Removal of Phenolic Residues

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Phenolic compounds, widespread applied in pharmaceutical-chemical and petrochemical industry, are often present in industrial effluents in toxic levels which can give rise serious environment problems. When compared with traditional techniques, the biological means are generally friendlier for the treatment of such residues, because they are usually cheaper and release less byproducts. Biofiltration has been increasingly proving to be particularly effective for the biological removal of contaminants by using microorganisms immobilized in solid and porous supports, which can be applied for the destruction either of gaseous or liquid pollutants. Biofilters have been also developed for the removal of phenols and chlorophenols, among others. The effluents under investigation were present either in liquid or in gaseous phase and the reported results seem to be satisfactory for large-scale application of such a technology for the treatment of these effluents.

Continuous and batch processes have suggested that the choice of the process and reactor configuration can be influenced by the residue to be treated or the microorganism employed. The most commonly used microorganisms (*Pseudomonas sp.*, *Acinetobacter sp.* and *Bacillus sp.*) are immobilized onto different types of inert supports, even if new immobilization procedures, employing cheaper materials, such as sugarcane bagasse, could be an interesting alternative to further reduce the operating costs of biofiltration. The aim of this work is to provide an overview on the main aspects of biofiltration for the treatment of different industrial effluents, with particular concern to those coming from pharmaceutical and petrochemical industry.

POSTER PRESENTATION 1A-21

Rail Transport of Biomass in Canada

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Biomass is a low density feedstock with a low energy content compared to coal or oil. Two issues arise with transportation of biomass: achieving lowest possible cost and avoiding road and community congestion during delivery. Train transport has promise in each of these areas.

All field harvested biomass starts its journey on a truck, and thus the key question for train transport is whether it is economic to transfer it to a train. Since train transport incurs fixed costs for loading and unloading, but also achieves lower distance variable cost, there is a distance above which it is economic to use truck plus train transport instead of truck only. This study evaluates the relative economics of truck only and truck plus train transport for two abundant sources of biomass available in western Canada: grain straw and wood chips from forest harvest residues (tree limbs and tops left after harvest for pulp or lumber). The minimum economic rail shipping distance is determined for each.

POSTER PRESENTATION 1A-22

Year One: Agronomic Experience in Growing No Tillage Switchgrass Focusing on Seeding Rate, Nutrient Needs, and Landscape

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Switchgrass has been planted at The University of Tennessee's Milan Experiment Station located in west Tennessee. Four different soil and physiographic positions were chosen to represent the main soils in the area. These soils were located on: 1.) a level floodplain that was well to moderately well drained, 2.) a level floodplain that was poorly to somewhat poorly drained, 3.) an upland sloping hillside that had a naturally occurring hardpan, and 4.) an upland level that was well drained with no hardpan. These situations represent a wide range of expected switchgrass productivity levels.

A seeding rate experiment is conducted on the four landscapes. The productivity of each landscape-seeding rate combination will be evaluated. In the second year, a nitrogen fertilization study will be superimposed upon the seeding rate experiment. Four nitrogen fertilizer rates will be applied to each seeding rate. The purpose of the study is to determine optimum seeding and fertilization rates for each soil and landscape position.

In an adjacent area on each field, a comparison is being made of the commonly planted variety of switchgrass to three other new hybrid varieties, two from the breeding program in Georgia and one from the program in Oklahoma. Preliminary testing has indicated that these new varieties have considerably higher yield potential as compared to the common variety. Initial year yield data for the soil/landscape and seeding rates will be reported along with information on trials and tribulations of growing switchgrass. These data will be useful in variety selection by producers and the potential commercialization by seed companies supplying switchgrass seed. These two studies were formulated after careful review of existing knowledge on switchgrass production to determine the key questions remaining.

POSTER PRESENTATION 1A-23

Comparison of Physical, Chemical, and Anatomical Characteristics of Corn Anatomical Fractions

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A major goal of the DOE Office of the Biomass Program's Sugar Platform R&D area is the discovery of the root causes of the recalcitrance of lignocellulosic biomass to deconstruction. To address this question from the perspective of the anatomical structure of corn, several individual field-grown corn plants from a single inbred variety (Mo17) were dissected into distinct fractions, including the internode, node, leaf sheath, cob, stalk rind, and stalk pith. The chemical composition, specific gravity, and relative porosity of each of these fractions were determined. In addition, quantitive analysis of cross-sections of the internal structural features of four of these tissues was performed using light microscopy and image analysis software. Each structural tissue in plants is known to exhibit a unique pattern of physical, chemical, and anatomical characteristics, which may help explain observed differential performance in dilute acid pretreatment reactions (see the poster by D. Johnson, et al). This work supports ongoing programmatic research addressing the relationships between feedstock ultrastructure and the effect(s) of various pretreatment chemistries on enzymatic digestibility.

POSTER PRESENTATION 1A-24

Estimated Economic Impacts on the Agricultural Sector and the Nation's Economy of Supplying Feedstock to an Energy Sector

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The objectives of this analysis are to estimate total national production possibilities, the geographic areas of concentration of the feedstocks and total savings from government payments to traditional crops resulting from following a bio-energy strategy based on the use of cellulose feedstock. This study will also provide estimates on the economic impacts a mature industry will have on state economies throughout the United States from utilizing feedstock – switchgrass and cellulose crop residues – under two different price guarantee scenarios —$40.00 and $50.00 per dry ton. The economic impact indicators used in the analysis include total industry output and employment. The analysis includes the acreage and price impacts that would occur to traditional crops as switchgrass acreage and residues energy use increases.

The analysis indicates that at a price of $40 per dry ton offered to both switchgrass and residues, U.S. agricultural lands could produce 53 million tons of switchgrass and 100 million tons of crop residues for a total of 153 million dry tons of biomass. Individual supply curves deviate slightly from these production levels, as there is very little competition for resources between the two feedstocks. Switchgrass production competes for land resources; therefore, raising traditional commodity prices, reducing government support payments and increasing net returns in all regions. When both switchgrass and residues are produced, farmers realize net gains similar to switchgrass produced individually. At $40 per dry ton, U.S. switchgrass and residue production transformed into ethanol could displace 5.3% of all domestic gasoline consumption.

In the Switchgrass Only Scenario, there is a projected economic gain of $6.6 billion at a residue price of $50/ton as a result of growing and harvesting switchgrass. As a result of increases in commodity prices coupled with decreases government payments, economic activity is projected to increase $6.3 billion, resulting in a net gain of $12.9 billion and 95 thousand jobs. If the residue price is set at $40/ton, $3.3 billion in economic activity is generated from the growing and harvesting of switchgrass, and is augmented by an increase in total economic activity by a projected $4.4 billion, resulting in an overall benefit of $7.7 billion and close to 55 thousand jobs.

POSTER PRESENTATION 1A-25

Potential Perennial Biomass Feedstocks for the Southern United States

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The majority of the research on lignocellulosic crop biomass for biofuels has been centered on corn stover and switchgrass (*Panicum virgatum* L). However, diverse farm practices and subtropical climates of the Southern Coastal Plains of the United States make it more conducive to other biomass feedstocks such as perennial forage and bunch grasses. This talk will review the present data and potential of these grasses for production and use as fuel, fiber and soil/water remediation tools. Bermudagrass (*Cynodon dactylon* L.) and bahiagrass (*Paspalum notatum* Fluegge) are already produced extensively in the Coastal Plain as a forage crop. Sugar cane relatives (*Saccharum* sp.) has been assessed as energy cane in parts of the south. Napiergrass (*Pennisetum purpureum* Schumach.) and giant reed (*Arundo donax* L.) have been shown potential to produce over 20 tons DM/acre/year. Giant reed produces fiber that is comparable to hard wood which could be useful in paper/pulp and fiberboard industries. Use of these grasses for ethanol production systems, syngas or in co-firing electrical plants will be reviewed. Assessment of most promising biomass species and suggestions for future research will be discussed.

POSTER PRESENTATION 1A-26

The Use of Organic Dyes in Biomass Storage

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It is estimated that by the year 2030, biomass will need to provide 5% of U.S. power. Because agricultural biomass is harvested in a narrow time window, facilities requiring a constant supply will force biomass storage. As chemical quality analyses of the biomass during storage are expensive, there is a need for inexpensive tools to optimize storage methods and parameters of biomass feedstock. A simple colorimetric indicator to trace water flow in the biomass, and ultimately to indicate sugar degradation, would be ideal. The goal of this project was to evaluate several dyes for these properties.

Using three dyes currently legal for human consumption, with corn stover and wheat straw, we found unique properties for each dye. All dyes were water soluble and adequately stained the biomass for visual observations. FD&C Blue #1 was found to be chemically and microbiologically inert, suitable for use as a water tracer in the field. FD&C Blue #2 was a reversible redox active dye, and FD&C Red #40 was an irreversible redox active dye, both changing to colorless in response to microbial reduction. Using dyes as inexpensive water flowpath indicators will greatly assist the optimization of large scale biomass storage systems supporting integrated biorefineries.

POSTER PRESENTATION 1A-27

Effect of Fertility Management on Composition of Plant Fractions from Wheat

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The costs associated with the collection, handling, and storage of wheat stover are a major hurdle to the implementation of biomass feedstocks. The variation in composition of wheat plant fractions has not been well quantified. During 2004, wheat was collected by hand prior to harvest and separated into anatomical fractions (nodes, internodes, leaves, and chaff). Samples were collected from fields that employed a fertility program based on a decision support tool (dss4ag) that took into account hypothetical straw prices of $10 and $50/ton, in addition to the producer applied rate in Idaho.

Plant fractions are being analyzed for lignin, ash, glucose, and xylose composition using acid hydrolysis. Previous data has indicated that nodes are high in ash and are not as desirable. Cellulase will be added to plant fractions in shaker flasks to evaluate the enzymatic digestibility and efficiency of the conversion process. Preliminary data indicated that the leaves and sheath released approximately twice the glucose than nodes, internodes, or chaff. This would indicate that systems that preferentially collected leaves and sheaths might require less severe pretreatment methods. The data will be useful for determining the value of plant fractions and designing alternative collections systems.

POSTER PRESENTATION 1A-28

Corn Stover Quantity and Composition as Influenced by Agronomic Practices

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Corn was grown on a silt loam soil during 2003 and 2004 using no-till practices. During 2003 side-dress nitrogen rates were varied between 0 and 360 kg/ha (0 and 320 lb/ac) constant plant population (74,130 seeds/ha, 30,000 seeds/ac). During the 2004 season the seeding rate was varied between 59,300 and 88,960 seeds/ha (24,000 and 36,000 seeds/ac) and side dress nitrogen application rates between 0 and 280 kg/ha (0 and 250 lb/ac). The total quantities of cobs, leaves, husks, stalks, and grain were measured in each plot.

The moisture content and total available dry matter for each stover component was measured. Samples were ground, dried, and scanned using near infrared reflectance spectroscopy. The quantity of nitrogen, carbon, phosphorous, and potassium was determined for each stover fraction and agronomic practice. Enzymatic digestibility of the stover components were evaluated using shaker flasks and cellulase.

Stalks were highest in lignin while husks were lowest and increased with increasing nitrogen application. Ash was highest in the leaves (>5.7%) and stalks (~4.0%) and less than 2.6% in the cobs and husks. Glucose concentration was highest in the cobs and husks (~42%) and lowest in the stalks (~36%). No difference in glucose concentration was noted with varying N application rates or population changes.

Poster Abstracts for Session 1B

Enzyme Catalysis and Engineering

POSTER PRESENTATION 1B-08

Expression of Nisin by *Lactococcus lactis* in a Fermenter and Detection by Two Nisin-Sensitive Bacteria

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Nisin, a bacteriocin produced during the exponential growth phase of *Lactococcus lactis*, inhibits the growth of a broad range of gram-positive bacteria. Gram-negative bacteria can also be inhibited by nisin if the outer membrane is first destabilized by EDTA. In this study, nisin expression was related to growth conditions (100 rpm/30°C/36h) and the effects of the media components (skimmed milk; M17 and MRS diluted 1:1 with liquid skimmed milk) through five transfers of *L. lactis* ATCC 11454 cultures into fresh media. Nisin production was assayed by agar diffusion using *Lactobacillus* sake ATCC 15521 and a recombinant *Escherichia coli* DH5α expressing green fluorescent protein (GFPuv), as the nisin-sensitive test organism.

The titers of nisin expressed and released in culture media were quantified and expressed in arbitrary units (AU. mL^{-1} of medium) and converted to standard nisin concentration (Nisaplin®, 25 mg of pure nisin with an activity of 1.0×10^6 $AU.mL^{-1}$). The 9.09% total solids in 100% milk media favored the expression of nisin in all transfers (maximum 20,000 $AU.mL^{-1}$ in the 5th transfer). At half concentration, 50% milk media, nisin activity was reduced from 10-fold to 300-fold, at the 2nd transfer (1,397 $AU.mL^{-1}$) and the 5th transfer, respectively. However the incorporation of 50% milk with 50% MRS (half the standard MRS formulation) provided similar nisin activity (3,497-8,755 $AU.mL^{-1}$) for all transfers. The expression and release of nisin by *L. lactis* in milk with MRS broth (1:1), at 30°C/24h/200rpm, was monitored in a 1.5 L New Brunswick fermenter with an air flow of 1.5 $mL.min^{-1}$, without pH control. The added milk confirmed a positive influence in the expression and release of nisin and was shown to be the best component to add to synthetic media.

POSTER PRESENTATION 1B-09

Stability of Green Fluorescent Protein (GFPuv) in Chlorine Solutions of Varying pH

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Green fluorescent protein (GFPuv) is an excellent biological indicator (BI) due to its ability to be easily monitored in a wide variety of applications. The purpose of this work was to study GFPuv stability in chlorinated water for injection (WFI) and chlorinated potassium phosphate buffer (10 mM; pH 6 and 7), with and without agitation, to determine (i) the exposure time required for the chlorine and pH to lower fluorescence intensity 90% (decimal reduction time, D-value, min, 25°C), and (ii) to determine which buffer system provides optimal conditions to analyze the effectiveness of chlorine upon protein denaturation, which may be related to disinfection efficacy.

Fluorescence intensity (Ex/Em_{max} = 394/509 nm) was measured immediately after the addition of GFPuv (8.0-9.0 μg/mL) to constantly stirred solutions with chlorine concentrations ranging from: (i) 40ppm-160ppm in WFI (pH=10.6-10.9), in which the initial loss of 20-68% fluorescence occured; (ii) 10ppm-200ppm in phosphate buffers (pH=6.0-7.0), in which no change of initial fluorescence was detected up to 50ppm chlorine. The stability of GFPuv drastically dropped for chlorine concentrations >100ppm, which coincided with lowest D-values, between 1.3 min (147.11ppm) and 1.7 min (183.9ppm). For concentrations ≤100ppm chlorine, the highest GFPuv stability was exhibited in phosphate pH=7.0, with D-values ranging from 555.6 min (51.8 ppm) to 83.33 min (93.56 ppm), decreasing 10-fold to 55.6 min with 110.33ppm chlorine. For not stirred solutions, a regeneration of GFPuv fluorescence was observed with chlorine at 30ppm-100ppm in WFI. Complete mixing of GFPuv in solution is essential and provides for more uniform contact of the protein to the solvent system throughout the assay and permits more accurate determination of D-values. GFPuv performed as a suitable fluorescent BI for monitoring disinfection effectiveness.

POSTER PRESENTATION 1B-10

Thermal Stability of Recombinant Green Fluorescent Protein (GFPuv) in Glucose Solutions at Various pH Conditions

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Large Volume Parenteral Solutions (LVPS), the most widely used medications in health care (about 300 million units used in Brazil per year) are used in the replacement of body fluids, nutrition and as delivery vehicle for the administration of other medications. Glucose-based LVPS (5-50%), largely employed, must be assured with a sterility level by thermal processing, which efficacy should be provided by an appropriate biological indicator (BI) that supplies fast, accurate and reliable results. The purpose of this study was to evaluate the influence of glucose concentrations upon the thermal stability of recombinant green fluorescent protein (GFPuv) and its adequacy as BI for moist-heating.

GFPuv (3.5-9.0 μg GFPuv/ml), expressed by *E. coli*, isolated by TPP extraction with HIC, was diluted in buffered (each 10 mM: Tris-EDTA, pH 8; phosphate, pH 6 and 7, and acetate, pH 5) and in unbuffered (water for injection, WFI; pH = 6.70±0.40) glucose solutions (from 1.5% to 50%) and exposed to temperatures between 80°C and 95°C. The extent of protein denaturation (measured as the loss of fluorescence intensity) was expressed in decimal reduction time (D-value, min), the exposure time required to reduce 90% of the initial fluorescence intensity of GFPuv. At 95°C, the D-value for GFPuv in 1.5%-50% glucose, respectively, ranged from: (i) 1.56 to 1.94 min in acetate, (ii) 2.18 to 3.06 min in WFI; (iii) 2.35 to 2.61 min in phosphate, pH 6.0; (iv) 2.91 to 3.77 min in phosphate, pH 7.0; (v) 2.37 to 3.91 min in Tris-EDTA, pH 8.0. By the convenient measure of fluorescence intensity, GFPuv can be used as an indicator to report the extent of denaturation rates of other proteins in glucose solutions undergoing thermal processing. The thermostability of GFPuv provides the basis for its potential utility as a fluorescent BI to assay the efficacy of moist-heat treatments of LVPS at temperatures lower than 100°C.

POSTER PRESENTATION 1B-11

Influence of Feeding Rate Type on Fed-Batch Cultivation to Produce Glucose-6-Phosphate Dehydrogenase by *Saccharomyces cerevisiae* W303-181

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The enzyme glucose-6-phosphate dehydrogenase (G6PDH) plays an important role in maintaining the level of NADPH, and in producing pentose phosphates for nucleotide biosynthesis. It is also of great value as an analytical reagent, being used in various quantitative assays. However, it is very important to study new and promising alternatives for its production, like genetically modified microorganisms. Then, the aim of this work was to study the G6PDH production by fed-batch cultivation, using *Saccharomyces cerevisiae* W303-181. These cultivations were carried out in a 3 L bench bioreactor under aerobic conditions, pH 5.7 and 30 °C. Different feeding rates were studied (crescent and decreasing exponential feeding), and the feeding profile was determined by values of the parameter K (time constant), namely: 0.2, 0.5, and 0.8 h^{-1}. The best enzyme production occurred on crescent exponential feeding rate established by $K = 0.2\ h^{-1}$, and using a medium composed by glucose 5 g/L, YNB 1,85 g/L, adenine 67 mg/L, uracil 47 mg/L histidine 66 mg/L and tryptophan 80 mg/L. All cultivations showed that G6PDH production was associated to cellular growth. The results also showed that this process is promising for producing this enzyme.

POSTER PRESENTATION 1B-12

Sub-Cellular Distribution of Alcohol Oxidase (Ao) Activity and Its Role in Aliphatic Hydrocarbon Biodegradation Pathway in Yr-1 Strain of *Mucor circinelloides,* a Potential Bioremediator

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Environmental awareness and, specifically, soil damage due to oil spills in the past and in recent times has focused on the need for friendly strategies for the remediation of contaminated sites. Based on the fact that biodegradation was the major process for non-volatile oil components to be removed from the environment, bioremediation has been projected to play an important role in environmental clean-up.

A wide range of studies have dealt with bio-transformation and bio-remediation of petroleum hydrocarbons. Metabolic studies were implemented on the aerobic pathways for alkane, cyclic-alkane and aromatic and poly-aromatic hydrocarbon (PHA) biodegradation. Actually, there is a continuous increase in the number and amount of toxic compounds generated by our own society. It is becoming increasingly important to develop new enzymatic or microbiological techniques to detoxify and degraded most of these waste products.

We are interested in the study of oxidoreductases involved in the first and second steps of hydrocarbon biodegradation in filamentous fungi. In aliphatic hydrocarbon oxidation, the second step involves the activity of an alcohol oxidase; this has been an important enzyme because it has many biotechnological applications most of them in alcohol detection and in our own study, it is central for future bioremediation processes.

In the present work, we describe sub-cellular distribution of alcohol oxidase in function of the presence of different carbon sources in the culture media. The results strongly suggest the existence of alcohol oxidase activity in both, soluble fraction and also in the membranous-mixed-fraction (MMF). So, the question is: ¿Are there two different AO activities? One of them located in the membranous fraction and the other in the soluble fraction. Or the activity in MMF is only a transitory step across the membrane and finally AO arrives to the lumen in specialized vesicles where aliphatic hydrocarbon biodegradation is made.

First of all, we made experiments using different carbon sources, including glucose, decane, hexadecane and glycerol as sole carbon sources to detect the enzyme in soluble fraction by homologous immunodetection using antibodies against purified soluble AO.

In cell-free extracts (soluble and membranous fractions), we immunodetect two different bands, one of them corresponding to the soluble AO (46 kDA) and other band (34 kDa) that could be the responsible for AO activity in membranous fraction. Purification procedures confirm the existence of only one AO activity (46 kDa) in this strain that is located in membrane as a transitory step in its final location fate in lumen of specialized vesicles. 34 kDa band perhaps corresponds to an oxidase that contains an epitope that could be shared by some oxidases including alcohol oxidase.

POSTER PRESENTATION 1B-13

Application of Lipases by Entrapment in Hydrophobic Sol-Gel Materials to Concentrate Polyunsaturated Fatty Acids

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Several polyunsaturated fatty acids (PUFA) belonging to the omega 6 series, such as linolenic acid are of considerable interest due to their nutritional and therapeutic properties. Lipases are known to have reactivity on PUFA and these acids can be enriched by selective hydrolysis. In this context, the application of entrapped *Candida rugosa* lipase into hydrophobic silica gels by sol-gel process for vegetable oil hydrolysis can be represented an alternative for the related attainment of fatty acids in near ambient temperature. Various hydrolysis parameters, including type of oil (soybean, sunflower, canola and corn), enzyme concentration (22-220 U/g) and reaction time (1- 24 h) were investigated. The enzyme was encapsulated into sol-gel matrix using tetraethoxysilane TEOS as precursor in the presence of polyethylene glycol. After 1 h reaction, all substrates examined could be hydrolyzed to the corresponding fatty acids with degree of hydrolysis of 13-25%. Better performance was attained when soybean oil emulsion was used and this reaction was taken as a model for further studies to measure the effect of lipase concentration and oil: water ratio upon the rate of hydrolysis and the formation of linolenic and linolenic acids.

POSTER PRESENTATION 1B-14

Preparation of Cyclodextrins from Starch Using Immobilized CGTase from *Thermoanaerobacter* on Glyoxyl-Agarose

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Cyclodextrins (CDs) are cyclic oligosaccharides that are produced by the action of the CGTase enzyme on liquefied starch and have countless applications in the pharmaceutical, food and cosmetic industries, because they have a nonpolar cavity, which favors the encapsulation of a great variety of organic molecules. The stabilization of CGTase via multi-point attachment to a solid matrix allow the preparation of CDs at high temperatures using soluble starch or dextrin. CGTase was immobilized on agarose activated with high density of linear aldehyde groups that allow the establishment of multi-attachment enzyme-support bonds and the immobilization conditions were 25°C, pH 10 and 5h of reaction. The immobilization yield was 100% and the activity recovery was ca. 32%. The biocatalyst was capable of producing CDs at 85°C more quickly than the soluble enzyme. In addition, the biocatalyst maintained 94% of the initial activity after 5h at 85°C. The maximum conversion of dextrin to CDs was 29% both for the soluble and immobilized enzymes. When starch was used as substrate, the maximum conversion was 29% and 38%, with the immobilized and soluble enzyme, respectively. The lower conversion observed with immobilized CGTase and starch as substrate, results from intra-particle mass transfer limitations owing to the high molecular weight of the starch molecules.

POSTER PRESENTATION 1B-15

Effect of Inhibitors Released During Steam-Explosion Treatment of Barley Straw on Enzymatic Hydrolysis

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Barley straw is a very attractive lignocellulosic substrate for bioconversion to ethanol because it has high carbohydrate content, and is present in large quantities in Europe. For efficient ethanol production from lignocellulose biomass by enzymatic hydrolysis and fermentation, the lignocellulosic substrate should be pretreated to more effectively recover the hemicellulose and, concurrently, to make the cellulose more accessible to enzymatic hydrolysis.

The aim of this study is to investigate the influence of the liquid fraction (prehydrolyzate) obtained after steam-explosion pretreatment of barley straw on cellulose conversion in the enzymatic hydrolysis step. The pre-treatment conditions, selected in a previous work, were 180ºC and 10 minutes. After pretreatment the slurry was fractionated in a solid fraction and prehydrolyzate. This prehydrolysate was analysed for degradation compounds and sugars released during pre-treatment and used as media for enzymatic hydrolysis tests of the solid fraction, after pH adjusting to 4.8.

First results showed that the cellulose conversion was reduced by up to 34% after 48 h of hydrolysis when compared to standard tests on acetate buffer. When prehydrolyzate was diluted 1:2 (v/v), the reduction of cellulose conversion was by 18%. So, to determine the inhibitory effect on cellulase activity of toxic compounds produced during barley straw pretreatment, further studies have been performed by supplementing the hydrolysis media with different concentrations of those compounds. The results from this study will be presented.

POSTER PRESENTATION 1B-16

Assessment of Effects of Process Variables on Enzymatic Activity of Three Lipases in Compressed Fluids

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The use of enzymes as catalysts, especially lipases is becoming important in many research areas such as the modification of oils and fats, in the production of high-value added products from thermo sensitive substrates, etc. The advantages of using compressed or near critical fluids over liquid organic solvents as reaction media has been a matter of intense research due to their favorable transport properties that can accelerate mass-transfer limited enzymatic reactions, ease of separation, recovery of products or reactants and reduction in side reactions. Among some interesting substances, carbon dioxide, propane and n-butane appear to be promising as alternative media to conventional organic solvents. In this sense, this work investigates the influence of temperature (35-75ºC), pressure (10 to 280bar), exposure times (1 to 6h) and decompression rate (10-200Kg.m^{-3}.min^{-1}) on the activity of three immobilized lipases, two commercial ones - Lipozyme IM and Novozym 435, and a lipase from *Yarrowia lipolytica*. Results show that, in general, for carbon dioxide and propane media, an increase in exposure time and high decompression rates led to enzyme activity losses. For n-butane, however, a positive effect on lipase activity for all enzymes was verified for almost all experimental conditions studied.

POSTER PRESENTATION 1B-17

Computational Modeling of the Interaction of the Binding Domain of *T. reesei* Cel7A with Cellulose

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Cellulase enzymes play a critical role in the conversion of biomass to ethanol. These enzymes hydrolyze the linkages between cellobiose units in cellulose and yield sugars for subsequent or simultaneous fermentation. The Cel7A cellobiohydrolase is known as a processive enzyme and is thought to extract a cellodextrin chain from the surface of microcrystalline cellulose. The cellobiose product is released from the largest of the Cel7A protein domains, the catalytic domain. A much smaller folded peptide domain of about 4.5kDa, the type 1 cellulose binding domain (CBM), is thought to be responsible for binding the enzyme to the cellulose surface. It has also been proposed that the CBM also plays a role in decrystallization of the target cellodextrin. However, the mechanics and energetics of the interaction of the CBM with the cellulose substrate poorly understood.

In this study, we used computational molecular modeling to investigate interaction of the *T. reesei* Cel7A CBM with the 1,0,0 surface of a cellulose 1β model. We used the NMR "average" structure of the CBM[a] and the recent crystallographic structure of cellulose[b] as starting points. Molecular dynamics was performed with the CHARMM software suite and was used to investigate the orientation and interaction of the type 1 CBM with cellulose, all enclosed in a large box of water molecules. Our simulations show how the aromatic and other surface residues of the CBM align with the surface strands of microcrystalline cellulose. Our simulations also indicate that the type 1 CBM disrupts the water boundary layer[c] recently shown to manifest above the surfaces of microcrystalline cellulose.

Footnote

Kraulis, P.J.; Clore, G.M.; Nilges, M.; Jones, T.A.; Pettersson G.; Knowles, J.; Gronenborn, A.M. Biochem. 1989, 28, 7241. [b]Nishiyama, Y.; Langan, P.; Chanzy, H. J. Am. Chem. Soc. 2002, 124, 9074. [c] Skopec, C.; Zuccato, P.; Brady, J.W.; Torget, R.; Himmel, M.E. Carbohydrate Res. 2004, In press.

POSTER PRESENTATION 1B-18

RSM Analysis of the Effects of the Oxygen Transfer Coefficient and Inoculum Concentration on the Production of Xylitol by *C. guilliermondii*

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The industrial production of xylitol is based on the catalytic hydrogenation of a solution with high xylan content, resulted of lignocellulosic material hydrolyzed at high temperature and pressure conditions. Biotechnology is an alternative process. The bioconversion takes place through oxidative-reductive enzymatic reactions.

In this work the yeast *Candida guilliermondii* IM/UFRJ 50088 was used to obtain xylitol from D-xylose. The aim was to study the behavior of the yeast when crucial process variables were modified. The KLa (between 18 h-1 and 40 h-1) and the initial cell concentration (between 4 g and 10 g) were considered as control variables. A response surface methodology (RSM) was applied. The effects were evaluated using statistical tools. A regression model was developed and used to determine an optimal value that was further validated experimentally. The optimal conditions determined were KLa of 32.85 h-1 and X0 of 9,86 g, maximizing the productivity value to 1.628 g/h and the xylitol yield value to 0.708 g/g.

POSTER PRESENTATION 1B-19

Renaturing Foam-Fractionated Cellulase with Artificial Chaperones

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Foam fractionation is a simple method which can be used to concentrate surface active chemicals like proteins. One major drawback to protein foam fractionation is that denaturation can occur at gas-liquid interfaces within the process. Artificial chaperones (such as a combination of a detergent and a cyclodextrin) can be used to restore a portion of the lost activity. Normally, the laboratory protocol for the use of artificial chaperones for renaturation suggests leaving the treated solution undisturbed overnight, but in an industrial environment such a long time requirement leads to a low rate of production. In this study, the kinetics of renaturation of degraded cellulase using the detergent cetyltrimethylammonium bromide (CTAB) and β-cyclodextrin (β-CD) as artificial chaperones is studied. The solution containing cellulase and CTAB in the foam column is denatured by aeration. Following denaturation, the collected foam is tested for activity before and after adding β-CD. The activity of the solution is then checked several times over the next 12 hours period. The kinetic results are examined in order to determine whether the treatment time can be reduced from the nominal 12 hours.

POSTER PRESENTATION 1B-20

Comparative Performance of *Candida rugosa* Lipase Immobilized on Non-Commercial Matrixes to Yield Alkyl Esters in a Solvent Free System

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Biodiesel produced by the interesterification of vegetable oils is a promising alternative fuel to diesel regarding the limited resources of fossil fuel and the environmental concerns. In this work, production of fatty acid alkyl esters by lipase-catalyzed alcoholysis of babassu oil with primary alcohols in solvent free system was investigated. Two non-commercially available matrixes with different properties (polysiloxane-polyvinyl alcohol particles and niobium oxide) were selected for immobilizing microbial lipase from *Candida rugosa*. Besides having low cost, previous work revealed the suitability of these immobilized derivatives to be used as a catalyst for organic synthesis purposes.

Reaction mixtures of alcohol (ethanol, propanol and butanol) and babassu oil were incubated with experimental preparations of immobilized lipase for a maximum period of 96 h at either 40 or 50°C and mechanical stirring in a closed reactor. The immobilized lipase preparation Lipozyme IM^{20} was used as comparative parameter for the other two immobilized systems.

All immobilized preparations showed acceptable conversion levels for babassu oil with butanol (40% average conversion). The reaction systems consisting of oil and ethanol or propanol strong affected the activity of the enzyme. The processes catalyzed by *Candida rugosa* lipase immobilized either on polysiloxane-polyvinyl alcohol particles or niobium oxide gave poor conversions to esters (lower than 18%) and with Lipozyme intermediate yields of ethyl and propyl esters (35%) were attained.

Better overall reaction yields should be achieved by optimizing the parameters that affects the interesterification reaction of babassu oil with butanol. To achieve this it is recommended that further studies should be carried out with the *Candida rugosa* lipase immobilized on either niobium oxide or polysiloxane-polyvinyl alcohol that besides showing similar performance as Lipozyme have lower cost, which is essential for this kind of process.

POSTER PRESENTATION 1B-21

Development of Lipase Immobilization on Silica Gel Using Cross-Linking Method for the Production of Biodiesel

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Lipase is the enzyme that can catalyze alcoholysis of lipids with alcohols to biodiesel (fatty acid alkyl esters). The objective of this study is to develop a suitable immobilization method of lipase for the production of biodiesel. Lipase produced from *Rhizopus oryzae* was immobilized on silica gel by using cross-linking method. Each step in the immobilization procedure was optimized to improve the efficiency of overall immobilization procedure of lipase. The optimal conditions of each step were determined as follows; pretreatment of silica gel by 35% hydrogen peroxide, silanization of silica gel surface by 15% 3-amino-propyltriethoxysylane in acetone, crosslinking by using 2% glutaraldehyde modified in aqueous solution at 65 for 25min. Lipase was immobilized on silica gel under the optimal conditions. As a result, 92% of protein added was attached to silica gel and 35% of lipase activity was recovered. Finally, the activity of immobilized lipase was remained over 80% after 20 times of reuse.

POSTER PRESENTATION 1B-22

Withdrawn

POSTER PRESENTATION 1B-23

Small-Scale Cellulose Conversion Assay for the Evaluation of Cellulase Mixtures

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The efficient conversion of cellulose to soluble sugars is a multi factorial problem. Solving this problem only at the large, application scale is impractical. A small-scale method for the analysis of conversion efficacy of acid treated cellulose by a defined set of cellulases allows for the exploration of a great number of variables such as pH, temperature, substrate nature, enzyme loading, etc. Reactions are carried out in sealed 96 well microtiter plates, quenched, and filtered in this format and loaded onto an HPLC system configured to sample from this same configuration. Peak area analysis and data concatenation are automated. Our analysis method is time intensive but is also data rich, allowing for the analysis of the sugar product profiles. From this method we explore substrate and enzyme dose dependence and observe synergistic activities of purified cellulases. This small-scale method can be adapted to high throughput for a multi factor analysis.

POSTER PRESENTATION 1B-24

Production of Enzyme Feed Additive from *Aspergillus oryzae*

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Enzyme feed additive is produced by solid fermentation with *Aspergillus oryzae* SZ09. Inoculated media in big tanks composed of wheat bran 94%, soybean oil meal 4%, corn powder 1%, Caco 31%, are incubated at a temperature of 28°- 30° for 36h, ventilated every 0.5h. Process is determined. The main ingredients of the product are crude protein 25.5%, fat 3.0%, crude fiber 8.2%, Ca 1.07%, P 0.47%, protease 2312U, cellulase(FP) 0.13U, pectinase 157U,amylase 80U, lipase 51.3U. Feeding results on milks cows, pigs, chickens are satisfying:

In a trial time of 45 days on pigs the enzymes are added to feed at rates of 0%, 0.2%, 0.4%, and 0.6%. Comparing with 0% group, pigs daily weight gains are increased by 18.30%, 23.30%, 23.70%; in a trial time of 30 days on milk cows the enzymes are added to feed at rates of 0%, 0.2%, 0.4%, 0.6%. Comparing with 0% group, milk yields are increased by 2.0%, 6.2%, and 6.4%; in a trial time of 20 days on laying egg hens, the enzymes are added to feed at rates of 0%, 0.3%, 0.5%, 0.7%, comparing with 0% group, total egg weights are increased by 1.80%, 3.20%, 30%.

POSTER PRESENTATION 1B-25

Energetics and Conformations of the Hydrated Cel7A Linker Peptide

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Plant biomass is the most abundant source of fermentable carbohydrates in the world, which when converted to fuels such as ethanol, holds the potential for significant environmental, economic, and strategic gains. Currently, chemical or biological conversion of biomass is too costly to permit ethanol to compete as a viable alternative fuel. For this reason, understanding the mechanisms of biological degradation of biomass, with special emphasis on the depolymerization of cellulose, is an active area of research.

In particular, cellobiohydrolase I (Cel7A), from *Trichoderma reesei*, is one of the most active cellulases known. Cel7A is a multi-domain enzyme, consisting of a large catalytic domain containing an active site tunnel and a small cellulose binding module joined to one another by a 27 residue linker peptide. Although the spatial conformation adopted by the linker domain is yet to be determined, it is thought to play an important role in the enzymatic hydrolysis of cellulose. This enzyme, which is found in fungal cellulase systems, is believed to hydrolyze cellulose in a "processive" manner, liberating cellobiose residues. Unfortunately, the exact mechanism is not known.

Since the linker peptide is relatively small, it is possible to study the energetic conformations adopted by the linker through molecular mechanics and molecular dynamics techniques. In this work, we will present results of computer simulations of the *O*-linked glycosylated linker peptide in an aqueous environment in order to gain insight into the role the linker may play in cellulose hydrolysis.

POSTER PESENTATION 1B-26

Characterization of the *N*-Linked Glycosylation Sites of Native and Recombinant *Penicillium funiculosum* 7A Cellobiohydrolase

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The filamentous fungi *Penicillium funiculosum* is known to produce a highly effective cellobiohydrolase that is homologous to the Cel7A enzyme from *Trichoderma reesei* (CBH I). The *P. funiculosum* Cel7A catalytic domain also has three putative *N*-glycosylation sites. In binary cellulase systems with the E1 endoglucanase from *Acidothermus cellulolyticus*, the *P. funiculosum* Cel 7A cellobiohydrolase out performs its *T. reesei* counterpart by a factor of two. Recombinant Cel7 from *P. funiculosum*, overexpressed in *Aspergillus awamori*, demonstrated a higher molecular mass than the native enzyme, suggesting higher extents of glycosylation. Recent studies in our lab have demonstrated strong correlations between *N*-glycosylation and cellulase activity. To better understand the roles of glycosylation in the stability and activity of this enzyme, we analyzed the glycosylation patterns of both the native and recombinant forms using proteolytic digestion, followed by reverse phase HPLC coupled with electrospray ionization mass spectrometry (ESI-MS). In this poster, we demonstrate the successful application of MS and tandem MS to characterize the *N*-linked glycans of the native and recombinant *P. funiculosum* Cel7A enzyme.

POSTER PRESENTATION 1B-27

Enzyme Immobilization onto Polydimethylsiloxane Using Layer-by-Layer Self-Assembly Techniques

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Enzyme immobilization onto polydimethyl siloxane (PDMS) substrates has been investigated using layer-by-layer self-assembly techniques. Since PDMS is quite inert, attaching enzymes to its surface is more challenging than immobilization onto other more traditional materials such as quartz or silicon. Previously, the only successful immobilization technique was incorporation of enzyme directly into the plastic matrix. Although successful, this technique consumes excessive amounts of enzyme and has limited useful lifetime. Layering techniques are showing themselves to be superior.

Polyethyleneimine (PEI), polydimethyldiammonium chloride (PDDA), and polystyrenesulfonate (PSS) are used as polycations and polyanions in the assembly. These immobilization procedures were evaluated using urease and analyzed by UV visible spectrometer. The setting for each test was a channel-based continuous flow microreactors fabricated from PDMS and run at room temperature. Various combinations of layers (architectures) of cations and anions were used to attach urease and were examined for activity and useful lifetime. Reactors run continuously for 30 days using architecture of PEI/PSS/PEI(urease/PEI)$_7$ maintained almost 1/3 of its original activity.

POSTER PRESENTATION 1B-28

Evaluation of the Transferability of Mutational Effects Between Homologous Glycosyl Hydrolase Family One β-D-Glucosidases

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We previously identified, using error-prone PCR, several amino acid replacements that confer higher thermal stability to the glycosyl hydrolase (GH) family-1 b-D-glucosidase (BglC) from *Thermobifida fusca*. In order to obtain a better understanding of the structural basis for the thermal stability improvements observed experimentally using BglC, we generated similar substitutions in a homologous GH family-1b-D-glucosidase from *Trichoderma reesei*. We hope to demonstrate that the underlying mechanisms of stabilization are transferable between these glycosyl hydrolases from different species. We will compare the thermal stabilities and kinetic parameters of these recombinant enzymes with their wild-type counterparts. Specifically, we will present differential scanning microcalorimetry, activity half-lives, and Michelis-Menten kinetics (K_m and k_{cat}) for these two groups of mutant b-D-glucosidases. The observed trends with respect to (1) the transferability of mutational effects between homologous proteins from different species, and (2) the interplay between mutational enhancement of mechanical/thermal stability and retention of enzyme activity will be discussed.

POSTER PRESENTATION 1B-29

Raw Starch Hydrolyzing α-Amylase from the Newly Isolated *Geobacillus thermodenitrificans* HRO10: Production Optimization Using 2^4 and 3^2 Factorial Designs

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α-Amylase production by *Geobacillus thermodenitrificans* HRO10 is exclusively extracellular irrespective of stage of bacterial growth. *G. thermodenitrificans* HRO10 produced a raw – starch digesting α-amylase which showed optimum activity at 80 °C and pH 5.5. The enzyme has a relative activity of 83 % on raw potato starch. The hydrolysis of the raw potato starch was carried out at temperature (60 °C) below gelatinization temperature of potato starch. The culture conditions for the production of α-amylase by *G. thermodenitrificans* HRO10 was optimized in 500 ml flasks using full 2^4 and 3^2 factorial designs. A full 2^4 *Factorial Design* was carried out in order to identify the parameters (temperature-T, pH, C_k-metal ion and C_s-starch concentration), which significantly influenced α-amylase production at the chosen confidence level (99 %). In order to determine the collective effect of significant variables on the production of α-amylase (within the experimental range, covering all the points), a full 3^2 *Factorial Design* was carried out. The equation ($Y = -594.206 - 0.178T^2 - 8.448pH^2 + 6.020TpH - 0.005T^2pH^2$) that quantitatively predicted the optimized conditions for α-amylase production was derived. When the traditional one-factor-at-a-time method was employed, the maximum α-amylase produced by *G. thermodenitrificans* HRO10 was 20 U/ml. However, under optimized conditions (using the above equation) α-amylase production was improved to 30.2 U/ml.

POSTER PRESENTATION 1B-30

Catalytic Performance of Invertase Adsorbed on Anionic Exchange Resin

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Invertase (EC.3.2.1.26; K_m =17.0 mM, V_{max} = 0.0240 U.ml^{-1}, stable in the 4.0-4.6 pH interval) was adsorbed on DOWEX® anion exchange resins (types: 1x8:50-400; 1x4:50-400 and 1x2:100-400). One hundred milligrams of each polystyrene beads were suspended in 25 mL buffered (pH 5.5) invertase solution (total activity = 0.65 U) at 32°C. The mixture was maintained under agitation of 100 rpm by 24h. Among the complexes prepared, adsorption and immobilization indexes of 100% occurred only with the complex DOWEX-1X4-200/Invertase (D1X4-200I). So, the catalytic performance of D1X4-200I was evaluated through the sucrose hydrolysis in a continuous membrane reactor coupled with UF-membrane (cut off 100 kDa) or MF-membrane (pore diameter 5μm). The assays were carried out at least by 30h under agitation of 100 rpm, dilution rate of 1.6 h^{-1}, substrate concentration of 2.5mM, pH 5.5 and 30°C. No leakage of enzyme from the support was detected and yields of 84% and 95% were attained with UF and MF membranes, respectively. It were determined for D1X4-200I the kinetic constants (V_{max} = 0.0450 U.ml^{-1} and K_m = 18.3 mM) and the stability against pH, which ranged from 4.6 to 5.5.

POSTER PRESENTATION 1B-31

Enzymatic Synthesis of Sorbitan Methacrylate According to Acyl Donors and Its Application

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Sugar polymer has been considered as a biomaterial for medical applications. These biomaterials are widely used in the preparations of burn dressing, artificial membranes and contact lens. In this study, the optimum conditions which effected the enzymatic synthesis of sorbitan methacrylate using Novozym 435(lipase acrylic resin from *Candida antarctica*) in *t*-butanol from sorbitan and several acyl donor(ethyl methacrylate, methyl methacrylate, vinyl methacrylate) was investigated. Also hydrogel(poly(sorbitan methacrylate)) was synthesized by free-radical polymerization with sorbitan methacrylate as monomer.

The enzymatic synthesis of sorbitan methacrylate catalyzed by Novozym 435 in *t*-butanol was reached approximately 68% conversion yield at 50 g/L of initial 1,4-sorbitan concentration, 5% (w/v) of enzyme content, 1:5 of molar ratio of sorbitan to ethyl methacrylate, 50 of reaction temperature for 36 hours using ethyl methacrylate as acyl donor. Using methyl methacrylate as acyl donor, sorbitan methacrylate was synthesized around 78% conversion at 50 g/L of initial 1,4-sorbitan concentration, 7% (w/v) of enzyme content, 1:5 of molar ratio of sorbitan to methyl methacrylate, 50˚ of reaction temperature for 36 hours. Hydrogel(poly(sorbitan methacrylate)) synthesis was performed in 70˚ water-bath with monomer, initiator and cross-linker. Hydrogel was well synthesized in the condition of 20% (w/v) of sorbitan methacrylate as monomer and 4% (w/w) of AIBN as thermal initiator within 60 minute.

POSTER PRESENTATION 1B-32

The Evaluation of Individual Cellulase Performance on Complex Biomass

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The efficient conversion of cellulose to soluble sugars requires multiple cellulase activities because individual cellulase activities alone do not produce significant conversion of cellulosic biomass. Evaluating the contribution of an individual cellulase activity to biomass conversion requires the combination of that activity with other cellulases. Defined cellulase mixtures evaluate synergy between enzymes in a controlled manner. We show the development and validation of this approach at a small scale using at enzyme and substrate concentrations relevant to large scale process.

POSTER PRESENTATION 1B-33

Effect of Surfactants and Polyethylene Glycol on Enzymatic Hydrolysis of Wheat Straw

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Adsorption of cellulases on steam pretreated spruce can be significantly reduced by addition of surfactants or polyethylene glycol (PEG) and this has proven beneficial for the hydrolysis rate. The hypothesis is that the surfactants and PEG adsorbs on or interacts with the lignin and thereby minimizes unproductive adsorption of enzyme on lignin, which results in more enzymes active on the cellulose. However, it is less known how the nature of the lignin influences the effect of surfactants and PEG.

Wheat straw differs from spruce with respect to both lignin amount and content of guaiacyl, syringyl and p-hydroxyphenyl components. Surfactants and PEG might therefore interact differently with different lignins. Another important aspect is how the pretreatment methods modify the lignin and thereby alter the interaction between lignin and surfactant/PEG. Sulphur dioxide impregnation before steam explosion may change the surface properties of lignin. In this study, the wheat straw was pretreated with different methods to produce substrates with different lignin characteristics. The effect of surfactants, PEG and pretreatment method on enzymatic hydrolysis of the wheat straw was then evaluated.

POSTER PRESENTATION 1B-34

Production and Characterization of a New Cyclodextrin Glycosyltransferase of Alkalophylic Bacilli Isolated from Brazilian Soil

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Cyclodextrin glycosyltransferase (CGTase) catalyzes the degradation of starch to form α-, β- and γ-cyclodextrins (CDs) that are capable of forming inclusion complexes and stabilizing an ample spectrum of substances. In Brazil, there is a strong incentive to produce CDs at reduced cost, due to the great availability of substrate starch.

A new CGTase was obtained from an alkalophylic microorganism isolated from Brazilian soil of oat culture, using a high alkaline pH medium containing 1% Na_2CO_3. The enzyme was characterized in soluble form, using as substrate 100 g/L of matodextrin in 0.05 M Tris-HCl buffer and 5 mM $CaCl_2$. It produced mainly β-CD and its dextrinizing activity, 2^8, was greater than that of the enzyme of *Bacillus firmus*, strain 37, 2^7, previously studied by our group. Enzymatic activity was determined as a function of temperature and pH. The enzyme exhibited an optimum temperature of 50°C and was most active at pH 6.0. Activation energy for the production of β-CD was 9.4 kcal/mol. Thermal deactivation began after 3 hours of assay at the temperature of 50°C, but in the range 30-45°C, it was highly stable. The influence of substrate or product level on the initial velocity of CD production was also studied.

POSTER PRESENTATION 1B-35

Beyond the Barriers: Bioconversion Studies of Transgenic Trees to Ethanol

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Crystallinity and recalcitrance of lignocellulosics are major obstacles that negatively impact the economics of biomass conversion technologies. To overcome such barriers, NCSU is genetically engineering aspen trees (*Populus tremuloides)* as a model in making ethanol. The fundamental goal is to demonstrate the potential of such techniques in producing more cost competitive and sustainable biomass resources. The project objective is to engineer specific traits and evaluate ethanol production using a 3 stage treatment process - dilute acid, enzyme hydrolysis & fermentation. The composition of the resulting transgenics will have significantly less lignin with a corresponding higher cellulose content; further modified to a lower degree of crystallinity. This presentation will disclose the strategy and developments in the*: (i.)* genetic engineering of biosynthetic pathways of aspen trees, *(ii.)* high-throughput screening of newly discovered, low cost cellulases (NREL Subcontract), *(iii.)* microanalytical characterization of the biomass substrates, and *(iv.)* fermentation of ethanol (yields and efficiency). The results from Phase 1 - focusing on the characterization and development of miniature application tests/assays for converting non-transgenic controls, will be given. The application and economics of this biomass-derived process technology using novel transgenic trees represents the conclusion of this 3 year program.

POSTER PRESENTATION 1B-36

Enzymatic Saccharification of Pretreated Corn Fiber for Production of Sugars

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Corn fiber is a low-value co-product of corn wet milling, which has good potential as a feedstock for ethanol fermentation because of its high carbohydrate content, proximity to existing ethanol facilities, and low-cost. Corn fiber xylan can be easily hydrolyzed by just treating with hot-water. However, fermentation of the resulting hydrolysate is problematic because the yield of simple sugars following saccharification with commercial xylanase preparations is low. To develop more effective enzyme mixtures, both *T. reesei* RutC30 and two *A. niger* strains were cultured on destarched corn fiber (DSCF) to induce the production of xylanolytic enzyme mixtures. The supernatants were collected and used to saccharify DSCF either alone or in combination with each other. Combining *T. reesei* Rut C30 with either *A. niger* preparation increased the yield of xylose and arabinose 180%. The best performing mixture of enzymes was subsequently used to saccharify DSCF that had been treated with 160°C water. The heat treatment alone yielded 25% of the xylan as free xylose. Very little of the glucans were converted to glucose. After enzymatic saccharification, 61% of the xylan sugars were released as monomers, as well as much of the glucans (up to 282 mg glucose per g DSCF, db). Current work is directed towards increasing this yield further by optimizing fungal culture conditions, corn fiber pretreatment conditions, evaluating other enzymatic preparations, and adjusting saccharification conditions.

POSTER PRESENTATION 1B-37

Inhibition of Cellulase, Xylanase and β-Glucosidase Activities by Lignin Fractions Derived from Softwood

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The bioconversion of lignocellulosic substrates to ethanol involves enzyme-catalyzed hydrolysis of the cellulose and hemicellulose components to fermentable sugars. Attempts to improve process economics include genetic engineering of cellulases, xylanases and related hydrolases to improve their specific activity and thermostability. However, it is recognized that enzyme performance is reduced during lignocellulose hydrolysis by interaction with lignin. Therefore, the selection or engineering of enzymes for reduced lignin binding offers an alternative means of enzyme improvement. This study demonstrates that various hydrolytic enzymes or enzyme complexes differ significantly in their inhibition by lignin fractions derived from softwood, thereby providing support for this approach.

A lignin fraction (L1) was obtained from Douglas-fir by ethanol organosolv extraction followed by precipitation with water. A second fraction (L2) was obtained from the extracted lignocellulosic residue by exhaustive hydrolysis of the cellulose component. The inhibitory effects of L1 and L2 on the activities of 7 cellulase preparations, 3 xylanase preparations and a β-glucosidase preparation were then determined. Enzyme preparations were obtained from commercial sources or produced in the laboratory. The cellulases differed by up to 3.5-fold in their inhibition by lignin; the xylanases showed less variability (≤1.7-fold). Of all the enzymes tested, β-glucosidase was least affected by lignin. Inhibition by L1 was significantly higher than L2 for all enzymes tested. This investigation is supported by structural analyses of L1 and L2 using ^{13}C NMR and other physical and chemical methods.

POSTER PRESENTATION 1B-38

Probing the Role of *N*-Linked Glycosylation in Stability and Activity of Cellobiohydrolases by Mutational Analysis

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To enable a new biorefinery industry in the U.S. based on lignocellulosic feedstocks, the current cost estimated for cellulase enzymes must be reduced. The filamentous fungi, *Trichoderma reesei* and *Penicillium funiculosum,* are known to produce highly effective glycosyl hydrolases (GH) that degrade the cellulosic component of plant cell walls. Both fungal species produce GH family 7 enzymes, or cellobiohydrolases, that are not only similar in structure, but have analogous *N*-linked glycosylation sites on the catalytic domain. Variations in the *N*-linked glycosylation of these two recombinant cellobiohydrolases, when expressed in *Aspergillus awamori,* were found to directly impact several important biological properties including thermal stability and specific performance on microcrystalline cellulose. The consequence of adding or removing *N*-linked glycans from the catalytic domains of both enzymes was investigated systematically by either adding or removing *N*-linked motifs using site directed mutagenesis. With this approach, we were able to correlate specific glycosylation sites on cellobiohydrolase catalytic domains with cellulase functionality and thus propose new roles for cellulase glycosylation.

POSTER PRESENTATION 1B-39

Withdrawn

POSTER PRESENTATION 1B-40

Production of LiP and MnP and Use of Cell-Free Culture Broth of *Phanerochaete chrysosporium* for Biodegradation of Phenolic Compounds

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The effect of the dissolved oxygen (DO) concentration on the production of lignolytic enzymes and isozyme distributions by Phanerochate chrysosporium was studied in the immobilized reactor system. The oxygen levels significantly affected the production of lignin peroxidase (LiP) and manganese peroxidase (MnP), as well as that of H2O2. Cell-free culture broth of *Phanerochaete chrysosporium* has been adopted to biodegrade chlorophenols as model recalcitrants. Two different media compositions, nitrogen-sufficient and limited, have been compared for distribution of isozyme and the production of lignin peroxidase,manganese peroxidase, and other metabolites such as oxalate. The isozyme distributions and their expression levels in both cases were significantly different based on SDS-PAGE data and changed with increasing the culture time and DO concentrations: H1, H2, H6, and H10 (Lignin peroxidases) appeared in nitrogen-sufficient medium but H3, H4, H5 (Manganese peroxidases) as well as H7 and H8 (Lignin peroxidases) were found in nitrogen-limited medium. Biodegradation efficiency, estimated by disappearance of the chemical as well as accumulation of chloride ion, of nitrogen-limited culture broth was found to be higher than that of nitrogen-sufficient culture broth. The culture broth of nitrogen-limited medium taken at 11 days showed the highest performance on the biodegradation of 2,4,5-chlorophenol. Usage of this cell-free culture broth may be easily applied to the biodegradation system for highly recalcitrant chemicals because this system would not be affected by the toxicity of the chemical as well as adsorption characteristics to the cells.

POSTER PRESENTATION 1B-41

Cloning and Characterization of Biomass Degrading Enzymes

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Biomass is an attractive candidate to replace fossil fuels as a source of many of our fuel and chemical feedstock needs. However, in order to fully utilize the potential of biomass, it is critical that technologies be developed that will hydrolyze this renewable material more effectively. We are interested in producing enzymes that will efficiently hydrolyze biomass. Our approach is to discover new genes encoding biomass-degrading enzymes from various environmental samples. We will report on the progress of the gene cloning and biochemical characterizations of these new enzymes.

POSTER PRESENTATION 1B-42

Simultaneous Liquefaction and Saccharification of Grain Mash: A Modified Process for More Effective Enzyme Utilization

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The traditional dry milling process is based on separate liquefaction and saccharification/fermentation steps, due to differences in the operating temperature and pH of the amylases and glucoamylases used in the process. However, advances in amylases for "cold hydrolysis", coupled with advances in enzyme technology that expand the pH and thermal-stability of these enzymes presents an opportunity to re-define the dry-milling process.

This work describes the performance of amylase and glucoamylase during a single-step liquefaction and saccharification process. The effects of pH and operating temperature on mash viscosity, starch hydrolysis, and ethanol yield will be discussed. The results show that a single stage process, particularly a temperatures at or slightly below the gelatinization temperature of starch, may be effective, leading to more rapid viscosity reduction and more efficient production of fermentable sugars than the traditional multi-step dry-mill process. The implications on enzyme use and energy consumption will also be discussed.

POSTER PRESENTATION 1B-43

Synthesis of the Enzyme Cyclodextringlucosyltransferase by *Bacillus firmus* for the Production of β-CD in Presence of Different Starch Concentration and Sources

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The synthesis of the enzyme cyclodextringlucosyltransferase (CGTase) from *Bacillus firmus* (strain #37) was studied in regard to the yield of cyclodextrin, CGTase activity and growth of bacteria by varing the starch concentration and sources like first step to the use of statistical experiment design. The CGTase is an enzyme with catalyzes intramolecular (cyclizing) and intermolecular (coupling and disproportionation) transglycolisation as well as having a hydrolytic action. A cyclic activity of accumulation and consuption of β-CD occurred during the bacterial growth. The differents starch sources studied were dextrin, α- and β-CDs, Acarbose, corn, potato, manioc and rice at 1.0 % (w/v). CGTase was more active when potato (0.341U/mL) and corn (0.336U/mL) starchs were used producing around 3.5g/L of β-CD in the medium. Low yield of β-CD was found with the use of simple carbon sources like α-CD and acarbose. Bacterial growth in these experiments used to be maximum when the consumption of substracts and products were total. When the concentration of corn starch was used at 2.0 % (w/v) the enzymatic activity was 0,60U/mL, the formation of β-CD was higher and cell growth was stable. These results suggest that there is a relation between CGTase synthesis and β-CD production.

POSTER PRESENTATION 1B-44

Enzymatic Hydrolysis of Water-Soluble and Insoluble Wheat Arabinoxylan

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Hydrolysis of arabinoxylan is an important prerequisite for improved utilization of wheat hemicellulose in the ethanol fermentation industry and other industries. This study investigated the individual and combined efficiencies of five commercial, cellulytic and hemicellulytic enzyme preparations, Celluclast 1.5 L, Ultraflo L, Finizym, Viscozyme L, and Cereflo in catalyzing the liberation of ferulic acid, arabinose and xylose from soluble and insoluble wheat arabinoxylan in a central composite design with two factors pH (3-7) and temperature (30-70°C). Ultraflo L, produced by *Humicola insolens*, was the best enzyme preparation in releasing arabinose, from both soluble and insoluble arabinoxylan and ferulic acid from insoluble arabinoxylan, whereas Celluclast 1.5 L, produced by *Trichoderma reesei*, was superior to the other enzyme preparations in releasing xylose. A synergistic cooperation in xylose release was found between Ultraflo L and Celluclast1.5 L on both soluble and insoluble arabinoxylan. Beta-xylosidase was purified from Celluclast 1.5 L to determine if that was the key enzyme activity from Celluclast 1.5 L that contributed to the synergism between Celluclast1.5 L and Ultraflo L. Based on the results the observed synergism between Celluclast1.5 L and Ultraflo L on soluble arabinoxylan is proposed to be a result of positive interaction between α-L-arabinofuranosidase and endo-1,4-β-xylanase activities present in Ultraflo L, and β-xylosidase activities in Celluclast1.5 L. For insoluble arabinoxylan the positive synergistic interactions are proposed to involve α-L-arabinofuranosidase, ferulic acid esterase and endo-1,4-β-xylanase activities present in Ultraflo L, and β-xylosidase activities in Celluclast1.5 L.

POSTER PRESENTATION 1B-45

Cloning and Characterization of Thermostable Esterase from *Archaeoglobus fulgidus* DSM4304

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New thermostable esterase from the hyperthermophilic archaeon *Archaeoglobus fulgidus* DSM 4304 (EST-3) was cloned, functionally expressed in *Escherichia coli* XL1-blue strain and biochemically characterized. The sequence of esterases were got from GeneBank and size of esterase gene is 744bp. The EST-3 gene was amplified by PCR and the gene was cloned into pQE 30 at *Bam* HI and *Sal* I restriction site for expression and purification. On positive clone exhibiting acquired esterase activity was directly detected by an in situ plate assay using a colony staining procedure with chromogenic substrate -naphthyl acetate and fast blue RR salt(esterase activity staining). A plasmid library of *A. fulgidus* esterse gene was screened for colonies showing thermostable enzyme activity against -naphthyl acetate. Positive colonies showed dark brown color after incubation at 75°C in presence of the substrate. It was also easy to purify the eaterase by 75°C heat treatment, which could denature proteins from *Escherichia coli*.

EST-3 is monomeric protein with a molecular weight of about 27.5 kDa. The enzyme is barely active at room temperature, displaying the maximal enzyme activity at about 75°C and after 180 min incubation at 95°C 10% activity still remains. Enzyme activity was analyzed with ketoprofen ethyl ester and the specific activity against ketoprofen ethyl ester is 1.2 mole / mg-protein/min.

POSTER PRESENTATION 1B-46

Enzymatic Digestibility of Lignin-Blocked Substrates

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To understand the heterogeneous kinetics of enzymatic hydrolysis of cellulose, enzymatic digestibility as a function of the initial hydrolysis rate and the specific accessible surface area of cellulose was investigated by interrupting and then restarting the hydrolysis of lignin-blocked substrates. The initial hydrolysis rate was measured at conditions that saturate cellulase adsorption on the accessible surface of cellulose. The specific accessible surface area was determined by lignin blocker pretreatment and subsequent addition of *T. reesei* CBH1 (Cel7A) mutant 212Q labeled with Alexa Fluor (AF594) at operational hydrolysis temperatures of 50°C. The results suggest that the rapid decreasing of the rate of enzymatic hydrolysis could be strongly related to substrate heterogeneity, enzyme inactivation, and end-product inhibition. We also found that the enzymatic digestibility measured by this approach for pretreated corn stover and other substrates could lead to a kinetic model to predict the performance of enzymatic hydrolysis of pretreated cellulose.

POSTER PRESENTATION 1B-47

Lactose Hydrolysis and Formation of Galactooligosaccharides by a Novel Intracellular ß-Galactosidase from *Talaromyces thermophilus*

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The kinetics of lactose hydrolysis by a novel intracellular ß-galactosidase from *Talaromyces thermophilus* were studied. Enzyme inhibition by products was evaluated using different concentrations of galactose and glucose. Both end products, galactose and glucose, were shown to be competitive inhibitors with glucose being the stronger inhibitor (K_i = 66 mM) than galactose (K_i = 370 mM). Discontinuous and continuous processes of lactose hydrolysis were compared. A wide experimental range of the main variables were evaluated, including the enzyme loading, lactose concentration, the lactose conversion and product (glucose, galactose and galactooligosaccharide) formation over the time. As was evident from these experiments, the enzyme showed a strong transgalactosylation activity. Hence, this novel enzyme can be useful both for the cleavage of lactose at elevated temperatures, and the formation of galacto-oligosaccharides, prebiotic sugars, which have a number of interesting properties for industrial food applications.

POSTER PRESENTATION 1B-48

Evaluation of Cell Recycle of *Pichia pastoris* GS115 on Fed-Batch Xylanase Production

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Cellulase-free xylanases are enzymes which have wide application in industrial processes, such as food, animal feed and mainly in the pulp and paper industries. These enzymes can be produced by both wild and genetically engineered microorganisms. The gene of *Thermomyces lanuginosus* IOC-4145 was cloned and expressed in *Pichia pastoris* GS115 with the objective to produce cellulase-free xylanases. Therefore, this work aims to evaluate biomass recycle on xylanase production in submerged fermentation. Experiments were carried out using 20mL BMM medium (in w/v: 0.5%methanol, 1.34%YNB, $4.0x10^{-5}$%biotine, pH6.0) in 125mL-conic flasks under orbital agitation (250 rpm), at 30 °C. Fed-batch processes were adopted by recycling biomass at each 24 and 72 hours and feeding the bioreactor with methanol at each 12 hours. Additionally, the influence of the initial cell concentration was investigated. Xylanase production was not reduced with the recycling time, during four biomass recycles, when the initial cell concentration was 2.3g/L, reaching a maximum activity of 174 IU/mL, corresponding a volumetric productivity of 6700 $IU.L^{-1}.h^{-1}$, in 24 hours. However, when the initial cell concentration of 0.23g/L was investigated, the enzymatic activity was reduced by 40% after the first recycle. Finally, it could be concluded that the initial cell concentration influenced the process performance, yet the feeding interval did not affect enzymatic production.

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POSTER PRSENTATION 1B-49

Cellulase and Lignin-Blocking Protein Adsorption on Cellulosic Substrates

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A better understanding of how to implement the use of lignin-blocking proteins to lower the enzyme requirement for hydrolysis of cellulosic substrates will be obtained through characterization of the adsorption and desorption of blockers and cellulase. Adsorption profiles will be determined for promising lignin blockers on pure cellulose, acid pretreated corn stover, and pure lignin. A number of promising lignin blockers will be evaluated in addition to bovine serum albumin as a reference material. Avicel from Sigma will be used for pure cellulose, and lignin-rich substrates will be produced through the complete hydrolysis of pretreated corn stover. The effect of pH, temperature, pretreatment temperature, and order of addition of blockers and cellulase on their adsorption will be explored. The desired result of this research is a set of operating conditions which enhance the uptake of lignin blockers by lignin, and of cellulase by cellulose.

POSTER PRESENTATION 1B-50

Production of Cellulases and Hemicellulases from Agricultural Wastes by *Aureobasidium pullulans* on Solid State Fermentation

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Cellulase and hemicellulase enzymes have application in the paper and cellulose, food and chemical industries. These enzymes are produced by several microorganisms, mainly from fungal species. In this work a selected strain of the yeastlike *Aureobasidium pullulans* was investigated for production of cellulases and hemicellulases in solid state fermentation (SSF). The substrates used were wheat bran, soy bran, soy peel and corn cob. Others parameters as the production of protease and the variation of the pH during the process of fermentation also were analyzed. Higher enzymatic production was obtained on wheat bran cultivation, which was: 1,05 U/ml of endoglucanase (96 hours), 1,3 U/ml of β-glucosidase (120 hours) and 5,0 U/ml of xylanase (96 hours). It was not observed the production of avicelase by the microorganism. The production of xylanase and endoglucanase obtained in the present work, when compared with some others *Aureobasium* species, was very expressive. Moreover, regarding with the scarcity of works on production of these enzymes by the microorganism *Aureobasidium pullulans* on SSF, the result stands out its importance, extending the source of these enzymes for future works.

POSTER PRESENTATION 1B-51

Activities Determination and Stability of Peroxidase and Polyphenol Oxidase Obtained from Maté Tea Leaves (*Ilex paraguariensis*)

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Maté (*Ilex paraguariensis*) is an important natural product of South Brazil. Peroxidase and polyphenol oxidase are the enzymes responsible for browning in maté tea leaves. However, the enzymatic extract can be used in biotechnological processes as catalyst of oxidation reactions. In this context, the objective of this work is to establish an experimental condition that leads to better extraction and measurement of the oxidases activities presented in maté leaves. The results showed that, the mass of raw material and polyvinylpyrrolidone had a positive effect on the process. On the other hand, the pH of measurement of activity had a negative effect for peroxidase and a positive effect for polyphenol oxidase. Related to the stability, the peroxidase presented thermal stability in the temperature range of 20 to 60°C and a residual activity of only 16% after a exposure time of 30 min at 80°C. The activity of polyphenol oxidase was affected by handlings of 20 to 60°C and the enzyme was entirely inactivated when exposed to 6 min at 80°C. The storage at –4 and –80°C did not injure the activity of polyphenol oxidase. The activity of the peroxidase which presented a decrease with freezing and was regenerated after 15th day.

POSTER PRESENTATION 1B-52

Obtaining Chelating Agents through Enzymatic Oxidation of Lignin by the Polyphenoloxidase

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Chelating substances are able to scavenge metallic ions from aqueous solutions. There is an increasing interest in the decontamination of industrial wastes, which contain lead and other ions. Lignin can form chelates with metals due to the existence of many aromatics rings and polar groups in the molecular structure. The oxidation of the lignin aims the introduction of carbonyl groups and become this macromolecule with improved chelating capacity. The enzyme polyphenoloxidase was extracted from potato by a process of purification using $(NH_4)_2SO_4$, the lignin was dissolved in 1:1 (v/v) 1,4-dioxane/phosphate butter solution at pH 7.6. The solution was oxygenated during 6 h and every 30 min an aliquot was samples, which was measured in UV and graphics of time X absorbance were obtained. Considering that the measure of absorbance was, theoretically, the increase of carbonyls and hydroxyls in the lignin, the graphics showed linear tendencies and after a constant behavior after 4 h, these results showed the lignin macromolecule was oxidized. The next step of this project will be the determination of chelating power using chromatography, the stationary phase beeing Sephadex G-10, and the mobile phase Cu^{+2} solution.

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POSTER PRESENTATION 1B-53

Reuse of the Enzyme Xylanase in the Biobleaching Process of Sugarcane Bagasse Acetosolv Pulp

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The use of xylases in the pulp & paper industry is increasing. Detailed studies made in laboratories showed that the necessary investments to adapt the enzymatic treatment to the mill conditions are not expensive, with exception to the pH adjustment. Despite several studies in this area, the reuse of the enzyme in pulp biobleaching process was not yet investigated. The reusing of the enzyme xylanase in the bleaching process of the sugarcane bagasse acetosolv pulp was studied. After enzymatic treatment with xylanase for 4-hours with stirring of the 85 rpm, the possibility of the enzyme recycle was evaluated by enzymatic activity, kappa number and viscosity of the obtained pulps. The preliminary results suggest the possibility of the reusing of the xylanase with maintenance of the pulp viscosity.

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POSTER PRESENTATION 1B-54

Characterization of a Thermophilic Lipase Produced by *Penicillium simplicissimum* in Solid-State Fermentation

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Lipases are very versatile enzymes that catalyse a range of hydrolysis and synthesis reactions in triacylglycerides and that can present regio- and enantioselective properties. They find applications in a large number of areas, ranging from wastewater treatment to the pharmaceutical industry. However, a broader use of lipases in industrial applications requires the availability of enzymes with interesting properties being produced at low costs.

In the present work, *P. simplicissimum* was grown on babassu cake, and lipase production was followed along fermentation time. After enzyme extraction with phosphate buffer, the temperature and pH profiles of lipase activity in the raw extract were investigated. With this purpose, a response surface experimental design was carried out to study temperatures in the range of 25-45°C and pH values in the range of 5-7. It was observed that the highest activities were obtained at slightly acidic (5-6) pH values and temperatures higher than 40°C. In view of these results, the temperature profile in the range of 35°C-60°C was further investigated for pH values equal to 5, 6 and 7. These experiments confirmed that this *P. simplicissimum* lipase is a thermophilic enzyme, since activities as high as 80 U/g were obtained for temperatures in the range of 50-60°C.

POSTER PRESENTATION 1B-55

Ab initio Study of the Interaction Mechanisms between Aromatic Amino Acids and Cellulose

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Cost effective enzymatic conversion of biomass to fermentable sugars relies heavily on the development of more efficient biocatalysts. In order to improve the efficacy of enzymes acting on cellulose, understanding the mechanisms of how they bind to and hydrolyze the substrate surface is critical. The binding domains of cellulases (and other glycosyl hydrolases) often display a trio of aromatic amino acids (usually tyrosine or tryptophan) positioned in spatial sequence. In order to better understand the enzyme-cellulose interaction, *ab initio* molecular dynamics simulations of the interaction mechanisms between tyrosine and tryptophan and the 1,0,0 surface of cellulose 1β were conducted. Particularly, the relative strengths of hydrophobic and CH-π interactions between the aromatic side chain and hydrophobic cellulose surface were examined. Furthermore, the roles of hydrogen bonding interaction between the cellulose hydroxyl groups and the aromatic side chains (e.g., the phenol hydroxyl group of tyrosine and the indole secondary amine group of tryptophan) were investigated. The energies of these aromatic amino acids binding to cellulose will be estimated computationally and discussed in terms of possible mechanisms of binding domain function.

POSTER PRESENTATION 1B-56

Adaptation of a Foam Fractionation Column for β-Glucosidase Purification Using a Crude Cellulase Solution

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Foam fractionation is a promising tool for enzyme purification and separation because it is simple, inexpensive, environmentally friendly and ready for scale up. Considering this may be a promising technique for separating microbial enzymes from a dilute solution. This was one of the reasons for utilization this technique in components purification of a crude cellulase nominated FILTRASE NLC provided by DSM Food Specialties, existing particular interest with respect to β-glucosidase activity.

The experimental bubble/foam fractionation column used was a two liters Nalgene tall graduate cylinder modifying the upper rim placing a cylindrical polyesthyrene (unicel) cup as cover. The initial volume liquid solution including the enzyme solution added, ranged from 1740 to 1990 ml, according to a typical batch experiment.

Air was introduced submerging an Aquarius domestic pump to create bubbles into the column fractionation, air flow rate was monitored by a rotameter using a kit for variable flow measure (Cole-Palmer) with scale of 0-150 mm/min with Tantalio floater. The air rate during the fractionation was maintained at 75-85 mm/min and the foam was withdrawn at various times in a manner similar to a batch distillation process.

The resulting volume of the liquid foamate "cut" was measured along with pH, total protein content, cellulase activity, using filter paper as substrate and β-glucosidase activity using p-nitrophenyl-β-D-glucoside as substrate. It was not possible the measurement of surface tension in the solution during foam fractionation but we are considering that this variable is a function of the bulk solution pH.

POSTER PRESENTATION 1B-57

Purification and Characterization of Two Thermostable Xylanases from Alcalophilic *Bacillus licheniformis* 77-2

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The alkalophilic bacteria *Bacillus licheniformis* 77-2 produces significant quantities of thermostable cellulase-free xylanases. In previous work, the crude enzyme was applied in the biobleaching of Kraft pulp, and it was demonstrated that the after enzymatic treatment the same Kappa number was obtained using 33% less ClO_2. An improved understanding of the hydrolytic properties of these enzymes is fundamental in the development of industrial application for these xylanases. Therefore, the crude enzymes was purified to apparent homogeneity by gel filtration (G-75) and ionic exchange chromatography (DEAE-sepharose and Mono Q), which resulted in the isolation of two xylanases. The molecular masses of the enzymes were estimated to be 17 kDa (X-I) and 40 kDa (X-II), as determined by SDS-PAGE. The xylanases demonstrated good stability up to pH 11 with optimum activity at pH 6.0 and retained more than 80 % of hydrolytic activity at pH 9.0. The purified enzymes were most active at temperature range of 70-75°C and the residual activities at 90°C after 1 hour were 50% for the X-I and 90% for the X-II. The predominant products of xylan hydrolysates indicated that these enzymes were endoxylanases.

POSTER PRESENTATION 1B-58

Conversion of Di- and Tri-Methoxybenzyl Alcohols by Laccases and Peroxidases

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Laccases and peroxidases are of interest as oxidants in analysis and detoxification of solutions containing aromatic compounds, in pulp bleaching, and in production of fiber boards and chemicals. The conversion of methoxy-substituted benzyl alcohols by *Trametes versicolor* laccase, *T. versicolor* lignin peroxidase and horseradish peroxidase was investigated. The compounds included in the study were: 2,4,5-trimethoxybenzyl alcohol, 3,4,5-trimethoxybenzyl alcohol, 2,3,4-trimethoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, and 2,3-dimethoxybenzyl alcohol. Enzymatic oxidation resulted in the formation of the corresponding methoxy-substituted benzaldehydes as the strongly predominant products. The observed reaction rates were, however, very different. Oxidation with laccase and horseradish peroxidase was very slow for some of the methoxy-substituted benzyl alcohols and was only observed when the reactions were carried out at low pH and with high substrate concentrations. The rates of oxidation observed with lignin peroxidase were in general high. The reaction rate order was the same for laccase and horseradish peroxidase and 2,4,5-trimethoxybenzyl alcohol was by far the best substrate. For lignin peroxidase, 3,4-dimethoxybenzyl alcohol was the best substrate and 2,4,5-trimethoxybenzyl alcohol ranked only second best. With 2,4,5-trimethoxybenzyl alcohol as substrate, horseradish peroxidase had the highest apparent k_{cat} value while lignin peroxidase had the lowest apparent K_m value. Lignin peroxidase displayed the highest catalytic efficiency and laccase the lowest.

POSTER PRESENTATION 1B-59

Heterologous Expression of *Trametes versicolor* Laccase in *Pichia pastoris* and *Aspergillus niger*

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Laccases are copper-containing phenol-oxidizing enzymes that are useful in many different applications, for example in lignocellulose processing and textile industry. Efficient and convenient systems for heterologous expression are needed for production and characterization of different laccases. The methylotrophic yeast *Pichia pastoris* has gained increasing attention as a host for fast and convenient expression of laccases under the control of the strong and methanol-inducible *AOX1* promoter. In this study, the strong and constitutive glyceraldehyde-3-phosphate dehydrogenase (*GAP*) promoter was investigated as an alternative to the *AOX1* system, which suffers from drawbacks such as the need for tight control of methanol levels. With filamentous fungi, such as *Aspergillus niger*, larger amounts of recombinant laccase can be produced, although the handling of the expression system is tedious in comparison with *P. pastoris*. Laccase cDNAs from the white-rot fungus *Trametes versicolor* were inserted between the glyceraldehyde 3-phosphate dehydrogenase (*gpd*) promoter and the glucoamylase (*glaA*) terminator and used for transformation of *A. niger.* The recombinant laccase was purified to homogeneity and its properties were compared with those of native *T. versicolor* laccase.

POSTER PRESENTATION 1B-60

Kinetic Investigation of Cellulase Enzyme Using Non-Crystalline Cellulose and Cello-Oligosaccharides

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Hydrolysis of cellulose by cellulase enzymes is a solid-liquid heterogeneous reaction. As such the reaction is strongly affected by the non-reaction resistances caused most notably by the crystalline structure. Other non-reaction factors include surface area, diffusion of enzyme, substrate and product, and adsorption of enzyme onto non-cellulosic components. These non-reaction factors mimic the true nature of the hydrolytic enzymatic reaction.

Recently we have invented a method to produce non-crystalline cellulose (NCC) from a commercial α-cellulose or cotton by a relatively simple process. This material is drastically different from natural cellulose in that the crystallinity is totally removed. We have also produced cello-oligosaccharides (COS) from the NCC.

With use of NCC and COS, we were able to study the true intrinsic kinetics of cellulose hydrolysis unaffected by the physical barriers. The most notable difference seen in this study is that the activity measured by initial rates against NCC is two orders of magnitude higher than that against crystalline cellulose. Since removal of physical barrier primarily increases the hydrolysis by endoglucanase, a significant amount of cellobiose was seen to accumulate in hydrolysis of NCC. Kinetic experiments conducted with addition of external cellobiose and glucose generated inhibition kinetic parameters vastly different from those previously reported. This paper reports the intrinsic kinetic model of cellulose hydrolysis and the associated kinetic parameters measured with NCC and COS.

POSTER PRESENTATION 1B-61

Rapid Measurement of Cellulase Activity Using Non-Crystalline Cellulose

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The current method of measuring the specific activity of cellulase is based on use of filter paper as the standard substrate. It involves incubation of the substrate with a given enzyme under a specified conditions followed by calorimetric measurement of released glucose. This method suffers from the fact that the overall procedure is very time-consuming (about 48 hours) and that it has low accuracy and consistency in replicate tests.

Recently we have invented a method to produce non-crystalline cellulose (NCC) from a commercial α-cellulose or cotton by a relatively simple process. This material is drastically different from the natural cellulose in that the crystallinity is totally removed. Because of non-crystalline structure, the initial rates of enzymatic hydrolysis of NCC are two orders of magnitude higher than that of natural cellulose. Using this material as a standard substrate, we have devised a rapid method to measure the cellulase activity. The procedure involves incubation of NCC for 20 minutes to determine the initial rate of hydrolysis. The resulting glucose and cellobiose measured by HPLC are taken as the index for cellulase activity. The results showed linearity with the amount of enzyme applied. From this a specific enzyme activity based on protein content could be determined. The activities thus determined were calibrated with the conventional filter paper units (FPU). Details of the proposed test method and the statistical analysis of the outcome are presented.

POSTER PRESENTATION 1B-62

Peroxidase Production and Application in the Isosafrole Biotransformation into Piperonal

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Isosafrole is the product of safrole isomerization (main component of essential oil extracted from *Piper hispidinervium*. Piperonal is one of its oxidation products, which can be used as important intermediate in the pharmaceutical synthesis such as L-Dopa and epinephrine, among others, being also helper in insecticides. However, the most economic importance of this substance is related to its use in the fragrance industry as a biodegradable fixer. Piperonal has more restricted distribution and it is less abundant in nature. In Brazil, piperonal is usually produced from isosafrole by ozonolysis.

The present work is based in two main scopes: peroxidase enzyme production by *Paecilomyces variotii* (IOC-4230) using modified Czapeck-Dox medium and its application in isosafrole biotransformation into piperonal, using hydrogen peroxide as oxidizing agent.

The methodology involved four steps: (i) spore production, (ii) microorganism growth in liquid culture, (iii) enzyme production, and (iv) biotransformation assay. Enzymatic activity assays were made daily through the method of guaiacol oxidation, and the samples that present the best results of enzymatic activity were used in biotransfromation experiments. The products were extracted and identified by gas chromatography and mass spectrometry. Allowing the quantification of piperonal with high yield and good selectivity.

POSTER PRESENTATION 1B-63

Production and Characterization of Thermo- and pH- Stable Cellulase from *Streptomyces sp* M23

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Present and forthcoming use of cellulase require enzymes that are stable both at high temperature and in alkaline environment. In the current work *Streptomyces* sp M23, isolated from Brazilian cerrado soil, was shown to produce pH and temperature stable cellulases. The culture supernatant, after incubation at 50ºC for two hours, retained 98% endoglucanase activity upon incubation at pH 6.0 and 90 % activity upon incubation at pH 8.0. Cellulases were concentrated through membrane filtration and partially purified using ammonium sulphate precipitation. The resulting protein pool was analyzed using zymogram and 2D gels.

Submerged fermentations were preformed using wheat bran, Distillers Grain (DG), crystalline - and amorphous (CMC) cellulose, lactose and glucose as carbon and energy sources and yeast extract, ammonium sulfate and casein amino acids as nitrogen sources. Contrary to the described pattern for other *Streptomyces* family members, our results indicate that the cellulase production by this particular strain was not negatively affected by glucose. This result may be of economical advantage in the fuel ethanol industry. The inducing effect of sophorose was also evaluated.

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POSTER PRESENTATION 1B-64

Immobilization of *Yarrowia lipolytica* Lipase by Adsorption on Hydrophobic Support

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Microbial lipases (acylglicerol hydrolases, E.C. 3.1.1.3) have been increasingly employed in a broad range of applications, such as in the manufacture of detergents, in the food, leather, pulp and paper and pharmaceutical industries, for the production of fine chemicals and for oily wastewater treatment. Lipase immobilization offers unique advantages in terms of better process control, enhanced stability, enzyme-free products, predictable decay rates, and improved economics. The aim of this work is evaluate the immobilization of *Yarrowia lipolytica* lipase on hydrophobic support. This enzyme was produced in 2000 liters fermentor on a medium constituted by glucose (1%), wheypowder (3%), ammonium sulphate (0.8%), cornsteep (1%) and olive oil (0.5%). At 30 hours of fermentation the culture broth was centrifuged and the supernatant was dryed by lyophilisation. The crude powder was solubilized in phosphate buffer (0.05M pH 7.0) and immobilized by physical adsorption on hydrophobic support (Accurel® MP 1000). The procedure was completed in 2 hours, with 98 % of protein adsorption. Enzymatic activity of immobilized lipase was 58 U/g assayed using olive oil as substrate and stability higher than 90% during 1 month of storage. These results clearly point out the potential of immobilized *Yarrowia lipolytica* lipase as catalyst.

POSTER PRESENTATION 1B-65

Characterization of Hemicellulose Degrading Enzymes Using Natural Substrates

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Our group has been pursuing gene discovery and improvement of biomass degrading enzymes through the use of directed evolution, whereby a library of mutated enzymes is screened to select for clones with improved performance under the screening conditions employed. For improved selection an *in-vitro* screen that emulates the anticipated final commercial-scale bioreactor process conditions was desired, thus requiring a screening assay that uses naturally occurring substrates. We present here work on the cloning of hemicellulolytic enzymes, and methods for the characterization of these enzymes using natural substrates.

POSTER PRESENTATION 1B-66

Docking Studies of Lipase Hydrolysis Applied to a New Prototype Anti-Asthma Drug

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Lipases constitute a class of enzymes of major importance in applied biocatalysis. The good stability of the lipases in organic solvents makes them suitable for reactions in intermediate stages of conventional organic chemical processes and in the catalysis of substrates which are insoluble in aqueous media. Three substrates that are precursors for pharmaceuticals building blocks, drifted to act as promising anti-asthmatic, were submitted to enzymatic hydrolysis: 4-ethyl-(2-(1,3-dioxo-1,3-dihydro-2-isoindoylyl))-phenoxyacetic ethyl ester (PHT-ET), 4-(1,3-dioxo-1,3-dihydro-2-isoindoylyl)-phenoxyacetic ethyl ester (*p*-PHT-ET) and 2-(1,3-dioxo-1,3-dihydro-2-isoindoylyl))-phenoxyacetic acid ester (*o*-PHT-ET). The reactions proceeded well in those cases in which *para*-substituted phenoxy groups were present (50% and 20% of conversion, respectively) and not for the *orto*-substituted substrate. This work addresses structural studies in order to understand the molecular reasons of *Rhizomucor miehei* lipase steric selectivity acting towards these substrates. Docking results concerned to interaction between these substrates and active site (Ser144-Asp233-His257) and oxianion hole (Ser82-Lys145) residues, revealed spatial hindrance between Tyr28 and phthalimide moiety of *o*-PHT-ET, suggesting that this interaction constitutes an impediment to enzymatic catalysis. On the other hand, *para*-substituted substrates did not show any hindrance, confirming the experimental results.

POSTER PRESENTATION 1B-67

Thermoacidophilic Cellulases and Hemicellulases from *Alicyclobacillus acidocaldarius*

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Hemicellulose and cellulose represent a large potential renewable reservoir of sugars that could be converted into useful fuels and chemicals. Many pretreatment techniques have been developed that allow near complete conversion of hemicellulose and cellulose into their component sugars; however, the harsh conditions required by these techniques can cause the formation of harmful byproducts that inhibit subsequent biologically-based conversions to fuels and chemicals. There is potential for the application of extremophilic hemicellulose- and cellulose-degrading enzymes to reduce the severity of pretreatments and reduce or eliminate these limitations. Of particular value could be heat and acid stable hemicellulase and cellulase enzymes. We screened numerous organisms from Yellowstone National Park and various culture collections for the ability to produce these enzymes at both high temperatures and low pH. One organism tested, *Alicyclobacillus acidocaldarius*, produced both extracellular hemicellulase and cellulase activities. Our initial characterization studies determined that both the cellulases and hemicellulases were active from 20-90°C, with the cellulases active over pHs from 2-6 and the hemicellulases active from pH 1-5. Data on the stability and kinetics of these enzymes at elevated temperatures and acidic pHs will also be presented.

POSTER PRESENTATION 1B-68

Evaluation of Solid and Submerged Fermentations for the Production of Cyclodextrin Glycosyltransferase by Thermophilic Bacterium H69-3: Charactization of Crude Enzyme

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Cyclodextrin glycosyltransferase (CGTase, E.C. 2.4.1.19) is an enzyme that produces cyclodextrins from starch by intramolecular transglycosylation reaction. Cyclodextrin is a important molecule for application in food, cosmetic, pharmaceutical and chemistry industries. CGTase production was studied in submerged and solid state fermentations by a thermophilic bacterium gram- namely H69-3 at 45°C. Different substrate sources as wheat bran, soybean bran, soybean extract, cassava solid residue, cassava starch, corn starch and other combinations were used in the enzyme production. Activity CGTase was higher in submerged fermentation with large production of activity at 48 to 60 hours of fermentation. Physical and chemical properties of this CGTase produced in submerged fermentation were determined. The optimum temperature was in range of 70-75°C. The thermostability was up to 55°C by 1 hour. The enzyme displayed two optimum pH, one at pH 5.5 and other at pH 9.0. The enzyme was stable in the range of pH 4.5 to 11.0.

POSTER PRESENTATION 1B-69

Cellulose Hydrolysis by *Penicillium echinulatum* Cellulases

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Preliminary studies have shown that *Penicillium echinulatum* is a good cellulase producer but little attention has been devoted to the characterization of its cellulase system. In this work, the hydrolytic potential of *P. echinulatum* enzymes has been compared to a commercial *Trichoderma reesei* preparation (Celluclast 1.5L FG®, Novozymes, Araucária, Brazil). The activity profile of the enzymes was determined against filter paper, carboxymethylcellulose (CMC), hydroxyethylcellulose (HEC), *p*-nitrophenyl-b-D-glucoside (PNPG), cellobiose (G2) and oat spelt xylan (XYL). The filter paper activity of 110 FPU/mL in Celluclast and 0.27 FPU/mL in *P. echinulatum* cellulases was used as a reference for comparison. For *T reesei* Celluclast, the ratio among CMC:HEC:XYL:pNPG:G2:FP volumetric activities was 2.83:12.64:12.43:0.39:0.16:1, whereas for *P. echinulatum*, this ratio corresponded to 12.07:17.34:12.17:1.14:0.69:1. These results indicated that the *P. echinulatum* system has greater endoglucanase and b-glucosidase activities than Celluclast. Hydrolysis experiments (2%,w/v) were also carried out with Sigmacell type 50 and two fractions of an eucalypt kraft pulp (bleached and unbleached) and, at equivalent enzyme loadings, Celluclast required supplementation with b-glucosidase activity (Novozym 188®) to display the same hydrolytic potential of *P. echinulatum*. We are currently evaluating the effect of both enzyme systems on the crystallinity and degree of polymerization of the above mentioned substrates.

Supported by: CNPq, CAPES, UFPR/Funpar, Araucaria Foundation

POSTER PRESENTATION 1B-70

Properties and Performance of Glucoamylases for Fuel Ethanol Production

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Glucoamylases are key enzymes utilized to produce ethanol from grain. They may be applied during a separate saccharification step, or added to the fermenters for simultaneous saccharification and fermentation. In either case, enzyme properties can have a significant impact on ethanol yield and production rate.

This work describes the characteristics and performance of various glucoamylases. Kinetics studies of maltodextrin hydrolysis were performed at 35°C and at 55°C, typically at pH 4.8. None of the commercial glucoamylases were able to completely convert maltodextrin into glucose, even if the reaction time was increased to 48h. Typically, about 85% maltodextrin conversion was obtained, and glucose yields were on the order of 75 to 80%. The reaction kinetics for most enzymes were biphasic, with a rapid initial conversion rate over about 1 hour, and then a significant reduction in rate until the dextrin, maltose and glucose profiles reached a plateau. The kinetics profiles were consistent with strong product inhibition and/or enzyme inactivation. Most of the glucoamylases were more active at 55°C than at 35°C; with one notable exception, which exhibited nearly equivalent activity at both temperatures.

POSTER PRESENTATION 1B-71

Purification and Characterization of a Novel Cyclodextrin Glycosyltransferase from Thermophilic Bacterium H69-3

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A new cyclodextrin glycosyltransferase (CGTase, E.C. 2.4.1.19) from thermophilic bacterium gram- H69-3, isolated from soil cassava crop, was concentrated by ultrafiltration and purified by gel filtration chromatography, ion exchange chromatography on Q-Sepharose, and ion exchange chromatography on Mono Q. The molecular weight of the pure enzyme was estimated to be 70 kDa with SDS-PAGE. The enzyme displayed optimum pH value at pH 6.5 The enzyme was most active in the pH range 6.0 to 11.5. The stability temperatures were up to 65°C by 1 hour heating in the presence of 10 mM $CaCl_2$. The optimal temperatures were at 55°C on pH 6.0. The enzyme was stimulated by Ba^{2+}, Ca^{2+}, Mg^{2+}, $Mn^{2+}$and DTT and it was strongly inhibited by Al^{3+}, Ag^{2+}, Cu^{2+}, Cr^{2+}, Fe^{3+}, Hg^{2+}, Sn^{2+} SDS and EDTA. Using maltodextrin as a substrate, the obtained values for K_m and V_{max} were 2.95 mg/ml and 36.0 µmol/min.mg respectively.

Supported by FAPESP

Poster Abstracts for Session 2

Today's Biorefineries

POSTER PRESENTATION 2-07

Optimization of Biodiesel Production from Castor Oil

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Biodiesel is an alternative biodegradable and nontoxic fuel, which is essentially free of sulfur and aromatics. It is usually produced by a transesterification reaction of vegetable or waste oil with a low molecular weight alcohol, such as ethanol and methanol. Industrially, the most common method for biodiesel production is a basic homogeneous reaction.

This work presents the transesterification of castor oil with ethanol in the presence of sodium ethoxide as catalyst. This is an exceptional option for the Brazilian biodiesel production, since the castor nut is quite available in the country. Chemically, its oil contains approximately 90% of ricinoleic acid, that gives to the oil some beneficial characteristics as its alcohol solubility at 30ºC. Although these advantages, it is known that the castor oil transesterification needs high excess of alcohol (650%) for a possible glycerin separation.

In this way, this study showed, through a star configuration experimental design with central points, that it is possible to achieve the same yield of esters carrying out the transesterification reaction with only a smaller alcohol quantity and a new methodology was developed to get high purity biodiesel. The studied variables were reaction temperature, catalyst concentration and alcohol oil molar ratio.

POSTER PRESENTATION 2-08

Liquefaction of Agricultural Residues to Biopolyols at Atmospheric Pressure and Low Temperature

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The traditional liquefaction process generally needs high temperature and pressure to convert biomass to black oil. The liquefaction of agricultural residues to biopolyols at atmospheric pressure and low temperature will be presented in this manuscript. The effects of solvents on the liquefaction rate of different kinds of feedstocks will also be presented.

Liquefaction of agricultural residues (corn stove, wheat straw, and rice straw) at ambient pressure and low temperature with three different solvents named ethylene glycol (EG), ethylene carbonate (EC) and polyethylene glycol (PEG) was studied. The analysis of experimental results showed that the acid catalyzed liquefaction process fit the pseudo-first-order kinetics model. The constants for liquefaction rate of corn stover with EG, EC and PEG were 1.34×10^{-3}/min, 5.29×10^{-3}/min, and 3.02×10^{-3}/min respectively. When EC was used as solvent, such constant of rice straw was 1.83×10^{-3}/min, lower than that of corn stover. When a mixed solvent of EG and EC with a ratio of 2:8 (w/w) was used, the constants of corn stover and rice straw were 2.79×10^{-3}/min and 1.06×10^{-3}/min respectively. The properties of the liquefied mixture such as hydroxyl numbers and the viscosities of biopolyols were also analyzed.

POSTER PRESENTATION 2-09

Fermentative Hydrogen Production from Animal Manure

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Hydrogen is a clean energy source with H_2O as its only combustion product. It has the highest energy content (122 KJ/g) among any known fuels. The current hydrogen production methods, such as steam reforming of fossil fuels, water electrolysis, and pyrolysis of biomass are energy intensive and expensive. Biological/fermentative production of hydrogen is a sustainable and attractive method, especially if waste agricultural residues could be used as raw materials.

As traditional fermentative hydrogen production is limited by the low yield and conversion rate, a hydrogen production followed by methanogenic anaerobic digestion to increase the energy yield of the overall process was developed in this work. Dairy manure was used as feedstock. Sequential anaerobic digesters were implemented to separate different stages of manure anaerobic digestion process, i.e., composite disintegration, carbohydrate/protein hydrolysis, acidogenesis/acetogenesis, and methanogenesis. In the acidogenesis/acetogenesis stage, heat-shock and low pH were used to inhibit the nonsporeforming bacteria (hydrogen consuming methanogens) and to enrich the sporeforming bacteria (hydrogen-producing acidogens). Also, nitrogen was spared into the reactor to reduce the hydrogen partial pressure. The produced volatile fatty acids in acidogenesis/acetogenesis stage were converted into methane in the subsequent methanogenesis reactor. Supporting media were used to enhance the biomass population and the consequent methane production. The sequential digesters enhanced both hydrogen and total biogas production significantly.

POSTER PRESENTATION 2-10

Production of Acetone Butanol from Corn Fiber Xylan Using *Clostridium beijerinckii* P260

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Acetone butanol (AB or solvents) was produced from corn fiber xylan (CFX) using *C. beijerinckii* P260. In order to perform these studies, individual components of CFX (glucose, xylose, galactose, and arabinose) were fermented using 60 g/L initial sugar. In approximately 72 h of fermentation, these sugars resulted in the production of 12.4, 11.1, 10.9, and 15.2 g/L total solvents, respectively. Under the same conditions, CFX was not fermented due to lack of bacterial growth and hydrolysis of CFX. Fermentation (pH 6.1-6.3) with 60 g/L CFX and the addition of a crude hemicellulase preparation was also unsuccessful, indicating that the hydrolysis of CFX was too slow to provide sufficient sugars for growth and solvent production. Fermentation with 60 g/L CFX and 5 g/L xylose resulted in the production of 5.2 g/L total AB, suggesting that the culture was able to partially hydrolyze CFX as 5 g/L xylose cannot produce more than 1.7 g/L AB. Further fermentation with 60 g/L xylan, 5 g/L xylose plus the crude hemicellulase preparation produced 9.6 g/L total solvents. These studies suggested that CFX can be fermented to AB by *C. beijerinckii* when hemicellulases and low amount of sugar are provided.

POSTER PRESENTATION 2-11

Process Modeling and Economic Analysis of MBI Biorefinery

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Detailed Aspen Plus™ based process modeling and economic analysis was developed to evaluate the performance of MBI's biorefinery concept. The biorefinery concept envisioned efficient use of raw materials to produce a variety of products in addition to ethanol and improve profitability of dry mill ethanol plants. Upstream corn milling equipment in conventional dry mill ethanol plants was replaced with germ and fiber separation equipment. Degermed-Defibered Corn (DDC) with higher starch content was fed to the existing saccharification and fermentation units resulting in higher ethanol productivity than with regular corn. The fiber was pretreated using Ammonia Fiber Explosion (AFEX) to enhance sugar separation. The separated sugars were used as feedstock for a variety of value-added products. Corn germ was processed into corn oil and deoiled germ using a mechanical expeller.

Aspen Plus™ based process modeling and economic analysis was developed using current experimental data to identify potential opportunities, process and economic bottlenecks, critical cost factors and provide research directions towards enhancing the overall economics of the dry mill ethanol plant.

POSTER PRESENTATION 2-12

Techno-Economic Assessment of Hemicellulose Extraction of Wood Chips for Ethanol Production

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The US Forest Products industry is a major contributor to the national economy as well as a major producer and consumer of energy. A series of projects have been proposed by the industry to examine ways to increase the value of their products coming from the wood feedstocks already being processed in their mills. One near term option is to carefully pre-extract the hemicellulosic sugars before the main pulping step and then be converting them to ethanol. A key difference to other lignocellulosic biomass conversion processes is that the solid "residue" left after the extraction is the highest value material since it will be further processed to make pulp and paper products. There is a trade-off in how much hemicellulose is to be extracted without adversely affecting the amount and quality of the pulp to be made.

A key aspect to the economic viability of such a process is the tradeoff of how much hemicellulose can be extracted vs. the costs to then produce a saleable ethanol product. A techno-economic assessment has been performed to determine realistic boundaries of hemicellulosic sugar conversions and other operating conditions by which such a process could be economic.

POSTER PRESENTATION 2-13

Extraction of Hyperoside and Quercitrin from Mimosa (*Albizia julibrissin*) Foliage

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Mimosa, an excellent energy crop candidate because of its high growth yield, also contains, on a dry basis, 0.83 percent hyperoside and 0.90 percent quercitrin. Hyperoside has been documented as having anti-inflammatory and diurectic properties, while quercitrin may play a role in intestinal repair following chronic mucosal injury. Thus, mimosa might first be extracted for important antioxidant compounds and then used as a feedstock for energy production. This paper presents results from studies aimed at determining the effect of extraction parameters (temperature, solvent composition, solids concentration, particle size, time) on concentration and yield of these important quercetin compounds. Conditions are sought which maximize yield and concentration, while complementing subsequent biomass pretreatment, hydrolysis and fermentation.

POSTER PRESENTATION 2-14

Evaluation of Soybean Hulls as a Source of Ethanol

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Soybeans are one of the largest crops in the United States. The soybean core is surrounded by a rigid hull that is approximately one quarter cellulose plus significant levels of highly substituted hemicellulose, pectin and uronic acid. Protein levels are relatively low compared to the soybean meal at about 10%. The hulls are separated from the meal and blended with and sold as animal feed. However, soybean hulls have been suggested as a source of carbohydrates for fermentation to ethanol. Work will be described regarding the ability of *Saccharomyces cerevisiae* as well as recombinant ethanologenic bacteria to ferment soybean hulls carbohydrates to ethanol. Important to this evaluation are the proportion of carbohydrates that can be converted to ethanol and the impact of the overall process on the protein value in the fermentation residue.

POSTER PRESENTATION 2-15

Development of New Antifoam Technologies for Fermentation Processes

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Fermentation is often accompanied by foam formation because of the high foaming tendency of solutions containing biomaterials such as proteins. The aim of our work is to better understand the foaming process and the properties of antifoam in fermentation processes. Production of lipase by *Yarrowia lipolytica* has been selected as model fermentation system, as we experienced it to be especially foamy.

We characterized how the yeast mass production increases dramatically the foaming tendency as well as decreases the capacity to control the foam with chemical antifoams and correlated it with the release of extracellular proteins. Ability to control the foam of a range of materials (from organic materials to silicone) was screened on the fermentation medium by a test protocol reproducing aeration conditions in the bioreactor. This demonstrated that dramatically different antifoaming efficiency are obtained, the best one being based on silicone material. The effects of selected antifoams on oxygen transfer, growing medium surface properties and downstream processes like ultrafiltration were also investigated.

Poster Abstracts for Session 3A

Plant Biotechnology and Feedstock Genomics

POSTER PRESENTATION 3A-08

Variation of S/G Ratio and Lignin Content in a *Populus* Family Influences the Release of Fermentable Sugars by Dilute Acid Hydrolysis

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Wood samples from individual progeny of a second generation *Populus* cross, grown in a common garden, were shown to have different lignin contents and S/G ratios (S: syringyl-like lignin structures; G: guaiacyl-like lignin structures). The lignin contents varied from 22.7 to 25.8% and the S/G ratio from 1.8 to 2.3. Selected samples spanning these ranges were hydrolyzed with dilute sulfuric acid (1% by weight) to release fermentable sugars (1% by-weight solids, 175°C maximum, 12-minute hydrolysis time). The conditions were chosen for only partial hydrolysis of the hemicellulosic fraction to maximize the expression of variation among samples. The results indicated that both lignin contents and S/G ratio significantly affected the yield of xylose. For example, the xylose yield of the 25.8% lignin and 2.3 S/G (high lignin, high S/G) sample produced 30% of the theoretical yield, whereas the xylose yield of the 22.7% lignin and 1.8 S/G (low lignin, low S/G) was 55% of the theoretical value. Significant interactions between lignin contents and S/G ratios also were observed. These results indicate that lignin content and composition among genetic variants within a single species can influence the hydrolyzability of the biomass.

POSTER PRESENTATION 3A-09

Enhanced Secondary Metabolite Production by Elicitation in Transformed Plant Root System

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Plants are a potential source of a large number of valuable constituents. Generally, plants produce secondary metabolites in nature as a defense mechanism against attack by pathogens and insects. In recent research of the root culture system, a wide variety of elicitors have been employed to modify cell metabolism in order to enhance the productivity of useful metabolites in plant cell cultures. Elicitation strategies are compounds or treatments that induce plants to synthesize phytoalexins at elevated levels. The active mechanisms of elicitors are considered to be different and complex. Since little is known of the biosynthetic pathways of most secondary metabolites in plants, the effect of elicitation on the plant root culture cannot be easily predicted. Therefore, elicitation approaches are performed by trial and error. The effect of elicitors depends on many factors, such as the treated concentration of elicitor, the growth stage of the culture at the time of elicitation and the contact time of elicitation. In this study, *P. ginseng* C.A. Meyer hairy root cultures, established by infecting of *A. rhizogenes* KCTC 2744, were used for improving the biosynthesis of secondary metabolites and growth rate by using several elicitors (selenium, nickel, salt stress and etc.).

POSTER PRESENTATION 3A-10

Identification and Characterization of Maize Mutants with Potential Use as Green Chemical Feedstocks

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In order to expand available germplasm resources with enhanced bio-processing characteristics, we are using near-infrared spectroscopy to identify secondary cell wall mutants of maize using the Uniform*Mu* population developed at the University of Florida. NIR reflectance spectra are acquired from dried leaf segments obtained from 1,000 F2 families (20 leaves per family) segregating for transposon-induced mutations. The field lay-out, data acquisition, and data analysis accommodates environmental and operator-related variation. Unusual spectra that may reflect variation in chemical composition are identified by principal component analysis. Based on our data approximately 10% of the families contain unusual spectra in a proportion consistent with a genetic mutation. Only 15% of these putative mutants have an obvious visual phenotype, indicating that the screening based on spectral features captures putative mutants that would otherwise go unnoticed. Pyrolysis-molecular beam mass spectrometry (Py-MB-MS) is performed as a secondary screen to characterize the chemical composition of the putative mutants in more detail. In addition, the genes affected by the mutations can be cloned after of the development of homozygous mutant lines. This project is funded by the NSF Plant Genome Program.

POSTER PRESENTATION 3A-11

Recombinant Cellulase Expression in the Chloroplast of Tobacco Plants

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A gene (*egIV*) encoding the endoglucasnase EGIV from *Ruminococcus albus* and its modified version containing the cellulose binding domain CBDII from *Clostridium stercorarium* xylanase A, were used to transform tobacco plants. Coding regions of the two genes were cloned into plant expression vectors under the control of an alfalfa *ssu* rubisco promoter containing or not a chloroplast transit peptide. Plants containing the constructs with no transit peptide accumulated the gene product in the cytosol, while those with the transit peptide accumulated the product in the chloroplast, as shown by microscopic examination and immuno-localization, indicating that the alfalfa transit peptide functioned in tobacco cells. Plants producing cellulases in the chloroplast showed no alteration of phenotype, while cellulases in the cytosol adversely affected plant growth and development. Recombinant cellulases extracted from leaves retained their activity. In view of a potential industrial lignocellulose bioprocess, autohydrolysis of transgenic tobacco plants expressing cellulases was examined.

POSTER PRESENTATION 3A-12

Direct Observation of The Maize Primary Cell Wall Using Atomic Force Microscopy

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Understanding the mechanism of plant cell wall deconstruction at the molecular level will provide valuable new insights to the nature of biomass recalcitrance. We have employed atomic force microscopy (AFM) to image the surface structure of different faces of maize parenchyma cell walls at the nanometer scale. In most cases, microfibrils are clearly distinguishable from background cell wall material and display an average diameter of 5-10 nm, with some small variance depending on the specific cell imaged and similar cells in different developmental stages. Under AFM measurement, the microfibril is rather smooth with no detectable periodicity at the subnanometer scale. Upper sheets of parallel microfibrils in the cell wall were clearly shown to be arranged or deposited in approximately 50 degree rotation with respect to lower sheets. An interesting discovery is that the macrofibrils appear only on the surface layer of the cell wall. Each macrofibril appears to be composed of a number of elementary fibrils, and eventually split or dispersed into a number of microfibrils towards the ends. The microfibril is believed to contain one elementary fibril, with various amounts of non-cellulosic polymers deposited on its surface during cell growth. Based on our direct AFM surface measurements and currently available literature data from plant cell wall biophysics, biosynthesis, and genomics, a new elementary fibril model and its possible biosynthesis are proposed. This new 36-chain elementary fibril model may also provide new insights useful for the plant cell wall biodegradation.

Poster Abstracts for Session 3B

Biomass Pretreatment and Hydrolysis

POSTER PRESENTATION 3B-08

Simultaneous Saccharification and Fermentation of Steam-Pretreated Corn Stover at High Dry-Matter Concentration for Fuel Ethanol Production

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In ethanol production from pretreated lignocellulosic materials, there are many advantages in utilizing simultaneous saccharification and fermentation (SSF) instead of separate hydrolysis and fermentation (SHF). For example, end-product inhibition of the enzymes in the enzymatic hydrolysis can be avoided, the risk of contamination is reduced (since the somewhat toxic hydrolysate after pretreatment tends to suppress bacterial growth.), and capital investments are lower as the total reactor volume is decreased due to higher productivity. However, a disadvantage of SSF is the fact that optimum temperatures for the yeast (35°C) and enzymes (40-50°C) are different.

SSF was performed at different concentrations (5, 7.5 and 10 %) of water insoluble substances (WIS) with 5 g/L compressed baker's yeast and 32 FPU/g cellulose. The results showed that SSF of steam pretreated corn stover at 10 % WIS was fully possible and resulted in an ethanol yield of 70 % of the theoretical based on the cellulose concentration in the raw material.

SSF was also performed with lower yeast concentration (2 g/L ordinary baker's yeast) and with yeast cultivated on the hydrolysate from the pretreatment (5 and 2 g/L). A decreased yeast concentration to 2 g/L ordinary baker's yeast did not decrease the final ethanol yield but the ethanol productivity decreased during the initial part of the SSF. The cultivated yeast showed a higher productivity during the initial part of the SSF but it did not result in higher final ethanol yield. These results indicates that the enzymatic hydrolysis was the limiting factor during the last part of the SSF at 10 % WIS.

POSTER PRESENTATION 3B-09

AFEX Pretreatment of Corn Fiber-Ethanol Fermentation and Animal Feed Analysis of Residue

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Corn fiber from a dry corn processing plant was pretreated using Ammonia Fiber Explosion (AFEX) to enhance digestibility of the fiber. In the AFEX process, corn fiber, ammonia and water are heated under high pressures. The pressure is suddenly released to expand the fiber and enhance enzymatic hydrolysis. The pretreated fiber was hydrolyzed with commercial enzymes to release monomeric sugars and fermented to ethanol using selected microorganisms. The residue from the fermentation was dried and analyzed for animal feeding values. Ethanol yields from starch and cellulose of the corn fiber were determined and compared to the yields from untreated corn fiber. The residues from the ethanol fermentation of both treated and untreated corn fiber were analyzed for fiber, protein, amino acid, fat and energy content. Preliminary economic evaluation of the residues as potential animal feed was comparatively performed.

POSTER PRESENTATION 3B-10

The Dilute Acid Pretreatment of Corn Stover Anatomical Fractions

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A key technical barrier to the commercialization of fuels and chemicals from biomass via a sugar platform route is the high cost and relative inefficiency of producing fermentable sugars from lignocellulosic biomass. Treatment of biomass with dilute acid has long been recognized as a promising technology for the saccharification of biomass feedstocks; however, the yields of solubilized sugars are less than quantitative. At lower treatment severities, the hemicelluloses are hydrolyzed and sugars are solubilized as monomers and oligomers. At higher treatment severities, cellulose depolymerization increasingly occurs; however, sugar degradation reactions also become more evident.

The purpose of this work was to characterize the changes in plant cell wall chemistry that occur in selected anatomical fractions of corn stover during dilute acid pretreatment. These fractions include corn nodes, internode stems, leaves, and sheaths. The release of sugars from dilute acid pretreatment of corn stover anatomical fractions will be described and related to the gross chemical composition of the fractions.

POSTER PRESENTATION 3B-11

Porosity Measurements on Dilute Acid Pretreated Corn Stover

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The porosity of lignocellulosic materials has been used by several workers as a guide to the enzymatic digestibility of pretreated biomass. It is known that the physical and chemical properties of pretreated biomass vary according to the source of the biomass and their pretreatment conditions. The main goal of our research is to develop characterization tools that can be used to predict the digestibility of corn stover after pretreatment and ultimately ethanol yields. With these tools, it is our aim to identify pretreatment conditions that increase the susceptibility of the cellulose in pretreated biomass to enzymatic hydrolysis, and consequently minimize the amount of enzyme required in the process, thereby increasing the yield and decreasing the cost of products from biomass feedstocks.

Two methods were identified as suitable for our objectives: (i) the solute exclusion method, originally developed by Stone and Scallan, and (ii) thermoporosity. The solute exclusion method showed differences in the enzyme accessible pore volume between the pretreated samples and the unpretreated corn stover. However, only very small differences were observed amongst the pretreated samples that gave ethanol yields ranging from 70 to 96%, and a poor correlation was found between accessible volume to an enzyme-sized molecule and digestibility. In addition, this method was found to be time consuming, laborious, and irreproducible. ^{1}H NMR thermoporosity was used to measure the amount of water in different pore sizes ranges. Several interesting correlations were found between the pore volumes for pores in the range from 20 to 200 Å and either digestibility, or xylan removal. The results from the NMR analyses were also much more reproducible than the results from the solute e xclusion method.

POSTER PRESENTATION 3B-12

Pretreatment of Waste Papers for Citric Acid Fermentation by *Aspergillus niger*

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Aspergillus niger is the most popular strain for citric acid production at an industrial scale. Carbon sources used should be cheap and available e.g. agricultural waste and waste papers. The waste papers are mainly composed of cellulose, which must be hydrolyzed to glucose before being used as carbon source. Therefore, the pretreatment is important and necessary. In this study, corrugated boxes, newspapers, office papers, books, magazines and miscellaneous papers were cut into 1 cm x 5 cm by paper cutter. The cut papers then were incubated at different temperatures under vapor pressure. It was found that the maximum temperature used to produce 22.8 g/L reducing sugar from corrugated boxes is at 120^0C. The papers were further hydrolyzed with 1M of sulfuric acid, acetic acid, and succinic acid, at the ratio of liquid and solid of 8:1 (V/W), 60 minutes. The results showed that the maximum citric acid production from sulfuric acid pretreatment is 14.3 g/L from newspapers, whereas from hydrochloric acid, acetic acid and succinic acid is 12.0 g/L, 18.2 g/L and 21.7 g/L, respectively, from corrugated boxes. Cellulase activities proportionally increased with citric acid production. Further study is to use base or enzymes to increase the reducing sugar released.

POSTER PRESENTATION 3B-13

Kinetic Modeling of Xylo-Oligosaccharides Production from Brewery's Spent Grains Autohydrolysis

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Autohydrolysis is a mild, economic and environmental friendly process, suitable for the selective fractionation of hemicelluloses, which can be used for the production of oligosaccharides. Among these, xylo-oligosaccharides (XOS) obtained from hardwoods and agricultural byproducts, such as brewery's spent grains (BSG), may have potential application in the pharmaceutical and functional food market.

In order to provide information to the development of technical and economic studies for XOS production, hemicellulose hydrolysis can be assessed by means of kinetic models based on pseudo-first order reactions. However, the large quantities of arabinose in some non-woody materials, may difficult the mathematical interpretation of the data.

Considering the interest of extending the kinetic modeling to lignocellulosic materials with a relatively high content of arabinose, and to provide a reliable kinetic interpretation of BSG autohydrolysis, in this study several kinetic models based on sequential pseudo-homogeneous first-order reactions were developed and tested. Xylan and arabinan were assumed to yield oligosaccharides, monosaccharides (xylose or arabinose), furfural and other decomposition products in consecutive reaction steps. The models proposed provide a satisfactory interpretation of the hydrolytic conversion of xylan and arabinan. The dependence of the calculated kinetic coefficients on temperature was established using Arrhenius-type equations.

POSTER PRESENTATION 3B-14

High-Solids Enzymatic Saccharification of Cellulose

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Batch enzymatic hydrolysis of pretreated lignocellulosic material was investigated at high insoluble solids levels (>25% w/w) in shake flasks reactors and a novel bench-scale reactor capable of handling high solids slurries. While high-solids enzymatic hydrolysis of cellulose is advantageous for reducing capital and operating costs, operating an enzymatic saccharification reactor at high insoluble solids levels presents a unique set of physical and reactor-dependent challenges such as mass transfer, temperature control, mixing, pH control, and sugar inhibition. This work partially focused on characterizing the effects of these problems. Specifically, we quantified cellulose hydrolysis rate limitations due to mass transfer and sugar inhibition showing that it is possible to achieve >80% cellulose conversion using enzymatic hydrolysis of dilute acid pretreated corn stover (PCS) at initial insoluble solids concentrations of 25% by weight (equivalent to 13% cellulose) using a Spezyme CP cellulase (40 mg protein / g cellulose or 10.7 FPU / g cellulose) and water-washed PCS solids at 45°C and pH 5. A final glucose concentration exceeding 140 g/L in the liquid fraction of the saccharified slurry was obtained.

POSTER PRESENTATION 3B-15

Factors Affecting Scale-Up of High Solids Saccharification from Shake Flasks to Stirred Tank Reactors

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Under process-relevant high-solids conditions, scaling up enzymatic cellulose hydrolysis from shake flasks to stirred tank reactors (STRs) can result in differences in both the rate and extent of reaction, with better performance often obtained in shake flasks. The goal of this work was to determine unambiguously the source of these differences and to demonstrate the ability to scale up the enzymatic hydrolysis reaction from shake flasks to laboratory scale fermentors. Batch enzymatic hydrolysis experiments were performed using unwashed dilute acid pretreated corn stover (PCS) at insoluble solids levels of 5% to 15% (w/w, dry basis). Results show that mixing problems in STRs become significant at insoluble PCS solids levels exceeding roughly 10% (as a consequence of the challenging PCS slurry rheology). However, with appropriate impeller selection and sufficient agitation, reasonably good mixing and heat transfer, and thus temperature control, can be obtained at PCS solids levels as high as 13.5-15.0%; effective mixing can be achieved at solids levels as high as 14-16% using predigested PCS particles. Importantly, despite literature reports to the contrary, impeller-induced shear produced using a range of marine impeller configurations and speeds did not result in significant levels of cellulase inactivation. Taken together, these results show that differences in the performance of the enzymatic saccharification reaction between reaction vessels of different scales and geometries can be minimized if proper attention is given to ensuring good temperature control.

POSTER PRESENTATION 3B-16

Bioethanol Production from Ammonia Pretreated Waste Oak Wood by SSF

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Oak wood and waste oak wood were pretreated with aqueous NH_3 in a flow-through a column reactor, Ammonia Recycled Percolation (ARP), for ethanol production through Simultaneous Saccharification and Fermentation (SSF). This pretreatment method is highly effective in delignification and in hemicellulose solubilization of lignocellulosic biomass. We have previously investigated on various pretreatment processes using a flow-through (percolation) reactor system in our laboratory. The primary purpose of this investigation is to assess the effectiveness of the ARP treatment as a pretreatment process specifically for oak wood. We were interested in verifying the changes in chemical composition and physical characteristics of biomass brought about by the pretreatment and how those factors affect the enzymatic digestibility. Most of the lignin removal occurred within the first 20 minutes of the reaction. The ARP process solubilizes 40-50% of the hemicellulose in liquor but leaves the cellulose content intact in solid. The solubilized carbohydrate exists in olgomeric form. Decomposition of carbohydrates during the pretreatment is insignificant. The digestibility of the treated waste oak wood is substantially higher than those of α-cellulose. The enzymatic digestibility is correlated with the extent of lignin removal and hemicellulose removal perhaps due to increased surface area and porosity. Conversion of cellulosic biomass to ethanol involves SSF.

POSTER PRESENTATION 3B-17

Two-Step Pretreatment of Corn Stover by White Rot Fungi and Dilute Sulfuric Acid for Ethanol Production

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Prior to enzymatic hydrolysis, a pretreatment step is needed to degrade the lignin and enhance the enzymatic accessibility of corn stover. Although physic-chemical methods have been extensively discussed, fungal pretreatment approach was also focused by some researchers in recent years because of its advantages of energy saving and environmental friendliness. In this study, a two-step pretreatment by *Phanerochaete chyrsosporium* and dilute H_2SO_4 was investigated to improve the overall sugar and ethanol yield.

In the first step corn stovers were pretreated with *Phanerochaete chyrsosporium* for 6 weeks without addition of any nutrients. In the second step, the washed solid material from the first pretreatment step was impregnated with dilute H_2SO_4 (0.5%) under 180°C for 8 min. The effects of pretreatment were evaluated by simultaneous saccharification and fermentation(SSF). The ethanol yield of pretreated corn stover using the SSF configuration reached 55% of the theoretical value, and that of control reached 41%. The results showed that two-step pretreatment is a promising method to increase the overall yield in the corn stover-to-ethanol process.

POSTER PRESENTATION 3B-18

Genus Reciprocity of White Rot Fungi on the Pretreatment of Corn Stover in Mixed Culture

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Fungal pretreatment of corn stover by mixed cultivation of *Pleurotus ostreatus* and *Coridus versicolor* was studied. The results showed that laccase was produced continuously and attenuated slowly than in corresponding single strain culture, and higher laccase activities were obtained by mixed culturing. Additional nutrients, such as glucose, nitrogen and inorganic salt can obviously reduce corn stover weight loss and promote lignin biodegradation. Compared with mycelium inoculation, the whole culture inoculation (mycelium and its broth from the pre-incubation) can accelerate lignin biodegradation. The optimized results showed a total 26.7% lignin removal in corn stover, and 17.2% in control pretreated with *Pleurotus ostreatus* after 4 weeks' pretreatment.

The experiments suggested that mixed cultures among different genus of white rot fungi have positive effects on laccase production and lignin biodegradation of corn stover. The pretreated corn stover can be used to ferment ethanol by simultaneous saccharification and fermentation.

POSTER PRESENTATION 3B-19

Bioethanol from Cellulose with Supercritical Water Treatment Followed by Enzymatic Hydrolysis

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Increased attention has been focused on bioethanol prepared from lignocellulosics as an alternative to fossil fuels due to its environmental friendliness. Recently, chemical conversion of cellulose with supercritical water has been studied to obtain sugars. In our previous studies, it was found that cellulose could be converted into glucose and oligosaccharides in the water-soluble(WS) portion and polysaccharides as precipitates which are composed of amorphous glucans with DP≥13. However, oligosaccharides and polysaccharides can not be fermented to ethanol by the yeast, *Saccharomyces cerevisiae*.

In this study, an effort was made on increasing the yield of glucose by supercritical water treatment followed by enzymatic hydrolysis on those cellulose-derived products with cellulase mixed with β-glucosidase. A comparative study on enzymatic hydrolysis between WS portion and precipitates were also made. The obtained hydrolysates were then studied on their fermentability to ethanol with the yeast.

POSTER PRESENTATION 3B-20

Effect of Pressure on Organic Acid Production from Japanese Beech Treated in Supercritical Water

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We have been investigating the use of supercritical water for the production of biofuels and useful chemicals from lignocellulosics. Previously, we reported that lignocellulosics were converted into various sugars and its decomposed products by hydrolysis,
dehydration and fragmentation reactions during supercritical water treatment. In addition, organic acids were found to be produced as the treatment is prolonged. Organic acids such as lactic acid, are of interest because they can be further processed into biodegradable polymers, or converted into gases such as methane, carbon monoxide and hydrogen by microorganisms. Therefore, an understanding of organic acid production is important for the development of biomass conversion process in supercritical water. In this study, the production of organic acids from Japanese beech (Fagus crenata) was investigated using a batch-type system and a flow-type system at various reaction pressure. As a result, it was suggested that the amounts of organic acids produced in supercritical water might be manipulated by controlling the pressure of supercritical water.

POSTER PRESENTATION 3B-21

Effects of Sulfuric Acid Loading and Residence Time on the Composition of Sugarcane Bagasse Hydrolysate Obtained in a 250-L Batch Reactor and Its Relation with the Xylose-to-Xylitol Bioconversion

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A 2^2 full factorial design was employed to assess the effects of sulfuric acid loading and residence time on the composition of sugarcane bagasse hydrolysate obtained in a 250-L reactor. The acid loading and the residence time were varied from 70 to 130 mg acid per gram of dry-bagasse and from 10 to 30 min, respectively, throughout 7 experiments where the temperature (121 ºC) and the bagasse-to-acid solution ratio (1:10) were kept constant. Both the sulfuric acid loading and the residence time influenced the concentrations of xylose and inhibitors in the hydrolysate. The highest xylose concentration (22.71 g/l) was achieved when using 130 mg acid per gram of bagasse and 30 min. These conditions also led to increased concentrations of inhibiting compounds in the hydrolysate. All the 7 hydrolysates were vacuum-concentrated, to increase and normalize the concentrations of xylose, and detoxified, by pH alteration and adsorption in active charcoal, before being used as fermentation media for xylitol production in a stirred tank reactor. Interestingly, the best xylitol production (37.46 g/l), productivity (0.85 g/l h) and yield (0.78 g/g) were not achieved in the hydrolysate obtained using the least severe conditions (70 mg/g and 10 min) or in the hydrolysate obtained using the most severe conditions (130 mg/g and 30 min).

POSTER PRESENTATION 3B-22

Development of a Multi-Stage Fermentation System for Maximizing Solids Concentration and Optimizing Enzyme Loading for Continuous SSF of Pretreated Biomass

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Continuous processes are typically favored for production of commodity products, and many process designs assume enzymatic hydrolysis of cellulosic biomass will be performed in such systems. Furthermore, it is often assumed that high solids levels can be employed in these processes. However, limited data is available on continuous hydrolysis and fermentation performance for pretreated cellulosic biomass, particularly at high solids concentrations. Thus, a new experimental operation is being developed at Dartmouth College for understanding the effects of high solids loading during continuous enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF) of biomass. The system consists of three, 3L stirred bioreactors connected in series with the entire system controlled by Delta V software to provide optimal operating conditions as well as data recording. This system can be run in the continuous or batch mode and enables us to follow liquefaction rates of substrate during hydrolysis and the effects of enzyme loadings (FPU:CBU), residence times, impellor configurations, and inhibitory levels of both sugars and ethanol on performance.

POSTER PRESENTATION 3B-23

Catalytic Mechanisms of Dilute Acid Depolymerization Hydrolysis of Xylan

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It is well known that hemicellulose sugar yields can be significantly improved when small amounts of acid are added to biomass in a hydrothermal pretreatment step. Yet the mechanism involved is not clearly understood. Our experiments show that xylose degradation can be modeled as a first order homogeneous reaction and that the rate constant drops initially with increasing pH until a pH = 2, consistent with results reported by others. However, when we consider higher pH values than previously studied, the rate constant begins to level off, Depolymerization rate constants were also estimated for data we developed with soluble birchwood xylan oligomers using a simple Flory depolymerization distribution model and found to fit the observed change in degree of polymerization well. Interestingly, the rate constants for oligomer degradation dropped faster with increasing pH than for xylose loss and continued to drop at a pH over 2. The Brönsted relation states that either acids or bases can participate in catalytic reactions, and catalysts can be either specific (hydronium or hydroxide ions) or general (any Lewis acid or base). Based on this information, we developed a rationale for determining the catalytic mechanisms involved.

POSTER PRESENTATION 3B-24

A Simple and Effective Pretreatment for Biomass Materials

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Athenix has developed biologically-friendly pretreatment methods for hydrolysis of lignocellulose. One representative pretreatment utilizes sodium carbonate, and is carried out at moderate temperature and pressure, and does not require specialized reaction vessels. The by-products of this pretreatment are compatible with enzymatic hydrolysis following simple pH adjustment, which allows pretreatment and saccharification to be carried out in the same reaction vessel and eliminates pretreatment waste streams. Using corn stover as a representative feedstock, we have hydrolyzed greater than 80% of total sugars by this method, with virtually all of the sugars being present as monomers. Mass reductions in excess of 70% are also observed, as well as reduced stover particle size. Sodium carbonate pretreatments also lead to significant hydrolysis of other feedstocks, including corn fiber, sugarcane bagasse, Distillers' dried grains, and spent barley malt. Hydrolyzates generated by this method are compatible with downstream fermentation without further cleanup, suggesting that toxic by-products are not being generated. Finally, an independent economic analysis of this process suggests that sugars can be produced at a cost that is economically competitive.

POSTER PRESENTATION 3B-25

Enzymatic Hydrolysis of Corn Stover Pretreated with Dilute Acid: Effect of Pretreatment Conditions on Enzyme-Substrate Interactions

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The type of pretreatment and substrate greatly affect subsequent enzymatic hydrolysis and fermentation operations, and considerable effort has been directed at understanding fundamental factors responsible for these differences. The choice of pretreatment configuration, temperature, and chemical influences the amount and type of biomass components removed, and at one level, changes in enzymatic hydrolysis have been related to removal of lignin, hemicellulose, and other constituents. Additionally, pretreatment alters physical features of the substrate that are difficult to characterize but affect enzymatic hydrolysis, and attempts have been made to understand their importance. We are engaged in a cooperative project to evaluate how enzymatic digestion of cellulose varies for several leading pretreatments and quantify the relationship between performance and key features of the pretreated substrates. We are first focusing on the impact of cellulase loadings and the level of beta-glucosidase supplementation on sugar release and on clarifying the extent of interaction between enzyme loadings and pretreatment conditions for dilute acid pretreated corn stover. We also seek to understand synergism among cellulase components and their interaction with the substrate. In the near future, we plan to perform similar experiments and modeling for corn stover and poplar pretreated by other leading technologies.

POSTER PRESENTATION 3B-26

Enhancement of the Enzymatic Digestibility of Waste Newspaper Using Tween

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Methods of increasing the enzymatic digestibility of waste newspaper by adding Tween-series surfactants as a pretreatment with or without subsequent enzymatic hydrolysis were investigated. Tween was selected because this surfactant increases cellulase activity during enzymatic hydrolysis and does not inhibit cell growth in downstream fermentation process. When surfactant was used in a pretreatment, a synergy effect was expected during hydrolysis. However, since it was necessary to wash the pretreated substrate with water to remove inhibitors produced during pretreatment, the synergy effect could not be obtained. When surfactant was used in the pretreatment, it was found that surfactant effect on digestibility was marked and this was higher at lower enzyme loading. The addition of enzyme and surfactant to substrate at the beginning of enzyme reaction was found to most effectively maximize digestibility. Compared to the digestibility of untreated sample, an increase of approximately 40% was observed in digestibility when surfactant was added into either the pretreatment stage or the hydrolysis stage, respectively, whereas an increase of only 45% was observed when surfactant was added to both stages. This results show that one addition of surfactant to either the pretreatment or hydrolysis stage is sufficient.

POSTER PRESENTATION 3B-27

Dilute Acid Hydrolysis Pretreatment of Agro-Food Wastes for Bioethanol Production

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Due to its environmental benefits, bioethanol is considered a promising biofuel for the transport sector. Thus it is necessary to reduce its production costs by using new alternative biomass feedstocks. Due to its high agricultural potential, Spain has a strong food processing industry that produces approximately 450.000 tons of organic waste per year. The canning industry has to be taken into account because of its increasing development and capacity in Spain. Besides its development, increasing amounts of wastes are being generated by this kind of industries, and solutions have to be developed in order to diminish its environmental effects.

Wastes from fresh and processed vegetables have been used as feedstocks for a diluted acid hydrolysis process using sulphuric acid as catalytic reagent. Specifically in this study tomato, red pepper and legumes (beans, lentils and chickpeas) residues have been chosen as feedstocks.

Firstly, a characterization of the wastes was done to determine its moisture, protein, carbohydrate, lignin, extractive, and ash contents.

Preliminary pre-treatments were carried out varying temperatures, from 100ºC to 120ºC and residence times, from 5 to 20 minutes. Assays were performed with a dry matter content of 5% in 100 ml flasks in the absence or presence of sulphuric acid (concentration ranged from 0.5 to 1.0 % (wt/wt)) in order to optimise the process depending on the feedstock employed.

Samples from both solid and liquid fractions obtained were analysed by different techniques (enzymatic, chromatography) in order to determine the sugar content and the presence of toxic compounds, which can inhibit the fermentative microorganism.

The results obtained from the dilute acid hydrolysis assays regarding simple sugar solubilization in the liquid fraction have been very successful reaching values of 3.61 and 7.83 g/L for tomato and red pepper residues, respectively. The suitability of this pre-treatment has also been evaluated by the low levels of sugar degradation compounds detected in the samples which are significantly lower than the concentrations reported in the literature.

POSTER PRESENTATION 3B-28

Steam Stripping as an Unwashed PCS Detoxification Method Proof of Concept

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One of the major problems associated with dilute-acid hydrolysis of lignocellulosic biomass is the poor fermentability of the acid hydrolysates. Different compounds are liberated and formed during hydrolysis, some of which are toxic to the fermenting *Saccharomyces cerevisiae*. These inhibitors include lignin degradation products (phenolic compounds), furan derivatives (such as furfural and HMF), and aliphatic acids (especially acetic acid).

Steam distillation is a process used to separate mixtures where one component has an appreciable vapor pressure. It is widely employed in industry for the isolation of many natural essences and oils. The high volatility of the inhibitors (furfural, HMF, acetic acid, etc.) makes steam stripping a promising way to separate them from the acid pretreated corn stover. Furthermore, in the conventional furfural production from biomass, steam distillation followed by fractionation is used for recovery of furfural.

In the present study, steam stripping was tested as a detoxification method for improvement of both cell growth and ethanol production by *S. cerevisiae*. Unwashed PCS from dilute-acid pretreatment was used and fermentability of the detoxified solids was assayed. As a proof of concept, water addition followed by evaporation was used as a way to simulate the effect of steam distillation.

POSTER PRESENTATION 3B-29

Xylose Fermentation with Non-GMO *Saccharomyces cerevisiae* Utilizing an Extra Cellular Xylose Isomerase

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In order to maximize the ethanol yield from lignocellulosic feedstocks it is commonly agreed that the hemicellulose fraction needs to be utilized along with the cellulose in order to obtain an economical viable conversion technology. The problem utilizing the pentose potential of the biomass feedstocks is that the commercial used S. cerevisiae production strains, which is used due to their resistance towards high ethanol concentrations, are unable of converting C5 sugars. Great efforts have been made in introducing genes into *S. cerevisiae* to enable the organism to co-ferment other sugars than glucose.

The presented work shows an alternative way to utilize the hemicellulose sugars in a *S. cerevisiae* fermentation without having to deal with GMO related production costs. External xylose isomerase is added to a *S. cerevisiae* fermentation in a combined glucose and xylose media. The fermentation dynamics and potential in converting the xylose into ethanol in the presence of an xylose isomerase is evaluated.

POSTER PRESENTATION 3B-30

Enzymatic Hydrolysis of Manure Fiber Pretreated by Metal Ions and Hydrogen Peroxide

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Animal manure is rich in carbohydrates derived from forages such as alfalfa, barley and corn. For instance, cattle manure is more than 50% fiber, which means, with proper treatments, it could supply a substantial resource of cellulose, hemicellulose to produce useful intermediate chemicals—mono-sugars thorough enzymatic hydrolysis. Ion and peroxide has been reported as an environmental friendly oxidative agent to depolymerize cellulose. Most of the research has focused on studying the mechanism of these microorganisms in relation to the H2O2-Fe radical system with mild experimental conditions such as low radicals concentration, moderate temperature and atmosphere pressure. This might have been one of reasons why all of these experiments typically took too long, ranging from several days to several months. In this research, transition metal ions such as copper and iron will be investigated at different levels of temperature, pressure and radical reagent concentrations in terms of achieving an effective pretreatment method to enhance following enzymatic hydrolysis of manure fiber.

POSTER PRESENTATION 3B-31

Identifying Relations between Pretreatment Parameters, Enzymatic Hydrolysis, and Inhibition of Thermophilic Xylose Fermentation Using Alkaline Wet Oxidation of Rice Straw

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In the production of bioethanol or other bio-based chemicals from lignocellulosics, the subsequent enzymatic hydrolysis and fermentation processes are known to be affected by the pre-treatment technology applied. It has been found that the enzymatic hydrolysis of cellulose are directly related to the removal of lignin during pre-treatment yielding a higher enzymatic convertibility at higher lignin removal. Also, a relationship between the pre-treatment technology and the inhibitory effect of the pre-treated biomass suspensions has typically been found. Using alkaline wet oxidation as a pre-treatment technology for herbaceous biomass has been found to generate lower amounts of inhibitory compounds compared to acid catalyzed process.

This presentation will include the results of manipulating the type (Na_2CO_3 or NH_3) and amount of alkaline catalysis in the wet oxidation of rice straw. The results presented will illustrate the effect of the tested combinations of pre-treatment parameters on the enzymatic hydrolysis of cellulose and the inhibitory effect on ethanol production during thermophilic batch fermentation. The results will also included, the presentation of a relationship between the enzymatic convertibility and the inhibitory effect on ethanol production.

POSTER PRESENTATION 3B-32

Development of a Technology Combining Thermal Hydrolysis, Wet Oxidation, and Steam Explosion for Pretreatment of Lignocellulosic Biomass

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During the last couples of decades, increased international focus has been directed towards renewable resources for production of biofuels and bio-based chemicals. However, because of the complex structure of lignocellulosic, primarily causing a blockage of the cellulose and hemicellulose by lignin, different physical and chemical technologies have been developed. These technologies include thermal hydrolysis, alkaline wet oxidation, and acid catalyzed steam explosion. This presentation will include a description of the development of a pre-treatment technology in which the three abovementioned technologies are combined in one low energy consuming batch-mode reactor.

The main benefits of the combined pre-treatment technology are: (a) no requirement for initial mechanical milling, (b) the ability to process high biomass concentration, (c) an easy controllable process, and (d) a high energy recovery.

The presentation will emphasize on the amount of fermentable sugars obtained after enzymatic hydrolysis of pre-treated wheat straw using different pre-treatment parameters and biomass concentrations.

POSTER PRESENTATION 3B-33

Test of Steam Explosion after Dilute Acid Percolation for the Maximum Recovery of Sugars from Agricultural Waste Feedstocks

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Lignocellulosic feed-stocks from agricultural wastes such as corn stalk, cobs, rice straw and reeds are pretreated to be hydrolyzed subsequently. The pretreatment of these feed-stocks before saccharification by steam explosion resulting in excessive loss of solids and especially of hemicellulose in the process. Hence in this study a consecutive dilute acid percolation and explosion process was tested for the maximum recovery of all sugars including xylan, glucan and the lignin. The operation parameters for optimization of this SEDAP (Steam Explosion after Dilute Acid Percolation) process were the time, and temperature of percolation. The percolation and explosion products were recovered and subsequently test hydrolysed to obtain maximum conversion of glucan by cellulase enzymes.

With the feed-stocks considered 83.5 – 99.5% of percolation and explosion yields, and 72.2 – 93.6% of saccharification yields were obtained. The corn cob and rice straw treated by SEDAP at 200°C for 4 minutes showed maximum sugar yields of 346g, 335g from 1000g of biomass respectively. The corn stalk and reeds produced 403g and 457g of sugars from 1000g of dried biomass using SEDAP at 200°C for 10 minutes and enzymatic saccharification. The result shows that the sugar yields of the SEDAP process is very high in comparison with conventional steam explosion pretreatment.

POSTER PRESENTATION 3B-34

Ethanol Production from Steam-Explosion Pretreated Wheat Straw

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Wheat straw, an important residue from grain industry in Spain, seems to be a promising substrate for bioconversion to fuel ethanol. The lignocellulosic nature of this material makes the pretreatment an essential step to increase the accessibility of hemicellulose and cellulose to enzymes. Among processes developed to pretreat lignocellulosic biomass, steam-explosion has been identified as a low cost and high yield technology to prepare the cellulose fraction to enzymatic attack.

The aim of this work was to determine the best operation conditions for steam explosion pretreatment of wheat straw and to evaluate the potential of using this feedstock in a Simultaneous Saccharification and Fermentation (SSF) process.

Steam explosion experiments were performed in a batch steam-explosion pilot unit at various experimental conditions: temperatures from 160 to 230ºC, residence times of 5 and 10 minutes, and sulphuric acid (0.9% w/w) as catalyst or water.The effectiveness of steam-explosion was evaluated in terms of hemicellulose-derived sugar recovery in the liquid fraction, cellulose recovery in the solid fraction and enzymatic hydrolysis (EH) yield. Finally, the SSF bioconversion process was tested using a themotolerant strain of *Kluyveromyces marxianus* CECT 10875

Results show that a maximum overall sugar yield of 85% of total sugars contained in raw material can be attained at 180ºC and 10 minutes when using 0.9% sulphuric acid as catalyst. SSF yield at this condition was about 70% of theoretical yield. Higher temperatures of 200-210ºC are needed to get maximum yields when biomass was preimpregnated with water. Detailed results of this study will be presented.

POSTER PRESENTATION 3B-35

Cellulose to Glucose Conversion Via Swelling-Decrystallization and Subsequent Low Severity Acid Hydrolysis

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Sequential steam-aqueous fractionation of biomass leads to the production of a hemicellulosic-rich liquor, a lignin-rich stream and a cellulosic-rich residue. The latter can be separated, if desired, into fines and fibers. This paper discusses an approach to decrystallize the cellulosic matrix (as fines or fibers) via swelling in a concentrated acid solution leading to the formation of a viscous hydrogel. A mediator is then used to change the ionic concentration of the viscous hydrogel which is converted into glucose at moderate severities (100 C and 30 min being typical). High yields of glucose are consistently obtained (> 80 % of theoretical). Recovery of acid and mediator permits to envisage their reuse in the process. The mediator facilitates the production of hydrolyzates with 10 – 12 wt% glucose concentration.

POSTER PRESENTATION 3B-36

Effect of Elevated Temperature Separation and Washing of Pretreated Biomass on Dilute Acid Pretreatment Conditions and Enzyme Requirements

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The dilute acid pretreatment of biomass has been shown to effectively hydrolyze hemicellulose to oligomeric and/or monomeric sugars, leading to significant enhancement in the enzymatic digestibility of the cellulose remaining in pretreated biomass using commercially-available cellulase preparations. In its most-practiced form, dilute acid pretreatment is conducted as a batch or continuous plug-flow operation and does not result in significant lignin solubilization, which could hinder the effectiveness of enzymatic digestion. Many studies have reported on percolation or flow-through dilute acid pretreatment approaches, which cause greater amounts of lignin removal by preventing re-precipitation of solubilized lignin upon the pretreated biomass when cooled. For many biomass feedstocks, this results in a further enhancement of enzymatic digestibility conversions and/or rates. However, such pretreatment approaches have been shown to be economically unattractive due to high liquid throughputs, resulting in high steam requirements and dilute process streams.

This work describes an approach that involves a high solids pretreatment immediately followed by a elevated temperature separation and possibly an elevated temperature washing with water or other dilute solvents. This approach is designed to achieve greater amounts of lignin removal to enhance enzymatic digestion with better water management than flow-through pretreatment approaches. The impact of this approach will be examined on hardwood and agricultural residue feedstocks using various separation and washing parameters. The ability of this approach to affect required pretreatment conditions and the amount and types of various enzyme activities in subsequent enzymatic digestion is also discussed.

POSTER PRESENTATION 3B-37

Steep Delignification of Biomass and Lignin Recovery

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Some pretreatment technology is required prior to enzymatic conversion of cellulosics to sugars. A variety of pretreatment processes are under development, and most require high temperatures and pressures, as well as pretreatment chemicals. These high temperature pretreatments of the biomass also result in the production of inhibitory chemicals and degradation products. There is a need to develop lower cost, efficient pretreatment processes to improve the economics of bio-refineries.

In this paper/presentation R&D efforts including design improvements, parameter optimization and performance assessments of the BPI 'Steep Delignification Pretreatment" (SDP) process are discussed. The SDP Process combines chemical recycle, separation/recovery of lignin and is designed to minimize energy requirements associated with pretreatment. The SDP process results in the production of a delignified 'pre-treated' biomass readily susceptible to enzymatic breakdown to xylans and glucans.

In this work, results of bench and small pilot scale lignin separations using the SDP process on corn stalks and wood chips will be presented. In addition results of bio-fermentation trials of the SDP process treated biomass will be presented.

POSTER PRESENTATION 3B-38

Sugar Degradation during Analytical Biomass Hydrolysis

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Acid hydrolysis is used to convert the polymeric sugars in solid biomass samples and oligomeric sugars in biomass pretreatment liquid samples to monomeric form prior to analysis. However, this procedure degrades some of the sugars to byproducts such as furfural and hydroxymethylfurfural. Sugar Recovery Standards (SRS) processed in parallel are used to correct the error due to sugar losses, but the magnitude of the losses depends on sugar type and concentration.

Eight different sugars at concentrations of 1-100 g/L were subjected to acid hydrolysis conditions. These sugars and their degradation products were analyzed using High Performance Liquid Chromatography. Results show a trend of increasing degradation with increasing sugar concentration, while the order of sensitivity to degradation was cellobiose < glucose, galactose, and mannose < arabinose < xylose << fructose.

The results of this work show that using a constant value to correct for sugar degradation may introduce significant error (± 5% w/w absolute of sample). Accurate analysis results require preparing SRS close to the actual sugar concentrations of the biomass sample. We recommend including glucose as an SRS for biomass pretreatment liquid samples, and including cellobiose as an SRS for solid biomass samples.

POSTER PRESENTATION 3B-39

Automating Biomass Analysis Using Accelerated Solvent Extraction

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Analytical methods that provide an accurate chemical composition of biomass and biomass-derived samples using rapid and inexpensive methods are an enabling technology, needed for the optimization, evaluation and commercialization of processes that convert biomass to bio-based materials, power, fuels and chemicals. One limitation of traditional wet chemical method is the slow, expensive, and labor-intensive nature of many of the analytical procedures.

This papers presents recent improvements in the NREL standard Laboratory Analysis Procedures (LAPS) that automate solvent extraction, solids analysis and sample preparation. These changes incorporate the use of a Dionex® Acceletrated Solvent Extractor (ASE), which minimizes labor, minimizes solvent usage, improves analysis reproducibility and improves sample throughput. For example, new methods will be presented that convert a two stage, 48 hour soxhlet extraction sequence to a 2 hour, hands-free, automated extraction. Improvements in both precision and accuracy are observed making this the method of choice for data that will be used to calibrate rapid spectroscopic analysis methods. Application of the ASE to automate the analysis of feedstocks, pretreated materials and saccharification slurries will be presented.

POSTER PRESENTATION 3B-40

Understanding Fluid Transport Barriers in Corn Stover Using Light Microscopy

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The transport of catalysts (enzymes or chemical) into biomass is believed to be a major bottleneck during thermochemical pretreatment and enzymatic hydrolysis. Using dilute acid pretreatment as a model system, we are examining bulk fluid flow and molecular diffusion mechanisms through image analysis to visualize solute transport through corn stover tissues. A range of molecular-sized dyes is being used to characterize movement of small chemical and large enzymatic catalysts as it varies with pretreatment temperature and pressure. Observations are made on the dyed fractions using confocal and stereo light microscopy. Along with providing a fundamental understanding of the transport barriers associated with the pretreatment and enzymatic hydrolysis of biomass, this work will further experimental efforts aimed at correlating structural changes in biomass during chemical pretreatment with its' susceptibility to enzymatic digestion.

POSTER PRESENTATION 3B-41

First-Principles Investigation of Sugar Degradation – The Effect of Solvent and Reaction Condition

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Optimizing reaction conditions to minimize sugar loss is a primary concern during pretreatment and key to enabling an effective biorefinery industry. Direct experimental analyses of these thermochemical reaction pathways is challenging and benefits from insights gleaned from computational modeling. For this reason, sugar degradation reactions at different acidities were investigated with first-principles calculations using explicit solvent water molecules in the simulation. At lower acidity, the rate-limiting step in sugar degradation was found to be protonation of the hydroxyl groups on the sugar ring. Hydroxyls with the highest proton affinity will initiate sugar degradation leading to the major degradation products. At high acidity, protonation is rapid and sugar degradation initiates at multiple hydroxyl groups on the sugar ring leading to multiple products. Furthermore, we found that water molecules play a significant role in acidic sugar degradation pathways and these reactions will be discussed. Firstly, a water molecule competes with the hydroxyl group on the sugar ring for protons. Secondly, water forms hydrogen bonding with the hydroxyl groups on the sugar rings, thus weakening the C-C and C-O bonds (each to a different degree). Note that the reaction pathways could be altered due to the change of relative stability of the C-C and C-O bonds. Thirdly, sugar hydroxyl–hydrogen-bonded water molecules could easily donate or extract a proton from the sugar reaction intermediate, thus terminating the reaction. Indeed, the sugar degradation pathway is complex due to multiple protonation probabilities and the surrounding water structure. Water structure is in turn affected by reaction conditions such as acidity, temperature, the presence of salts and cosolvent, and the flow dynamics of the reactor.

POSTER PRESENTATION 3B-42

Simultaneous Saccharification and Fermentation of Spruce Wood Using a Fed-Batch Technique for Higher Ethanol Concentrations

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Ethanol from lignocellulosic materials is considered to be an attractive alternative for partial replacement of gasoline. It can be produced from a variety of materials, such as forest waste or agricultural waste, which, in the latter case, can add to the overall process economy from starch-based ethanol production.

Downstream processing costs, such as that of distillation, make up a large part of the production costs. Therefore, it must be strived towards a minimum in energy requirements. One of the most important parameters is the concentration of the ethanol coming from the fermenter tank. The higher the concentration, the less energy is required for distillation.

Simultaneous Saccharification and Fermentation (SSF), using a fed-batch technique, was used to increase the dry-matter load (and thus the ethanol concentration) in the fermenter vessels. The concentrations of Water-Insoluble Substances (WIS) were varied between 5 and 15%. Yeast, cultivated on wood hydrolyzate to improve the tolerance towards inhibitors, was used to ferment the sugars. This made it possible to reduce the added amount of yeast, while still reaching concentrations above 4%, which have an impact on the final production cost. Results from the on-going study will be presented.

POSTER PRESENTATION 3B-43

Cellulase Mimetic Catalyst for Lignocellulose Hydrolysis

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Developments in the understanding of the nanoscale structure and molecular mechanism of cellulolytic enzymes provide insights that may guide the development of a cellulase mimicking catalyst that efficiently hydrolyzes cellulose and hemicellulose. In this paper, characterization of the catalytic module of the mimetic is presented. Maleic acid, a dicarboxylic acid, the most promising catalytic mimetic exhibits improved selectivity toward the generation of fermentable sugars by reduced sugar degradation when compared to mineral acids. Maleic acid is compared against sulfuric acid for the hydrolysis of microcrystalline cellulose, corn stover, and hybridized sorghum. At optimized conditions, maleic acid yields at least equal amounts of glucose and 40% more xylose that sulfuric acid in the best case. Additionally, corresponding xylose degradation products were reduced in the maleic acid hydrolysis conditions. Cellulose binding domain mimetics that selectively concentrates maleic acid near the cellulose surface for improved performance are also presented.

POSTER PRESENTATION 3B-44

Effect of Biomass Concentration on Enzymatic Hydrolysis of Sugar Cane Bagasse

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Sugars were produced by enzymatic hydrolysis from sugar cane bagasse at three solids concentrations (5, 7.5 and 10 %, dry weight basis) with 2 IU of cellulolytic and xylanolytic enzymes per gram of dry substrate. The biomass was treated with an ammonia pressurization depressurisation process (PDA). Enzymatic hydrolysis was performed at 50ºC and 100 rpm for 48 h, using a pH 4.8 citrate buffer with 0.15% sodium azide. The sugar production was determined by the dinitrosalycilic acid (DNS) method and the sugar profile was determined by HPLC. As the solids content increased, the concentration of the sugars increased. The highest sugar conversion was achieved at 7.5 % treated biomass and it was almost four fold of that found with the untreated biomass used as a control.

POSTER PRESENTATION 3B-45

Separation of Cellulose from Hemicellulose and Lignin from Sugarcane Bagasse and Cane Leaf Matter

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Significant research effort has been devoted to producing low cost glucose from cellulose. However, the hemicellulose and lignin fractions together are probably equal in weight to the cellulose in any given biomass. These fractions may have multiple uses. Thus, it is critical to develop technologies that utilize all the major components of lignocellulosic biomass.

In a joint research effort, Michigan Biotechnology Institute (MBI) and Audubon Sugar Institute (ASI) investigated several combinations of ammonia fiber explosion (AFEX), chemical oxidation (Ox-B) and dilute caustic washs to separate cellulose, hemicellulose and lignin from sugarcane bagasse and cane leaf matter (CLM). ASI has shown that using a singlet oxygen complex ion (Ox-B treatment) can remove both lignin and hemicellulose from sugarcane bagasse. Sugarcane bagasse and CLM samples were treated by the Ox-B process either before or after AFEX treatment, followed by a dilute caustic wash, in order to evaluate the combined effects on the fractionation of the biomass. The comparative data will be presented.

POSTER PRESENTATION 3B-46

Conversion of Municipal Solid Waste to Carboxylic Acids by Anaerobic Countercurrent Fermentation: Effect of Using Intermediate Lime Treatment

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Municipal solid waste (MSW) and sewage sludge (SS) were combined and anaerobically converted to carboxylate salts by using a mixed culture of acid-forming microorganisms. MSW is an excellent energy source and sewage sludge is a good source of nutrients. In this research, MSW and SS were combined, so they complemented each other. Four fermentors were arranged in series for a countercurrent fermentation process. In this process, the solids and liquid were transfer in opposite directions, with addition of fresh biomass to Fermentor 1 and fresh liquid media to Fermentor 4. An intermediate lime-treatment of solids exiting Fermentor 3 before entering Fermentor 4 was applied to improve the product acid concentration from the untreated MSW/SS fermentations. All fermentations were performed under anaerobic conditions at 40°C. Calcium carbonate was added to neutralize the carboxylic acids and to control the pH. Iodoform was used as a methanogen inhibitor. Carboxylic acid concentration and gas composition was determined by gas chromatography. Substrate conversion was measured by volatile solid loss, and carboxylic acid productivity was calculated as function of the total carboxylic acids produced, the amount the liquid in all fermentors, and time. Addition of intermediate lime treatment increased product concentration and conversion by approximately 30% and 15%, respectively. The highest carboxylic acid concentrations for untreated MSW/SS fermentations with and without intermediate lime treatment were 22.17 and 17.74 g carboxylic acid/L liquid respectively. These results confirm adding a treatment step between Fermentor 3 and Fermentor 4 would increase the digestibility and acid productivity of the fermentation.

POSTER PRESENTATION 3B-47

Recyclable Biocatalyst Conjugates for Cellulose Hydrolysis

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One of the principal deterrents for economical conversion of cellulose to sugars via enzymatic hydrolysis is the high cost of the cellulase enzymes. One approach to decreasing the cost is to reuse/recycle the enzymes. Western Research Institute (WRI) is addressing recycling of the cellulase enzymes. We are developing a concept to immobilize the enzymes on reversibly soluble polymers (biocatalyst conjugates), which allows the enzymes to be recovered and recycled. Cellulase enzymes are immobilized on a reversibly soluble polymer to produce the biocatalyst conjugate, which in the soluble form is used to hydrolyze several fresh batches of cellulose. In the soluble form, the biocatalysts mediate the desired reaction, thus overcoming steric hindrance and mass transfer limitations encountered with insoluble matrices. After completion of the hydrolysis reactions, the biocatalyst conjugates are made reversibly insoluble by changing the pH of the solution, causing full precipitation and recovery of the enzymes. The biocatalyst conjugates can be recycled to hydrolyze a fresh batch of cellulose by changing the solution pH. This presentation will provide a summary of the preparation of the biocatalyst conjugates and a discussion of the results demonstrating the significance of the concept. Results will be presented demonstrating the repeated hydrolysis of crystalline cellulose and cellulose produced from wheat straw using the WRI fractionation process. These results show the concept is a powerful approach that is scalable and can become a core technology in biorefineries.

POSTER PRESENTATION 3B-48

Evaluation of Cellulase Preparations for Hydrolysis of Hardwood Substrates

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Seven cellulase preparations from *Trichoderma* and *Penicillium* spp. were evaluated for their ability to hydrolyze the cellulose component of substrates prepared from poplar and maple by ethanol organosolv pretreatment. Hydrolysis was quantified using two indices: mean specific rate and specific conversion. The activities of the cellulase preparations on model cellulosic substrates (filter paper, carboxymethylcellulose and Avicel) were also determined, together with their β-glucosidase, xylanase, pectinase, galactomannanase and β-glucanase activities. The ability of a cellulase preparation to hydrolyze pretreated hardwood showed little correlation with its activity on filter paper, carboxymethylcellulose and Avicel; however, there was a significant correlation with its levels of endogenous β-glucosidase and xylanase activity. Differences in the performance of the various cellulase preparations were substantially reduced following supplementation with a commercial β-glucosidase preparation from *Aspergillus niger*. This β-glucosidase preparation was shown to contain significant levels of endogenous xylanase activity. These data suggest that the levels of endogenous β-glucosidase and xylanase are important factors in determining the ability of a cellulase preparation to hydrolyze pretreated hardwood, and that deficiencies in the levels of both enzymes can be compensated by supplementation with corresponding activities present in the β-glucosidase preparation. Presumably, β-glucosidase improves cellulose hydrolysis by preventing cellobiose accumulation, while xylanase activity assists removal of residual hemicellulose, thereby increasing the accessibility of cellulose to cellulases.

POSTER PRESENTATION 3B-49

Analysis of Cellulase Performance on Hardwood Substrates Using a High Throughput Micro-Assay

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Hardwoods are considered prospective feedstocks for production of bioethanol and associated co-products. Poplar is currently the major focus of investigations by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) in its effort to expand the range of potential feedstocks for bioconversion. Development of a commercially-viable bioconversion process for poplar includes the optimization and evaluation of several different pretreatment processes. Attempts to improve enzyme formulations for hydrolysis of pretreated poplar are proceeding in parallel. In order to expedite the assay of the large volume of enzyme and substrate samples required in this study, we have developed a high thru-put micro-assay applicable to hardwood substrates. To test the effectiveness of this method, substrates were prepared from poplar and maple using steam explosion or organosolv pretreatment. The ability of the method to detect and further analyze differences in the performance of various cellulase preparations is demonstrated. The conclusions of this analysis are verified by comparison with data generated using a standard flask-based assay procedure.

POSTER PRESENTATION 3B-50

Biorefining of Poplar for Fuel Ethanol and Co-Product Manufacture Using Ethanol Organosolv Pretreatment: Process Optimization by Response Surface Methodology

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The development of biorefineries to produce fuels, commodity chemicals and other materials from renewable lignocellulosic feedstocks offers a promising alternative to continued reliance on petrochemicals. Among several biorefinery options currently under investigation is an organosolv process, using aqueous ethanol and an inorganic acid catalyst, to pretreat hardwood, softwood and agricultural residues. This process generates a cellulose-rich fraction for subsequent hydrolysis to glucose, a high-quality lignin fraction for co-product manufacture, and various other chemicals derived from hemicellulose and other biomass components. In this study we have investigated the influence of key process parameters (temperature, reaction time, ethanol concentration and catalyst dosage) on the yields and properties of the principal product streams. The study emphasizes the application of response surface methodology to optimize process parameters for cellulose hydrolysis while evaluating effects on co-product yield and quality.

POSTER PRESENTATION 3B-51

Recovery of Enzymes Following Hydrolysis of a Lignocellulosic Substrate

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Lignocellulosic residues from forestry and agriculture are potential substrates for bioconversion. Most processes currently under consideration involve a pretreatment step to improve substrate accessibility, followed by enzymatic hydrolysis to produce soluble sugars for subsequent fermentation. However, despite recent price reductions, the high cost of hydrolytic enzyme remains a significant impediment to full-scale process commercialization. As part of a research effort to develop a commercial process for bioconversion of softwood residues, we have examined the potential for enzyme recycling following hydrolysis of mixed softwood pretreated using an ethanol organosolv process. Following hydrolysis, part of the enzyme complex is found free in solution but a significant fraction remains bound to the residual substrate. We have used response surface methodology to determine temperature, pH, ionic strength, and detergent (Tween 80) concentration for optimal release of bound protein and enzyme activity from the residual substrate. These conditions were then used to evaluate a recycling strategy involving both free and released enzymes.

POSTER PRESENTATION 3B-52

Determination of Surface Area of Ammonia-Treated Dwarf Elephant Grass by a Physisorption Process

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The adsorption isotherms were obtained for untreated and ammonia treated dwarf elephant grass at 20°C. The surface area of the samples was determined by the method of moisture sorption isotherms and by a physisorption technique, applying the BET theory. The surface area of the ammonia treated samples turned out to be lower than that of the untreated sample, however, the ammonia treatment increased the water holding capacity of the grass, indicating changes in the structure of the fibers, as shown by scanning electronic microscopy. The surface area determined with the method of moisture sorption isotherms was higher that that determined with the physisorption technique. The latter technique was not appropriate to determine the surface area of the ammonia treated samples.

POSTER PRESENTATION 3B-53

Composition and Glycan Reactivity of Commercial Grass Seed Straws

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The study was designed to ascertain the enzyme susceptibilities, and potential monosaccharide yields, of the major glycan fractions in commercial grass seed straws. Results for Kentucky bluegrass (KBG), perennial ryegrass (PRG), and tall fescue (TF) were compared with those of a representative switchgrass (SG). Commercially available xylanase and cellulase preparations were used to test enzyme susceptibility. Treatments intended to enhance enzyme efficacy were limited to aqueous-based extractions deemed appropriate for small-scale, on-farm, low initial-capital, operations. Macrocomponent contents of KBG, PRG and TF were similar: glucans 33, 38 and 32 percent; xylans 20, 20, and 18 percent; and lignin 22, 21, 25 percent; respectively. Representative (KBG) heated aqueous treatments extracted >20 percent of total feedstock solids; significant decreases in feedstock (solid)-associated glucan (~25%), compared to relatively small decreases in the analogous xylan fraction (~5%), were observed. Other fractions, most notably ash (~ 5% of feedstock solids), were more easily extracted (~50% for KBG ash). Relatively mild treatments significantly increased feedstock enzyme susceptibilities; cellulose saccharification values approached 34% of theoretical. Near maximum glucose yields were obtained within 16 hr saccharification at relatively low enzyme loads (5 FPU per g biomass).

POSTER PRESENTATION 3B-54

Optimization of Pretreatment of Barley Straw for Ethanol Production: Comparison of Two Impregnation Techniques

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In the biomass to ethanol production process cellulose-rich raw materials are pretreated at high pressure and temperature to facilitate the enzymatic degradation of the cellulose fraction into fermentable sugars. When barley straw is used as a substrate a common catalyst in the pretreatment step is H_2SO_4.

Submerging the material into the liquid catalyst is a common lab-scale impregnation technique. However, this technique is not an option in a full-scale ethanol plant as the consumption of chemicals would be unnecessarily large. Furthermore, there are difficulties in increasing the dry-matter content in the raw material after impregnation. This would lead to large water streams throughout the whole process and result in high energy demand in the distillation and evaporation steps. A more realistic impregnation technique would be to add the catalyst thru nozzles i.e. spraying the material with the catalyst. This would decrease the consumption of chemicals and increase the dry-matter content in the process.

A study on pretreatment of H_2SO_4-impregnated barley straw using the two different impregnation techniques, i.e. soaking and spraying, revealed that harsher conditions are required in the pretreatment step implementing the latter technique. Thus, the study was complemented with an optimization of pretreatment on barley straw using spraying as an impregnation technique. Results from this study will be presented.

POSTER PRESENTATION 3B-55

Biomass Fractionation to Yield Cellulose, Lignin and Hemi-Sugar Streams

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Lignocellulosic biomass represents a low value resource that, in the future, can provide fuels, chemicals and energy to replace petroleum and meet our nation's energy needs. Development of biorefining technologies is ongoing and several technical approaches are in different stages of development. One of the processing approaches is to pre-treat the raw biomass in order to fractionate it into the individual biopolymers (cellulose, hemicellulose, and lignin). This approach yields a purified cellulose product that is easily hydrolyzed to yield monomeric sugars for down stream processing. The biomass fractionation unit is the center of a biorefinery and is required to be efficient, economical, robust and reliable. Western Research Institute has evaluated a number of pretreatment approaches and has initiated development of a novel fractionation concept, which is effective, reliable, robust, and appears to be economical. The process takes advantage of reagent recycling and uses simple reaction vessels to increase reliability and improve the economics. This presentation will provide a description of the technology and present results from the fractionation of wheat straw. The results demonstrate that a high-quality cellulose product, amenable to enzymatic hydrolysis, is produced in high yields. The process also yields separate streams of purified lignin and the hemicellulose sugars as co-products. These co-product streams are easily obtained without the use of sophisticated separations schemes.

POSTER PRESENTATION 3B-56

Corn Fiber as a Raw Material for Hemicellulose and Ethanol Production

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Corn fiber is a byproduct of the corn wet-milling industry and a huge amount of it (app. 130 t/day) is produced in Hungary. The major component of corn fiber is the pericarp that consists of 35% hemicellulose, 18% cellulose and 20% remaining starch (protein, fiber oil and lignin are also present in this material). Corn fiber is presently used as animal feed. However with the begin of biofuel production corn processing would increase and than there might be problems with the utilization of the surplus fibrous by-products. The major problem with lignocellulosics is their complex structure: cellulose, hemicellulose and lignin are built together to resist the environmental effects. However if we would like to utilize their carbohydrate content via enzymatic and microbial processes we have to use some kind of pretreatment. In case the pretreatment has been done to get a valuable product like hemicellulose the process might be more economical.

In this work destarched corn fiber was pretreated by using different alkaline solutions and the water-soluble hemicellulose was dissolved and precipitated with ethanol. The residual material consisting mostly of carbohydrates could be hydrolysed by cellulolytic enzymes and fermented into ethanol by using *Saccharomyces cerevisiae*.

POSTER PRESENTATION 3B-57

A Comparison of Hot Water Pretreatment of Corn Stover by Batch and Flowthrough Reactors

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Fluid flow through the solids during pretreatment of corn stover with just water enhanced removal of total mass, xylan, and lignin; total sugar recovery in the hydrolyzate; and cellulose digestibility; and decreased degradation of xylan oligomers, as evidenced for operation over the temperature range of 160 to 220°C with corn stover. However, the variation in xylose reaction rate with flow is inconsistent with traditional first order kinetic models. We have recently found that reaction of prepared xylan does not change with flow rate to a significant extent. Furthermore, increasing flow rate enhanced solubility of long-chain xylan oligomers and total xylose yield in the hydrolyzate of corn stover. These results imply that the solubility of lignin-xylan-oligomers could play a key role in the hydrolysis of corn stover and suggest that solubility limitations and lignin-xylan complex configuration might be the most important factors in controlling hemicellulose hydrolysis of lignocellulosic biomass such as corn stover.

POSTER PRESENTATION 3B-58

Covalent Cross-Links in Corn Stover and their Impact on Hemicellulose Hydrolysis and Cellulose Digestibility

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Lignin shields hemicellulose and cellulose in biomass, restricting access of hydrolytic enzymes to both of these components. Pretreatment is essential to reduce this barrier to high sugar yields, but development of lower-cost and higher-yield technologies could be accelerated by improving our understanding of the interplay between lignin and hemicellulose. In this study, the linkages of ferulic acid and p-coumaric acid to lignin and hemicellulose are investigated by determining hemicellulose sugars, lignin, and ether- and ester-link phenolic acids in solutions and solid residues following treatment of corn stover with dilute sodium hydroxide at room temperature. Ferulic acid and p-coumaric acid are measured using an Inertsil ODS-3V column via HPLC, and the soluble polysaccharides are determined by HPLC with a Bio-Rad Aminex HPX-P column after post hydrolysis. Based on this information, a model of cross-links between hemicellulose and lignin is proposed. In addition, the impacts of covalent cross-links on hemicellulose hydrolysis by dilute acid and cellulose digestion by enzymes are evaluated by comparing base-treated corn stover at low and high content of ester-link phenolic acids. Total hemicellulose sugar yields and cellulose digestibility are also related to the ether-link phenolic acid content in the base-treated corn stover.

POSTER PRESENTATION 3B-59

Rapid Composition Monitoring for the Biorefinery: An Overview

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This presentation gives an overview of process analytical chemistry (PAC) tools that are currently available, or already being used, to improve biomass-based processes that produce fuels or chemicals. Tools such as near-infrared spectroscopy (NIR) allow the chemical composition of process streams to be monitored in essentially real-time, enabling improved process understanding, improved process design and control, and improved process performance and product quality. PAC tools can add value at many points in a biorefinery ranging from incoming feedstock qualification, to pretreatment monitoring and control, through fermentation monitoring, to monitoring of product separation and purification. This presentation describes the capabilities and limitations of the available tools and how they can add value to biomass-based processes.

POSTER PRESENTATION 3B-60

White-Rot Fungus *Ceriporiopsis subvermispora* Used in Delignification of Sugarcane Straw

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The white-rot fungus *Ceriporiopsis subvermispora* was cultivated on sugarcane straw, six-gram samples of straw in Erlenmeyer, without additional carbon source or salts solution. The biologic pretreatment can be used together with pulping process and has a high economic on the use of chemicals on pulping. Experiments were realized using three different systems:

- **Solid state fermentation (a)**: Cultures were grown at 27°C for 30 d using 250 and 500 mg/kg (dry mass of fungus/straw) of inoculum. **(b)** 7, 15 and 30 d of treatment at 27°C using 250 mg/kg of inoculum.
- **Semi-solid state fermentation**: 30 d at 27°C using only 250 mg/kg of inoculum.

Table 1: Loss of mass and components (%).

	250 mg/ kg solid	500 mg/kg solid	250 mg/kg semi-solid	7 d	15 d	30 d
mass	22.4	19.8	3.1	4.8	12.1	15.2
glucan	5.1	7.8	3.9	3.9	8.4	8.7
polyoses	25.7[1]	24.8[1]	3.5[1]	3.3[2]	11.2[2]	17.4[2]
total lignin	42.8	41.9	3.5	-	-	-
Klason lignin	-	-	-	2.0	25.8	26.7
Selectivity*	8.4	5.4	0.9	0.5	3.1	3.1

[1]Polyoses = xylan + arabinan. [2]Polyoses = xylan + arabinan + glucuronic acid+acetyl.
* loss of lignin/loss of glucose

The results presented in the table showed that this fungus is adequate for the pretreatment of sugarcane straw, causing delignification and preserving the fibers properties. After biological pretreatment, the use of chemicals, on pulping, can be reduced significantly. The best condition of biodegradation was using 250 mg/kg of inoculum for 15 d of solid state fermentation, with selectivity higher than 3.0.

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POSTER PRESENTATION 3B-61

Selectivity and Action of White-Rot Fungus *Ceriporiopsis subvermispora* on Sugarcane Bagasse: 20-L Fermentation

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In this work the action of the white-rot fungus *Ceriporiopsis subvermispora* in the fibers of the sugarcane was monitored by periods of time from 7 to 60 days using varied inoculum charge (100, 250, 500 and 750 mg of fungi per kg of bagasse). The solid state fermentation of 800 g bagasse was carried out in 20-L bioreactors. The action fungal of *Ceriporiopsis subvermispora* was evaluated by chemical analysis and also by enzymatic activities measured as lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac) and xylanase (Xyl). Table 1 shows the results of losses of mass and components of bagasse samples treated with 250 mg/kg and also that the *C. subvermispora* was selective for the degradation of lignin. The results of enzymatic activities showed that after 30 day of *C. subvermispora* incubation in the fibers the values of Xyl activities were in the range of 4500 UI/kg independently of the inoculum charge. The MnP has a maximum of production until 30 days and Lac and LiP were not detected.

	Biodegradation time (days)				
Losses (%) / selectivity	7	15	30	45	60
Mass	10	12	13.3	17.1	19.1
Xylan	11.2	7.9	6.9	12.1	13.4
Glucan	9.7	12.9	17.7	25.4	24.7
Lignin	19	22.1	25.6	21.6	33.1
Selectivity	1.7	2.8	3.8	1.8	2.5

" Selectivity is the loss of lignin / loss of glucan; values are related to control

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POSTER PRESENTATION 3B-62

Biofuel Production from Crop Byproducts and Residues

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The objective of this study was to evaluate the feasibility of converting crop residues (e.g. corn stover) to biopolyols, and making the biopolyols into biofuels. Our atmospheric pressure low temperature liquefaction (APLTL) process was optimized to produce biopolyols suitable for biofuel production. Effect of liquefying agents and catalysts on liquefaction and use of liquefied materials as liquefying agent were evaluated. To make biofuel from the biopolyols, the biopolyols were filtered to remove solid sediments, hydrogenated to reduce molecular weight and possibly opening ring-structure, and fractionated using vacuum distillation column. The obtained biofuels were burnable. Preliminary tests showed that the biofuels have the similar atomization characteristics as biodiesel. Further study is necessary to evaluate the chemical and physical properties of biofuels as affected by biomass type, liquefaction, and oxidation, and to develop and optimize the molecular weight reduction and ring opening methods.

POSTER PRESENTATION 3B-63

Liquefaction of Corn Stover and Preparation of Polyester from Its Liquefied Polyol

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This research has been focused on the preparation of bio-polyols from crop residues using the atmospheric pressure, low temperature liquefaction process, and the making of bio-polyester from the prepared bio-polyols. First, corn stover was liquefied in organic solvents such as ethylene carbonate and ethylene glycol with catalysts (such as sulfuric acid) at moderate temperature under atmospheric pressure. The liquefied corn stovers are rich in polyols, which can be directly used as feedstock for making polymers without further separation or purification. Secondly, polyester was made from the liquefied corn stover by cross-linking with multi-functional carboxylic acids and/or cyclic acid anhydrides. The strength of polyester is acceptable. The polyester is stable in cold water and organic solvents but readily biodegradable indicated by 82% weight loss when buried in damp soil for ten months.

POSTER PRESENTATION 3B-64

Production of Xylo-Oligosaccharides from Corn Stover and Corn Cobs Pretreated with Aqueous Ammonia

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Xylooligosaccharides (XOS) in purified form are currently marketed as a high-value food additive in Asian countries. We have investigated on a novel method of producing food grade XOS from corn cobs and stover, which is simpler than the conventional methods. This method is based on pretreatment of substrates with aqueous ammonia followed by selective enzymatic hydrolysis of the xylan fraction and charcoal purification. The process starts with SAA (soaking in aqueous ammonia) treatment of a feedstock which results in clean and xylan-rich substrates. The pretreated substrates are then subjected to selective enzymatic hydrolysis of xylan fraction using endoxylanases for production of mostly XOS and small amount of xylose. Fractionation and refining of xylooligosaccharides were accomplished by charcoal adsorption followed by ethanol elution. When properly operated, all of monomeric sugars and impurities were removed and the XOS were collected in high yields. Ethanol precipitation of the enzyme hydrolyzates without use of charcoal proved to be ineffective in refining and fractionating xylooligosaccharides. Xylanolytic hydrolysis of the SAA treated corn stover maintained high digestibility of the remaining cellulose, above 80% with 10 FPU/g-glucan. As a feedstock for XOS production, corn cobs are better than corn stover because of high xylan content and high density. The high packing density of corn cobs reduces water use in both SAA treatment and enzymatic digestion, which eventually leads to high XOS concentration.

POSTER PRESENTATION 3B-65

Optimal Conditions for Alkaline Detoxification of Dilute-Acid Lignocellulose Hydrolysates

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Alkaline detoxification strongly improves the fermentability of dilute-acid hydrolysates in the production of bioethanol from lignocellulose with *Saccharomyces cerevisiae*. New experiments were performed with NH_4OH and NaOH to define optimal conditions for detoxification and make a comparison with $Ca(OH)_2$ treatment feasible. Since too harsh conditions lead to sugar degradation, the detoxification treatments were evaluated through the balanced ethanol yield, which takes both the ethanol production and the loss of fermentable sugars into account. The optimisation treatments were performed as factorial experiments with 3 h duration and varying pH and temperature. Optimal conditions were found roughly in an area around pH 9/60°C for NH_4OH treatment and in a narrow area stretching from pH 9/80°C to pH 12/30°C for NaOH treatment. In addition, a comparison between optimal conditions for NH_4OH, NaOH, and $Ca(OH)_2$ treatment was made. By optimising each treatment, it was possible to find conditions that resulted in a fermentability that was equal or better than that of a reference fermentation of a synthetic sugar solution without inhibitors, regardless of the type of alkali used. The chemical transformations behind the improvement in fermentability are discussed. The considerable difference in the amount of precipitate generated after treatment with different types of alkali appears critical for industrial implementation.

POSTER PRESENTATION 3B-66

Identification and Quantitation of Organic Degradation Products in Dilute-Acid-Catalyzed Corn Stover Pretreatment Hydrolysates

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A variety of degradation products are produced upon pretreatment of lignocellulosic biomass with dilute acid. To date, the complexity of these samples has significantly limited the scope of efforts to perform summative analyses of degradation products. Qualitative and quantitative interrogation of hydrolysates is also paramount to identifying potential correlations between pretreatment chemistry and microbial inhibition in downstream bioconversion processes.

Chromatographic techniques have been used in combination with mass spectrometry (*i.e.*, HPLC-MS/MS and GC/MS) to qualitatively identify dozens of organic degradation products (*e.g.*, organic acids, phenols, aldehydes, etc.) in corn stover pretreatment hydrolysates. Additionally, a developing suite of analytical methodologies based on chromatographic separation of analytes with ultraviolet, MS, and/or flame ionization detection modes has been applied to perform quantitative assessments of a variety of hydrolysate components as a function of pretreatment time, temperature, and pH.

POSTER PRESENTATION 3B-67

Reactive Extraction of Organic Acids and Phenols from Aqueous Solution Using Quaternary Ammonium Hydroxide Ion Exchangers

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A persistent challenge in biomass processing involves balancing the severity of pretreatment chemistry with the production of microbial inhibitors. While a comprehensive molecular understanding of microbial inhibition in fermentation processes is yet to be elucidated, there is general consensus that organic acids and phenols derived from the lignin and extractive fractions of biomass are among the more inhibitory compounds present in pretreatment hydrolysates. A novel redox-recyclable process was recently developed in our group enabling: 1) simultaneous extraction of organic acids and phenols from aqueous solution, 2) quantitative recovery of extracted components in a minimal volume of secondary waste, and 3) regeneration of strong-base anion exchangers using redox chemistry. As compared with traditional ion exchange, the new process offers an alternative stripping method affording quantitative regeneration of quaternary ammonium hydroxide ion pairs without the use of a high concentration of displacing anion.

In the present study, the process has been proven over multiple extraction-recovery-regeneration cycles. Additionally, a fundamental investigation of the extraction step in the repeatable cycle has been performed, evaluating strong-base anion exchangers ($R_4N^+X^-$; $X^- = Cl^-$ or OH^-) in a side-by-side comparison.

POSTER PRESENTATION 3B-68

Multi-Step Detoxification of Pretreated Corn Stover Slurries

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The liquor separated from pretreated corn stover is rich in fermentable sugars but also contains harmful byproducts such as acetic acid that are toxic to most fermentation organisms. A concentration of 16 grams per liter acetic acid is measured in a batch of corn stover liquor. Detoxification of the liquor prior to fermentation will maximize ethanol production. The solids are necessarily separated from the slurry prior to detoxification of the liquor and contain approximately 60% (w/w) moisture. When the solids are returned to the liquor for the ensuing fermentation, contamination of already detoxified liquor occurs due to the diffusion of toxins from the moisture entrained in the porous structure of the corn stover solids. Further separation and detoxification steps are required to purge residual toxins that were entrained in the moisture in the solids.

A multi-step detoxification process was developed involving an extraction of the liquor and treating it with either activated carbon or over-liming with calcium hydroxide. The solids are then rinsed with treated liquor in order to withdraw entrained toxins. This rinse and treat method is repeated until the concentration of acetic acid falls below a known inhibitory level of 2 grams per liter.

POSTER PRESENTATION 3B-69

Modeling of a Horizontal Pretreatment Reactor Using Computational Fluid Dynamics

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CFD simulations are employed to model flow in an existing horizontal reactor designed to pretreat biomass. A slurry containing a high concentration of biomass solids will exhibit a high viscosity which poses unique mixing issues within the reactor. The viscosity of a 17 percent corn stover solids mixture may be on the order of 20,000 centipoise depending on operating conditions. A well mixed slurry will prevent over or under acid exposure in isolated regions, caking on the walls of the reactor, and consistently provide a uniform final product. A validated model will provide time and cost savings for optimizing reactor operating conditions and be useful in troubleshooting in the event of product defects. Results of the modeling effort are visually represented in various views from within the simulated reactor.

POSTER PRESENTATION 3B-70

Solubility Limitations in the Optimization of Water Only Pretreatment of Hemicellulose

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We propose that oligomer dissolution and solubility limitations play important roles in pretreatment of cellulosic biomass by hemicellulose hydrolysis; however we have no method of directly measuring solubilities at reaction temperatures. To test our hypothesis, commercial xylans and corn stover were pretreated with water in batch tubes at 180°C then cooled in a water bath to 80°C and filtered into two vials: one containing dilution water to preserve the solubilized species at 80°C and one without added water. Both were then cooled to 26°C. We found that for xylan at short reaction times, a significant concentration of high molecular weight oligomers precipitated at the cooler temperature. In addition, we tested different initial solids loading and found that the concentration approached an asymptote at higher solids, indicating that it was reaching saturation and consequentially the yields dramatically decreased. From this information, we inferred the solubility at reaction temperatures and were able to determine the amount of water needed to solubilize hemicellulose oligomers at different reaction severities to maximize recoveries. We also used this knowledge to explain the enhanced performance of water only flowthrough pretreatment compared to batch.

POSTER PRESENTATION 3B-71

Optimization of Pretreatment Parameters for the Ammonia Fiber Explosion (AFEX) Process on Poplar

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The ammonia fiber explosion (AFEX) process has been found to be an effective pretreatment for subsequent enzymatic hydrolysis of a wide variety of crop residues, grasses and hays. However, the AFEX process has never been optimized or even well-studied for pretreatment of hardwoods or softwoods. This paper presents our first intensive study of the effects of AFEX treatment on hardwood, namely a poplar sample provided by the National Renewable Energy Laboratory (NREL). This research is in conjunction with the Biomass Refining CAFI, a cooperative research team comprised of universities and NREL.

AFEX treatment parameters include temperature, moisture content, ammonia loading and time. Hydrolysis parameters include enzyme loading and the total amount and relative ratios of cellulase and xylanase activities. We have found sets of pretreatment and hydrolysis conditions that provide very high conversions of poplar glucan and xylan contents to monomeric sugars. These pretreatment/hydrolysis conditions will be compared and contrasted with conditions giving very high conversions of structural carbohydrates for corn stover, as determined in a previous CAFI project.

POSTER PRESENTATION 3B-72

Expansins Act Synergistically to Enhance Crystalline Cellulose Breakdown by Microbial Cellulases

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Expansins comprise a novel class of plant cell wall proteins that loosen plant cell walls without detectable breakdown of wall polymers. They increase stress relaxation and creep of isolated cell wall specimens and act by disrupting noncovalent bonding between cellulose microfibrils and wall matrix polysaccharides. Expansins play important roles in plant cell growth and other developmental processes where wall loosening occurs (see *www.bio.psu.edu/expansins/*) .

Although expansins do not hydrolyze wall polysaccharides, we found that they significantly enhance cellulose breakdown by fungal cellulases. We used *Trichoderma* cellulase in combination with expansins and monitored the hydrolysis of various substrates, using release of reducing sugars as the indicator of cellulytic attack. Synergism by expansin was negligible with soluble cellulose derivatives, but was substantial with crystalline celluloses. The greatest synergistic effects were seen with the most crystalline forms of cellulose, where up to 2X stimulation was seen after the initial, rapid phase of cellulose breakdown. Further experiments are underway to test new forms of expansins and additional wall materials and enzymes.

Because expansins had optimal synergism at a rate of 1:10 (expansin:cellulase), this protein might serve as a significant additive bio-alcohol production from plant cell walls. We will briefly discuss several possible scenarios for commercial applications, including use of transgenic plants added to the feeder stock and genetic engineering to modify plant cell walls for improved degradation by microbial enzymes.

POSTER PRESENTATION 3B-73

Chemical Analysis of Extracted Maple Wood Chips and Hemicelluloses Useful for Ethanol Production

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Traditional FTIR spectroscopy, FTIR microscopy, and pyrolysis molecular beam mass spectrometry (py-MBMS) in combination with multivariate statistical tools, were all used to examine the residues from hot water extracted maple pulp chips. Environmental Scanning Electronic Microscopy (ESEM) was used to examine changes in the wood structures as a function of extraction time. Both FTIR and py-MBMS provide some detailed molecular information on the samples without need for an extensive calibration set determined using traditional wet chemical analyses. FTIR spectroscopy provides additional information on macromolecular properties such as cellulose crystallinity. FTIR microscopy of residues provides information on the heterogeneity of the individual particles present in the sample down to the 10-micron level. As expected all of the spectroscopic tools show a monotonic change in the composition of the samples consistent with a loss in hemicellulose sugars. These results also highlight the value of using MVA tools for evaluation of pretreated biomass materials.

POSTER PRESENTATION 3B-74

Determination of Brewer's Spent Grain Chemical Composition and Production of Pentose Sugars as an Alternative for Its Use

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Brewer's spent grain (BSG), the solid residue separated after the wort preparation step, is a material that can be derived only from malted barley or from malted barley and adjuncts (other cereal grains that are added to act as source of fermentable sugars). In the present work, samples of BSG obtained from a process employing 100% malted barley were chemically characterized. The material was initially washed with water to remove brewery residues, dried at 50 ± 5°C to reach a 10% moisture content, and characterized following standard methods.

According to the results, BSG contains in dry weight percent, 16.8% cellulose, 27.8% lignin, 28.4% hemicellulose, 1.35% acetyl groups, 4.6% ash, 15.25% proteins and 5.8% extractives. Some variation in the composition of this material can occur when adjuncts are added in the brewing process. After hydrolysed with diluted sulfuric acid under adequate conditions, BSG generated a solution rich in xylose and arabinose, pentose sugars with potential uses in the food industry and that can be employed as substrate in biotechnological processes to obtain value added products such as xylitol or arabitol, for example.

POSTER PRESENTATION 3B-75

Ethanol Production from Pretreated Olive Wood and Sunflower Stalks by an SSF Process

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Olive wood and sunflower stalks are two of the most abundant, renewable lignocellulosic residues occurring in mediterranean countries. To date, these crop residues have no specific use but being burnt for domestic use (olive wood) or grounnd and scattered in the fields. As an alternative, these residues could potentially serve as a low cost feedstock for the production of fuel ethanol. In this work, the potential of using these lignocellulosic biomass residues in a Simultaneous Saccharification and Fermentation (SSF) process to produce bioethanol was evaluated.

As pretreatment, steam explosion was employed since it has been demonstrated to be an efficient technology to increase cellulose susceptibility to enzymatic attack. Different pretreatment temperatures were tested to determine the best conditions leading to the maximum ethanol yield from water-insoluble residue obtained after pretreatment These residues were then subjected to SSF tests using *Saccharomyces cerevisiae* at 35ºC and a commercial cellulase complex. Ethanol concentrations up to 30 g/L were obtained after 72 h fermentation. SSF yields, based on the glucose available in pretreated materials, were determined and the potential of both feedstocks for bioethanol production was assessed.

Poster Abstracts for Session 4

Industrial Biobased Products

POSTER PRESENTATION 4-07

Semi-Continuous Production of Lincomycin Using Immobilized *Streptomyces* Cells

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This presentation demonstrates that immobilized *Streptomyces* cells can prolong the activity for the production of secondary metabolites, which is a critical issue for the commercialization of the immobilized-cell process for production of secondary metabolites. Repeated transfer of immobilized cells into fresh medium every 10 days increased productivity of immobilized cells and maximum concentration of lincomycin, about 820 mg/L, was obtained since 8th batches. This is a 2.4-fold higher volumetric productivity than that obtained by suspended cell culture. Long-term immobilized-cell culture with high productivity was operated for more than 180 days.

We also investigated the suitability of immobilizing method using mycelia instead of spores, which was previously used for most cell immobilization of *Streptomyces* cell or fungal cells. Mycelia was successfully immobilized on beads. Considering difficulty of making spores of Streptomyces in liquid medium and also requiring new unit for preparing spores if spores are used. This is a very important point for scale-up of immobilized *Streptomyces* cells.

POSTER PRESENTATION 4-08

Lignin as an Antioxidant in Petroleum Asphalt

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Lignocellulosic biomass is a renewable resource that can provide fuels, chemicals and energy to decrease our Nation's dependence on petroleum. Lignocellulosic conversion processes produce sugars for fermentation as the principal product and yield lignin as a low-value co-product stream. Economic models assign lignin a fuel value comparable to that of coal ($50/ton) and anticipate the lignin will be combusted on site to provide energy for the process. The low value of lignin does not enhance the economics of these conversion processes since its value is not significantly greater than feedstock costs. Research efforts are underway to identify and develop added-value uses for lignin in order to provide additional revenue streams for these processes. Western Research Institute has conducted investigations on the utilization of lignin in asphalt and the results show lignin has potential as an antioxidant in asphalt pavement. Oxidation is one of the principal factors that cause asphalt pavement to deteriorate, which increases highway maintenance costs. Decreasing the rate of oxidation of the asphalt decreases the rate at which the asphalt hardens and prolongs the service life of highways. This presentation will provide results demonstrating the antioxidant activity of lignin in asphalt and will discuss processing scenarios to produce lignin for this application.

POSTER PRESENTATION 4-09

Low-Cost Sugarcane Bagasse Hydrolysate Supplementation for Xylitol Production in Repeated-Batch Immobilized Cell Cultivation Systems

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Xylitol bioproduction from different lignocellulosic residues has been a subject of intense research in the Laboratories of the Group of Applied Microbiology and Bioprocesses at the Chemical Engineering College of Lorena. A major part of these studies has been carried out in sugarcane bagasse hydrolysates (SBH), envisioning adding value to the surplus of bagasse generated by the Brazilian sugar-alcohol producing industries every year. Recently, the use of repeated-batch immobilized cell cultivation systems to produce xylitol from SBH was evaluated in our laboratories with satisfactory results. The major benefit brought by the use of immobilized cells was the ease of reuse at the end of the batches, while the major benefit brought by the use of a repeated-batch fermentation system was the non-necessity to grow a new inoculum for each batch. This presentation will describe the use of low-cost nutrients (ammonium sulfate and rice bran extract) that improve the xylitol bioproduction when Ca-alginate entrapped *Candida guilliermondii* FTI 20037 cells are cultivated in SBH using repeated-batch fermentation systems. Special attention will be given to a series of bioconversions carried out in a stirred tank reactor, which provided an average xylitol production of 51.6 g/l in each one of five repeated fermentations.

POSTER PRESENTATION 4-10

Production of Lactic Acid from Xylose by *Rhizopus oryzae*

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Lactic acid and its derivatives form an important category of chemicals for industries producing food, chemicals and pharmaceutical products. Optically pure lactic acid can be manufactured by carbohydrate fermentation processes and can be used for the production of polylactic acid (PLA) which is a biodegradable alternative to plastics derived from petrochemical materials. The filamentous fungus *Rhizopus oryzae* produces optically pure lactic acid from sugars such as glucose and requires mineral medium composition. In this study, we focus on the conversion of the pentose sugar xylose into lactic acid by *R. oryzae*. Xylose is one of the most abundant sugars in nature. The ease of isolation of xylose from lignocellulosic materials makes it an important potential feedstock for the production of lactic acid.

Lactic acid was produced from xylose by ten different *R. oryzae* strains. Fermentations were performed in batch flask erlenmeyers. Main by-products were glycerol and ethanol. In comparison with the lactic acid production of glucose, the yield of lactic acid on xylose was comparable but the productivity was significantly lower. Bi-phasic growth of *R. oryzae* occurred when both glucose and xylose were available in the medium.

POSTER PRESENTATION 4-11

Novel Enzyme Sensor Using Mediator Bound to Nanoporous Titanium Dioxide Film for Nitrate Detection

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Novel enzyme sensor for monitoring nitrate in various aquatic pollutants was investigated. Nitrate reductase requires cofactor like NADH for its biocatalytic function. Since cofactor functions electron transfer from electrode to the nitrate reductase enzyme, nitrate was then reduced to nitrite in the enzyme system. Mediator, an artificial electron transferring agent, was used instead because NADH is expensive. Novel biosensor system using the meditor and nitrate reductase was constructed for nitrate detection. Gallocyanine-bound nanoporous titanium dioxide(TiO_2) system was used as a mediator for electron transfer from electrode to nitrate reductase. Large surface area of a nanoporous TiO_2 provides the favorable environment in binding the mediator. Gallocyanine was bound directly and indirectly through an aminopropylsilane linker to nanoporous TiO_2 film. The electrode using an aminopropylsilane linker showed higher efficiency of electron transfer at the same potential than directly linked. Nitrate reductase was also immobilized by crosslinking with glutaraldehyde. Linear relationship was obtained with nitrate ion concentration up to 1mM. The novel enzyme sensor system investigated in this study can be used for other enzyme sensor requiring cofactor regeneration.

POSTER PRESENTATION 4-12

Production of *Bacillus sphaericus* Entomophatogenic Biomass Using Brewery Residues

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The utilization of brewery residual biomass and trub has been evaluated aiming to minimize the costs of the industrial production of *Bacillus sphaericus*-based bioinsecticide. The production media were formulated on the basis of its protein content in order to present an initial concentration of 7,0 g/L. The brewery residual biomass and trub promoted growth and sporulation of the three *B. sphaericus* strains that were isolated from Brazilian soils (S1, S2, S20). However, distinct growth and sporulation behaviour was observed in relation to the different nutritional conditions that were tested. Of the three bacterial strains, the medium containing trub favoured the growth of the most, in particular that of the S1 strain. Yet, the maximum sporulation percentage was obtained through the cultivation of the S20 strain in the medium containing brewery residual biomass. The obtained results were equal or superior to those determined in the media that are conventionally used for the production of *B. sphaericus* entomopathogenic biomass. The data for toxicity (LC50) study using *Culex quinquefasciatus* larvae also varied with the medium composition. A considerable reduction in the organic matter content occurred, although the residual values were still superior to that considered appropriate for effluent discharge.

POSTER PRESENTATION 4-13

A Fungal Biotechnology Core Research Program for Biobased Products

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The Fungal Biotechnology Core at Pacific Northwest National Laboratory (PNNL) is an integrated multi-task research program aimed at developing enabling technology around filamentous fungal fermentation processes for the production of various products from biomass. Filamentous fungi are capable of degrading complex biomass such as agricultural wastes into simple sugars which can subsequently be used by these organisms for highly efficient production of organic acids or other chemicals. In recent years there has been a rapid increase in the rate of generation of filamentous fungal genome sequences. Our group in particular is leading the genome sequencing project for *Aspergillus niger* in collaboration with the DOE's Joint Genome Institute and other researchers. The availability of fungal genome sequence databases enables advanced genetic tools and high throughput proteomic analysis to be applied to important questions in fungal morphology, organic acid production, biomass degradation and fermentation process development in organisms such as *A. niger, Phanerochaete chrysosporium* and *Trichoderma reesei*. We will outline our core biotechnology program and recent progress in genetic and proteomic research in a variety of filamentous fungal systems.

POSTER PRESENTATION 4-14

Biomasics: The Concept and Its Materialization (Sustainable Value Creation from Biomass Wastes)

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This work introduces the concept of *biomasics* and presents advances on its implementation for the production of biofuels (bioethanol) and chemicals (biodegradable polymers). Biomasics, as its inspiring concept petrochemistry, was born by the use of the raw matter (lignocellulosics and petroleum respectively) as provider of energy. Nevertheless, as petroleum industry soon realized, a rational use of the material requires maximization of added value, and thus the petrochemical industry begins its development. Biomass, instead is still widely used as an energetic. Sometimes this basic use of biomass is not sustainable (traditional domestic consumption for cooking and heating), and sometimes (as is the case of the Program *Pellets for Europe*) combustion is optimized through technological inputs. Nevertheless, despite optimized combustion, a raw matter of such a complex, rich and varied, calls for an approach of value creation before burning the final residues. We have put into practice this notion of a chain of added value by developing integrated bioprocesses able to competitively and efficiently recuperate phenols and modified phenols (used in the formulation of biopolymers) from the lignin fraction, before subsequently use the other two main components of biomass (celluloses and hemicelluloses) to produce ethanol. Thus, the process we have developed achieves maximum sustainability by producing a biofuel which does not affect the carbon cycle, biopolymers of high added value and ecologically friendly and a final residue for combustion, all of which are obtained from a raw matter (i.e. agricultural wastes) that have a negative impact if not treated. We will present advances on raw materials pretreatment (solid substrate fermentation), hexoses and pentoses fermentation, and a prefeasibility analysis for a given production capacity.

POSTER PRESENTATION 4-15

Potentiality of Bacteria Strains Isolated from Landfarming as Biosurfactants Producers

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Flavobacterium thalpophilum, *Acinetobacter* sp and *Bacillus subtilis* strains, isolated from contaminated soil from a Landfarming site (REDUC-PETROBRAS-Rio de Janeiro), were selected for the production of biosurfactant using different raw materials (commercial sugar, sugarcane juice and cane molasses, glycerol, lactic acid and mannitol). The three strains were able to produce biosurfactants, however, the production varied depending on the bacterium and the utilized carbon source. In some conditions, the decline in the surface tension did not occur. In general, sugarcane juice has generated the lowest values of surface tension. The best results of approximately 31 mN/m were achieved for 48-h of *F. thalpophilum* and *B. subtilis*. The raw materials also influenced the emulsifying efficiency of the produced biosurfactants; maximum emulsification index varying from 60 to 80% were obtained. These results confirm the potentiality of the isolated strains to produce biosurfactants with different peculiarities and, consequently, its utility in different industrial sectors.

POSTER PRESENTATION 4-16

Citric Acid Production by *Aspergillus niger* Using Date Base Medium Fortified with Whey, Methanol and Tricalcium Phosphate

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Citric acid is widely used by the food, pharmaceutical, and chemical industries as a major substrate for the production of a variety of products. There have been increased interests in using natural resources such as fruit sugars for the production of citric acid. Research has shown that dates, figs, carob pods, kumara, taro and sugarcane are good substrates for citric acid production by *Aspergillus niger.* The objective of this study was to determine the ability of *A. niger* to produce citric acid from natural carbohydrate sources; date and sweet whey. The uses of other supplements to increase productivity were evaluated. Two strains of *A. niger* (ATCC 6275 and 9642) were grown in media containing different concentrations of date extract or molasses fortified with whey, methanol or tricalcium phosphate. The fermentation experiments were conducted at 25 °C for 12 days and samples were withdrawn at different time intervals and analyzed for citric acid content.

Results showed that high citric acid was produced by *A. niger* ATCC 6275 in 20 % molasses in whey with citric acid concentration of 32.4 g/L. When methanol and tricalcium phosphate were added, significant increase in citric acid production was recorded. Citric acid concentrations were 38.4 and 42.4 g/l, in media fortified with methanol and tricalcium phosphate respectively. Our result indicates that the addition of sweet whey, methanol and tricalcium phosphate date or molasses could have a significant influence on the production of citric acid by *A. niger.*

POSTER PRESENTATION 4-17

Combinatorial Approaches for Developing New Catalysts for Polyols

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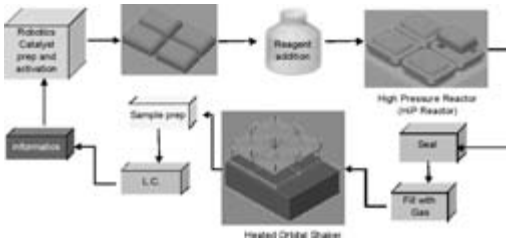
Formation of ethylene glycol (EG) and propylene glycol (PG) from sugars offers potential renewable resources for solvents, deicers, antifreeze and other products. The mechanism for hydrogenolysis is complex. At the minimum it involves dehydrogenation, accompanied with isomerization, retro-aldol (C-C cleavage), dehydration (C-O cleavage) and re-hydrogenation. Numerous precious metals and mixed metal systems on various supports along with various base or acid promoters have been used to catalyze the reaction. The complexity of the mechanism and the variety of metals, supports and promoters possible in optimal catalytic systems makes combinatorial methodology ideal for advanced catalyst development.



We have employed high-throughput combinatorial techniques for advanced catalyst discovery. The combinatorial approach has allowed us to rapidly prepare and screen in excess of 4,000 individual experiments in a matter of months. Surprising results were found on preferred supports, metal combinations and promoters. Catalysts systems screened included one or two central metals with up to two additional promoter metals (i.e. up to quaternary metal systems). The exact catalyst compositions will not be presented at this time. Rather, the presentation will focus on the process of catalyst discovery using mechanism-based design and statistical tools.

POSTER PRESENTATION 4-18

Batch (One- & Two-Stage) Production of Biodiesel Fuel from Rapeseed Oil

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Biodiesel fuel as renewable energy is an alternative that can reduce energy dependence on petroleum and air pollution. Several processes for the production of biodiesel have been developed. Alkali catalyzed transesterification with short-chain alcohols give high yields of methyl esters in short reaction times. In this research, batch (one- and two-stage) transesterification of rapeseed oil was investigated to produce the rapeseed methyl ester. The conversion of rapeseed oil in 30 L reaction system was showed similar reaction pattern and yield compared with the results obtained in 1 L reaction system. The rapeseed oil was converted about 98% at 400 rpm within 20 min in the condition of 1% (w/w) KOH, methanol molar ratio 1:10 and 60°C. For searching of optimum amount of added KOH and methanol amount of 2nd transesterification in 1 L reaction system, the 1st reaction was performed with 1:4.5 molar ratio and 1% (w/w) KOH, and obtained about 71% conversion yield. In the 2nd reaction, the conversion yield did not highly affected by the added amount of KOH above 0.2% (w/w) with different methanol molar ratio. In 0.2% (w/w) KOH addition, rapeseed oil was converted over 98% with 1:1 to 1:3 methanol molar ratios. In the 30 L two-stage transesterification, rapeseed oil was converted to about 98.5% in the 2nd reaction condition of 1:1 methanol molar ratio and 0.2% (w/w) KOH at 60_ for 30 min. From the refined fatty acid methyl ester product (biodiesel), the purity was obtained above 99% through post-treatment such as washing and centrifugation.

POSTER PRESENTATION 4-19

Continuous Acetone-Butanol-Ethanol (ABE) Production from Degermed Corn Using *Clostridium beijerinckii* BA101

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Use of degermed corn as feed for continuous ABE fermentation employing *C. beijerinckii* BA101 may have added economic advantage over the use of glucose. Corn (Yellow dent variety) contains 61% starch, 3.8% corn oil, 8.0% protein, 11.2% fiber and 16.0% moisture. Since the corn oil and fiber content of the corn are not needed in ABE fermentation, they were removed and these allowed for byproduct credit. The degermed corn solution and glucose (as control) were supplemented with P2 medium nutrients in order to support growth of *C. beijerinckii* BA101 and ABE production. The bioreactor was fed at a dilution rate of 0.03 h^{-1} and saccharified degermed corn solution/feed volume (4 L) was replaced every 72-84 h. The continuous reactor fed with degermed corn solution produced a maximum of 14.0 g L^{-1} total ABE while the reactor fed with glucose (control) produced a maximum of 12.0 g L^{-1} total ABE. The productivity recorded with degermed corn and glucose solutions was 0.42 and 0.36 g $L^{-1}h^{-1}$, respectively. The residual glucose concentrations of the effluents associated with degermed corn and glucose were 20.1 and 29.1 gL^{-1}, respectively. Interestingly, decreasing the P2 nutrient supplementation of the degermed corn solution in half did not result in a decrease in ABE productivity. *C. beijerinckii* BA101 apparently utilized the nutrients in the degermed corn to compensate for the reduction in the P2 medium supplements. It is anticipated that reduction of P2 medium supplements in half would significantly impact the butanol price.

POSTER PRESENTATION 4-20

Optimization of Distilled Monoglycerides Production

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Monoglycerides (MG) are emulsifiers widely used in food and pharmaceutical industries. Current industrial processes for MG production consist on the interesterification of triglycerides (TG) with glycerol (GL), in the presence of inorganic catalysts at high temperatures (above 200°C). This reaction is known as glycerolysis and produces an approximately 50 % MG content mixture. This level of concentration is suitable for many applications, although for some specific uses like margarine, shortening, icing and cream filling, distilled monoglycerides are required, which are purified MG (minimum 90%) obtained by the molecular distillation process.

Therefore, in this work, a 2^3 factorial design was employed to evaluate the effects of reaction parameters in the MG content after the interesterification reaction of refined soybean oil with glycerol in the presence of sodium hydroxide as catalyst. After that, the MG content in the reaction product was enhanced through the molecular distillation process in order to obtain distilled MG. Surface response methodology was applied to find the best distillation conditions for the MG enhancement.

POSTER PRESENTATION 4-21

Lactic Acid Production from Cheese Whey and Corn Steep Liquor by *Lactobacillus sp.* RKY2

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Biological production of lactic acid has attracted a great deal of interest due to its potential uses in many industries and the production of polylactide polymer. Although the biological production of lactic acid has some advantages over chemical synthesis, it still requires the cheaper substrates for industrial feasibility. From this point of view, cheese whey and corn steep liquor seem to be good substrates as carbon and nitrogen sources for biological production of lactic acid because cheese whey is a by-product of the cheese manufacturing industry and corn steep liquor is also a by-product of the corn steeping industry.

Lactobacillus sp. RKY2 is a newly isolated homofermentative lactic acid bacterium. We tried to investigate the fermentative production of lactic acid from cheese whey and corn steep liquor as cheap raw materials using *Lactobacillus* sp. RKY2. When the cheese whey containing 100 g l^{-1} of lactose and 15-60 g l^{-1} of corn steep liquor were used as carbon and nitrogen sources, respectively, lactic acid was produced up to 91 g l^{-1}. Lactic acid yields based on consumed lactose in cheese whey were above 0.90 g g^{-1}, and lactic acid productivities increased with the increase of corn steep liquor supplementation.

POSTER PRESENTATION 4-22

Optimization of Bacterial Cellulose Production Using *Gluconacetobacter sp.* RKY5 Isolated from Persimmon Vinegar

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Cellulose is the most abundant organic polymer in nature, where it plays an important role in integrity of plant. Plant cellulose is being intensively used in the paper and textile industries, which leads to a significant demand on wood biomass. Bacterial cellulose (BC) differs from plant cellulose with respect to its size, purity, and crystallinity. Recently, there have been interests in new fields of application of BC such as food, healthcare, cosmetic, environmental, clothing, and aircraft industries due to its distinguished physicochemical properties. Especially, the use of BC is promising in many industrial fields such as thickners for foods, medical materials for protection of burned skin, selective permeation membranes, and sensitive diaphragms for stereo headphones.

Recently, we have newly isolated a BC-producing microorganism from persimmon vinegar, dubbed *Gluconacetobacter* sp. RKY5. In this work, we tried to optimize the culture media and conditions for BC production using *Gluconacetobacter* sp. RKY5. *Gluconacetobacter* sp. RKY5 produced the highest amount of BC from glycerol as a carbon source and acetic acid as a secondary substrate. Under the optimized culture conditions, the maximum amounts of BC were obtained at 4.6 g l^{-1} and 5.6 g l^{-1} by static culture and shaking culture, respectively.

POSTER PRESENTATION 4-23

Natural Compounds Obtained through Centrifuge Molecular Distillation

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The raw materials used in food, pharmaceutical and cosmetics are substitutes for products of natural sources. The soybean oil deodorized distillate (SODD) is a product derived from refining edible soybean oil. Deodorization is to remove compounds that give odor and flavor to the oil. This step removes the unsaponifiable matter like sterols and tocopherols.

Molecular distillation is the most important process to obtain tocopherol concentrates, where compounds are processed at reduced pressure and temperature. Then it can be used to separate and purify thermosensible material as well as vitamins. The centrifuge molecular distillator was operated at high vacuum. The variables studied were feed flow and evaporator temperature.

Experiments were done at 140-220°C. The feed flow varied from 5 to 15 ml/min., With an increase in the feed flow and temperature of evaporator, the residue flow of distiller would be increasing. The objective of this study was to remove the maximum amount of fatty acids and to obtain the major tocopherol concentrate. The % FFA at distillate stream is bigger at low feed flow and evaporator temperature at 140°C. At high temperatures and feed flows, %FFA in the distillate stream is high, but there is loss of tocopherol.

POSTER PRESENTATION 4-24

Biosurfactant Production by *Pseudomonas aeruginosa* FR Using Palm Oil

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The use of low cost raw matter and production media appears as natural choices to biosurfactant process overall economy. Brazil is one of the world largest producers of plants oil, e. g., palm oil. Recently various studies have been published on biosurfactant production by some bacterial genus. However, to our knowledge no reports have been published on the biosurfactant production from palm oil by *P. aeruginosa* bacteria. The scope of this investigation was biosurfactant production from palm oil by *P. aeruginosa* FR using three low cost mineral media.

The tests were conducted using 250 mL Erlenmeyer containing 100 mL of mineral medium and 1mL of sterile palm oil, at 29ºC and 150 rpm. Tested mineral media presented these compositions in g/L: MM1 (KH_2PO_4 = 0.5, Na_2HPO_4 = 4.5; NH_4Cl = 2.0 and $MgSO_4.7H_2O$ = 0.01). MM2 ($(NH_4)_3PO_4$ = 1.0) and MM3 (N:P:K commercial fertilizer = 3.0). In all tests, the initial pH were adjusted to 7.0. Monitoring program included biomass, surface tension and critical micelal dilution of the media and emulsification index.

In best process condition (MM3) surface tension of the media decrease from 68 to 32 mN/m and the emulsification index using hexane, benzene and jet fuel were about 70%.

POSTER PRESENTATION 4-25

Novel Approach of Corn Fiber Utilization

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The corn wet milling procedure results in 10% (based on the processed corn) by-product called corn fiber, which is utilized worldwide as a low value feedstock for cattle. The aim of this study was to find a more rational way of utilizing corn fiber. Its main fractions are: 20% starch, 40% hemicellulose, 14% cellulose and 14% protein. Extraction of the highly valuable, cholesterol-lowering corn fiber oil is not feasible due to its low (2% w/w) concentration in the fiber.

The developed technology is based on simple and cheap procedures, like washing with hot water, dilute acid hydrolysis at 120°C, enzymatic hydrolysis of cellulose, screening, drying, extraction. The main fractions are sharply separated (1.: starch, 2.: hemicellulose, 3.: cellulose, 4.: lipoprotein, 5.: lignin). The lipoprotein fraction adds up to 10% of the original dry corn fiber, and contains 45% corn fiber oil, thus more oil is yielded than via direct extraction of the fiber.

It is concluded that the defined method makes the extraction of the corn fiber oil economically feasible. The fractionation process also significantly increases the yield of cholesterol-lowering substances (sterols and sterol-esters) at the same time clear and utilizable fractions of monosaccharides, protein and lignin are produced.

POSTER PRESENTATION 4-26

Poly-β-Hydroxyalkanoate Copolymers from *Burkholderia cepacia* Utilizing Renewable Forestry-Based Substrates

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Burkholderia (formerly *Pseudomonas*) *cepacia* was found to produce poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (P(3HB-co-3HV)) from xylose and levulinic acid, at levels ranging from 40-56 % (w/w) of cellular dry mass. Shake-flask cultures containing detoxified, maple-derived hemicellulosic hydrolysate (1.8-2.2 % reducing sugar) and concentrations of levulinic acid ranging from 0-0.62 % (w/v) were used to generate P(3HB-co-3HV) films comprised of 0-67 mol % 3HV. Pilot production in a 7-liter fermentor has demonstrated the potential to increase yields and improve the associated physical characteristics of the copolyesters. Levulinic acid was found to positively affect cellular growth, PHA accumulation, and copolyester composition, with periodic addition of this cosubstrate acting to increase PHA contents by 25 % compared to single-dose addition. Characterization of representative samples by ^{1}H and ^{13}C NMR and differential scanning calorimetry revealed a reduction in melting temperatures (T_m) and glass transition temperatures (T_g) to 90-100°C and –14.8°C, respectively, as a function of the mol % 3HV composition. Considering that the average molecular mass of the P(3HB-co-3HV) films was in excess of 600 kDa and that the temperature for thermogravimetric decomposition was well-above (>100°C) the corresponding T_m, there exists great potential for these value-added copolymers in thermoplastic applications.

POSTER PRESENTATION 4-27

Biological Upgrading of Recycled Paper Sludge: Ethanol Production Using Enzymatic Hydrolysate

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A process for biological upgrading of recycled paper sludge (RPS) was developed based on the enzymatic conversion of major sludge components (cellulose and xylan) and fermentation of the resulting sugars to ethanol. These two steps can be performed sequentially (SHF) or simultaneously (SSF). In the first step of the SHF process, when using Celluclast® 1.5L supplemented with Novozym® 188, a degree of saccharification of 100% was achieved. Concerning fermentation, when using the yeast *Pichia stipitis* CBS 5773, an ethanol concentration of 20 g L^{-1} was attained by the SHF process. However, this conversion required 179 hours, whereas the SSF process produced the same concentration only after 48 hours of incubation (overall conversion yield: 51% of the available carbohydrates on the initial substrate).

The results of this work clearly demonstrated an efficient ethanol production from RPS without pre-treatment and/or supplementation of the sludge material. The biological conversion process may thereby represent an opportunity for the reduction of an important waste stream generated from the recycling process, having direct benefits in the reduction of landfill costs while producing a commercial product from a substrate of negative cost.

POSTER PRESENTATION 4-28

Stimulation of Nisin Production from Whey by a Mixed Culture of *Lactococcus lactis* and *Saccharomyces cerevisiae*

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The production of nisin, a natural food preservative, by *Lactococcus lactis* subsp. *lactis* (ATCC 11454) is associated with the simultaneous formation of lactic acid during fermentation in a whey-based medium. Due to the low concentration and high separation cost of lactic acid, recovering lactic acid as a product may not be economical. On the other hand, the accumulation of lactate in fermentation broth inhibits nisin biosynthesis. The approach that we took in this study was to remove lactate biologically. A mixed culture of *Lactococcus lactis* and *Saccharomyces cerevisiae* was established in order to stimulate the production of nisin via the in situ consumption of lactate by the yeast strain which is capable of utilizing lactate as carbon source. The *Saccharomyces cerevisiae* in the mixed culture does not compete with the nisin producing bacteria because the yeast doesn't utilize lactose, the major carbohydrate in whey for bacteria growth and nisin production. The results showed that lactate produced by the bacteria was almost totally utilized by the yeast and the pH of the mixed culture could be maintained at around 6. Nisin production by the mixed culture system reached 135 mg/l, which is 1.5 times higher than that by a pure culture of *L. lactis*.

POSTER PRESENTATION 4-29

Production of Hydrogen from Waste Fryer Grease

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Hydrogen is an attractive energy source from environmental and economic stand point as it can complement conventional fossil fuels. Currently hydrogen is produced via steam reforming of natural gas and liquefied petroleum gas (LPG). However, these processes are not environmentally friendly and consume non-renewable sources. Biomass is a renewable resource and can produce high quality hydrogen. High cost of biomass is a major hurdle in utilizing these materials for hydrogen production. Waste fryer grease is an economic source for energy. It is well known that waste fryer grease is used for the production of biodiesel. Literature suggests that waste cooking oil is a good source for hydrogen production. The chemical composition of waste fryer grease changes depending on the nature of treatments used in restaurants. This will ultimately affect the gas product composition after pyrolysis and/or gasification.

Pyrolysis of waste fryer grease was carried out in a continuous down flow fixed bed microreactor made of inconel alloy, and packed with materials such as quartz chips. Temperature was maintained in the range of 650 to 850°C while varying inert gas (N_2) flow rate from 30 to 70 mL/min. Product gas was analyzed using HP 5890 and 5880 HP gas chromatographs. Preliminary results of the present work showed that major products are hydrogen, carbon monoxide, carbon dioxide, methane, ethane, ethylene, propylene, 1- butylene. The presentation will include the process optimization study for selective production of hydrogen from waste fryer grease via pyrolysis and steam reforming processes.

POSTER PRESENTATION 4-30

Biochar as a Precursor of Activated Carbon

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Biochar is one of products of fast pyrolysis process of biomass (including forest residue such as bark, sawdust and shavings; and agricultural wastes such as wheat straw and bagasse). This char is a high heating value solid fuel that is commonly used in kilns and boilers. Biochar can be considered as a precursor for production of activated carbons that are used as adsorbents of gases and vapours, catalysts supports, and separation media. The objective of the present investigation is to examine the possibility of production of activated carbon from biochar and the related adsorptive capacities of the product. Two activation methods (physical and chemical activation) were used. Experiments were designed by central composite design (CCD) method to study the effects of various operating conditions on BET surface area and yield of the product.

The surface area of Biochar was less than 10 m^2/g. Results show that it is possible to produce activated carbon from biochar with a BET surface area more than 660 m^2/g and a yield more than 38 wt %. The microprous area of the product is more than 50 % of BET surface area. The methylene blue number, as an indication of mesoporous area, was more than 300. The average pore diameter was 21.5 ^{O}A that is comparable with that of commercial activated carbons. The pH of product was 11.7 that shows the basic characteristic. Increasing BET surface area to more than 66 times of biochar surface with a relatively high yield, low ash content ($\leq$ 7 wt %) and suitable percentage of micropous and mesoporoes area show the high potential of this plentiful precursor to produce activated carbon.

POSTER PRESENTATION 4-31

Catalytical Oxidation of Lignins with Molecular Oxygen: Reactivity of Steam Explosion Lignin in Acidic Medium

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Lignin is a crosslinked and highly complex macromolecule obtained as a by-product from the cellulosic and pulp industry, using wood and agricultural residues as sugarcane bagasse. Oxidized lignin has very strong chelating properties used in industrial effluent treatment, used for the removal of heavy metals. Oxidation of the lignin obtained by steam explosion of sugarcane bagasse was performed using acetic acid and the Co/Mn/Br catalytical system, at 50-115°C for 5 h. Activation energy (Ea) was calculated ranging from 12.8 to 16.4 $kJ.mol^{-1}$ showing a reduction in Ea of 50% in average in comparision with the non-catalyzed system, indicating that the catalyst was efficient. FTIR data of samples collected during oxidation were very similar and Principal Component Analysis (PCA) applied to the spectra shows only slight structure modifications in lignin after 2-4 h of reaction. Oxidized lignins have been evaluated as chelating agents for the removal of heavy metals from aqueous solutions.

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POSTER PRESENTATION 4-32

Identification of Microbial Flora from Mosto mezcal Fermentation

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The Zimatlán of Alvarez located in the state of Oaxaca, México, is one of the main producers of mezcal, an alcoholic beverage obtained by fermentation after cooking the pineapples of *Agave angustifolia* (*Haw var.*). In this work we describe the isolation and identification of different microbial species obtained using the liquid fermentation named mosto.

Using the glass rod microbiological technique: some samples of 0.1 ml from mezcal fermentation were plated on nutritive agar (NA), dextrose Sabouraud agar (SA) and dextrose potato agar (PDA), after 48 h of incubation at 29°C 31 different yeast strains were obtained verifying the unicellular clones existing by colony and microscopic observations.

The pure strains obtained were identified by microscopic morphology and biochemistry tests. The purified yeast strains mainly corresponded to *Candida* and *Saccharomyces* genera, the other microbial strains that were found in mosto mezcal fermentation possibly participate in forming different substances giving a particular taste and bouquet to the final product.

This screening of different yeast strains with particular characteristics, is very important for the quality of Mexican mezcal beverages.

POSTER PRESENTATION 4-33

Moisture Sorption, Transport, and Hydrolytic Degradation in Polylactic Acid

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Polylactic acid (PLA) is a biodegradable, highly versatile, aliphatic polyester which can be produced from 100% renewable, natural resources offering a wide range of commodity applications. Management of moisture penetration and hydrolytic degradation of PLA is extremely important during manufacturing, shipping, storage, and end-use of PLA products. The research presented here investigates how degradation depends on polymer structure parameters, temperature, and water content.

Moisture transport, crystallization, and degradation have been measured in this project through a variety of experimental techniques including Size-Exclusion Chromatography (SEC), Differential Scanning Calorimetry (DSC), X-Ray Diffraction (XRD), and Karl-Fischer titration. As anticipated, hydrolytic degradation reduces the molecular weight of the polymer and is more severe with increasing temperatures. Nuclear Magnetic Resonance (NMR) has been investigated to measure degradation kinetics and moisture content. Quartz Crystal Microbalance (QCM) and Dynamic Vapor Sorption (DVS) experiments have also been used to measure moisture sorption isotherms and diffusion coefficients in PLA films after being subjected to varying heat treatment to produce films with varying crystallinity. A surprising result is that crystalline and amorphous PLA films exhibit identical sorption isotherms, within the accuracy of the experiments.

Poster Abstracts for Session 5

Microbial Catalysis and Metabolic Engineering

POSTER PRESENTATION 5-08

Construction of a Cellulase Hyper-Producing Strain, *Trichoderma Reesei* JS-1, for Laboratory Use by Active Nuclear Shuffling Techniques

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It was considered that construction of a cellulase hyper-producing strain for laboratory use is effective in order to accumulate knowledge for efficient enzymatic saccharification of cellulose. So, we attempted to produce such a strain by our nuclear shuffling techniques.

In this work, *Trichoderma reesei* Rut C-30 (ATCC 56765) was used as a model strain. In swollen conidia of the model strain, autopolyploid nuclei were formed. These swollen conidia were treated with haploidizing reagent followed by generating multiple smaller nuclei from a larger autopolyploid nucleus in a swollen conidium. From such swollen conidia, *Trichoderma reesei* JS-1 was selected using Avicel and filter paper. This strain could break down a filter paper more rapidly in comparison with the model strain. In the enzyme reaction mixture with filter paper, a larger amount of reducing sugar existed in the reaction mixture of the strain, JS-1. Moreover, it appeared that Avicel hydrolyzing activity and Salicin hydrolyzing activity were increased more in the strain, JS-1. This strain, JS-1 is sent to ATCC as a strain for laboratory use.

POSTER PRESENTATION 5-09

Validation of Opening Reading Frames with Possible Influence on the Xylose Growth Rate of *Saccharomyces cerevisiae*

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Bioethanol is a renewable energy source produced through fermentation of sugars. The raw material is a major contributor to the total production cost of bioethanol, and efficient utilization is needed to make the process economically feasible. An ideal microorganism for ethanol production from lignocellulosic material must ferment all the different sugars in the hydrolysate with a high fermentation rate and ethanol yield.

Since *Saccharomyces cerevisiae* strains overexpressing the genes for xylose reductase (XR) and xylose dehydrogenase (XDH) from *Pichia stipitis* and the endogenous *XKS1* gene encoding xylulose kinase (XK) grew poorly on xylose, a number of mutant, hybrid or adapted strains with enhanced xylose growth have been generated. Microarray transcription analyses of these strains have shown a limited number of ORFs with altered expression levels in all the investigated strains. In this study, the effect of these ORFs on the xylose growth rate of *S. cerevisiae* was investigated. This was accomplished by using strain collections in which the *S. cerevisiae* ORFs were either overexpressed or deleted. A functional xylose utilization pathway was introduced in all the tested strains by transformation with an integrative plasmid carrying XR, XDH and XK genes.

POSTER PRESENTATION 5-10

Zymomonas mobilis as Catalyst for the Biotechnological Production of Sorbitol and Gluconic Acid

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The bioconversion of glucose and fructose into gluconic acid and sorbitol was conducted in a 250mL-batch reactor with whole cells of *Zymomonas mobilis* treated or not with the fatty acid remover cetyl trimethyl ammonium bromide (CTAB). The initial substrate concentration (glucose plus fructose) (S_0) employed was ranged from 100 g/L to 600 g/L, meanwhile the other test conditions were set at pH 6.4, 39°C, agitation of 300 rpm and cell concentration of 30 g dry mass/L. Using either CTAB-treated or not treated cells, yield over 90% and productivity around 1.50 g/h.g cell for gluconic acid and sorbitol were attained at S_0 = 600 g/L. In all tests realized no cell growth was detected. In tests carried out with $S_0 \leq 300$ g/L occurred an ethanol production lower than 22 g/L. Bioconversions conducted with cells of *Zymomonas mobilis* entrapped into calcium alginate beads were also evaluated. Although using the same test conditions as for free cells, the yield for both gluconic acid and sorbitol was about 80% at S_0 = 600 g/L. The reason would be the restraint to the movement of low MW molecules in and out of the beads imposed by the jellylike nature of the calcium alginate envelope.

POSTER PRESENTATION 5-11

Effect of Substrate Feeding Strategy on Glucose-6-Phosphate Dehydrogenase Synthesis in Fed-Batch Culture of Recombinant *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae W303-181 is an adenine-dependent strain containing the plasmid YEpPGK-G6P, which was built by associating the vector YEPLAC 181 with the promoter PGK1 (phosphoglycerate kinase 1). This strain can produce high amount of glucose-6-phosphate dehydrogenate (G6PDH) if cultured properly. The G6PDH is an enzyme largely used as diagnostic reagent (mainly in enzyme immunoassay technique), being normally imported by developing countries (such as Brazil). Its local production should help to establish appropriate health programs as far as more accurate diagnostic methods can be used. Thereby, the microorganism was cultured by fed-batch process (the volume of the medium was increased from 2L up to 3L during 5h) at pH 5.7, aeration of 2.2 vvm, agitation of 400 rpm, 30°C, inoculum volume of 0.4L, initial medium volume of 1.6L and 20 mg/L of micronutrients (histidine, tryptophan, uracil and adenine). The feeding of glucose solution (10 g/L) followed the constant, linear or exponential mode. The highest G6PDH specific activity (250 U/mg of protein; 1U= 1 μmol of $NADP^+$/min), which is comparable with those presented by commercial preparations (150-500 U/mg protein), occurred when the substrate was fed into the fermenter through the decreasing linear mode.

POSTER PRESENTATION 5-12

Heterologous Expression of the *Clostridium cellulolyticum* Cellulosome in *Clostridium acetobutylicum*

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The cellulosome gene cluster of *Clostridium cellulolyticum* has been cloned and heterologously expressed in *Clostridium acetobutylicum*. This presentation will include a description of the vectors and promoters used, levels of expression of the various cellulosomal proteins, assembly of the cellulosome and its activities against carboxymethyl cellulose and Avicel.

C. cellulolyticum is able to degrade crystalline cellulose; however, it is not able to produce large amounts of ethanol. *C. acetobutylicum* is a very well characterized organism which has traditionally been used to produce solvents in acetone-butanol-ethanol fermentations. Production of a cellulosome in *C. acetobutylicum* therefore constitutes a major advance towards consolidated bioprocessing of cellulose to ethanol. Expression of the cellulosome in other organisms capable of producing useful end products such as butyrate is also being explored.

POSTER PRESENTATION 5-13

Efficient Production of L-Lactic Acid by Metabolically Engineered *Saccharomyces cerevisiae*

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Polylactic acid is being developed as a renewable alternative for conventional petroleum-based plastics. For mass production of lactic acid, we developed a metabolically engineered *Saccharomyces cerevisiae*, which produces lactic acid efficiently. In this recombinant, the coding region of *pyruvate decarboxylase 1* (*PDC1*) is substituted for that of the *L-lactate dehydrogenase* gene (*LDH*) through homologous recombination. The expression of mRNA for the genome-integrated *LDH* is regulated under the control of the native *PDC1* promoter. The difference in L-lactate production was compared among two different recombinants expressing *LDH* gene, i.e. either the *bovine LDH* or the *Bifidobacterium longum LDH*. The combination of *bovine LDH* and the *PDC1* promoter was proved to be effective for the efficient production of lactic acid.

Next, we constructed transgenic strain including 6 copies of the *LDH* gene on the genome under the control of the *PDC1* promoter. The L-lactate productivity was improved by increasing the number of copies of the *LDH* gene. On fermentation in inexpensive cane juice-based medium using 1 liter jar-fermenter, L-lactate production of this recombinant reached 122 g/L, with up to 61.0% of the sugar being transformed into L-lactate finally. The optical purity of this L-lactate was 99.9% or over.

POSTER PRESENTATION 5-14

A Unique Feature of Hydrogen Recovery in Endogenous Starch-to-Alcohol Fermentation of Marine Microalga, *Chlamydomonas perigranulata*

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A marine green alga, *Chlamydomonas perigranulata,* was demonstrated to synthesize starch through photosynthesis, stored once in a cell, and ferment it by an incubation in anaerobic and dark. Fermentation products are alcohols, ethanol and 2,3-butanediol (BD), acetic acid and carbon dioxide (CO_2). Previous fermentation data of algal biomass cultivated outdoors by using a tubular photo-bioreactor showed (i) good C recovery in fermentation balance, and (ii) higher ratio to alcohols and therefore lower ratio to CO_2 in C distribution among products than those expected from Embden-Myerhof-Parnas pathway, which led to propose a concept of CO_2-ethanol conversion system (CDECS).

In this study, previous data are evaluated in terms of H recovery and results obtained are presented as follows: C recovery 105% was well balanced, while H recovery was as high as 139%, which means additional gain of much H through fermentation. The above results were almost reproduced by a set of experiments carried out in the same month, October, next year, while another set of experiments done in June gave ordinary fermentation results with C and H recoveries. Further analyses of these data elucidated that BD is equal to ethanol in position as a product from CDECS, which led to revise the CDECS concept to a CO_2-alcohol conversion system (CDACS). The conversion factor and process of CO_2 to alcohols will be discussed in relation to the cultivation conditions employed by chance.

POSTER PRESENTATION 5-15

Withdrawn

POSTER PRESENTATION 5-16

Continuous Ethanol Fermentation from Acid Hydrolyzed Corn Stover by Extremely Thermophilic Anaerobic Bacteria

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Corn stover is an attractive, abundant and renewable feedstock for production of bioethanol as an alternative to transportation fuels. Corn stover can be pretreated by weak acid hydrolysis to fermentable sugars, predominantly xylose and glucose. The use of acid hydrolyzed corn stover (PCS) as a feedstock for bioethanol production is limited by presence of inhibitory compounds released during the pretreatment affecting the fermentation. Industrial scale ethanol production from corn stover will demand that both glucose as well as xylose are converted. Anaerobic thermophilic bacteria have been considered for ethanol production, predominantly because of their abilities naturally to ferment the whole diversity of sugars found in lignocellulosic biomass.

This study deals with the prospect of using thermophilic anaerobic bacteria for bioethanol production from PCS. Fermentability and inhibitory effect of PCS was investigated in a lab-scale continuous reactor operated at 70°C. The results have demonstrated substrate concentrations of PCS up to 15% g-TS/L was possible without inhibition of sugar utilization and ethanol production. Both xylose and glucose sugars were simultaneously and effectively converted to ethanol. The sugar utilization was higher than 95%, and an ethanol yield of approximate 0.34 g/g was achieved.

POSTER PRESENTATION 5-17

Natural Cashew Apple Juice as Fermentation Medium for Hyaluronic Acid Production by *Streptococcus zooepidemicus*

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Hyaluronic acid (HA) is a polysaccharide which exists in many connective tissues fulfilling functions such as flexibility and structure maintenance. In bacteria, HA forms a extracellular capsulae in streptococci culture. HA has been used in cosmetic, pharmaceutical and medical areas due to its physico-chemical properties and biocompatibility. In general, culture media for production of HA by *Streptococcus zooepidemicus* are expensive due to the presence of various aminoacids and vitamins. Furthermore, innoculum is prepared using Brain Heart Infusion (BHI medium) which may add immunogenic characteristics to the product. This work investigated the performance of cashew apple juice as medium for innoculum preparation and fermentation. Similar levels of cell mass were obtained in innoculum when cashew apple juice supplemented with yeast extract was used, as compared with BHI medium. High concentration of HA, 17,60g/L, was obtained in a batch fermentation with 2vvm aeration when the same natural medium was used. The product was viscous but not viscoelastic. These results show the feasibility for production of HA using cashew juice. Furthermore, it is a promising process that offers the advantage of using an agricultural residue generated in large amount in Brazil (cashew juice) in order to obtain a high added value product.

POSTER PRESENTATION 5-18

A Genetic Transformation System Accommodating the Alternative Genetic Code of *Pichia stipitis*

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The yeast, *Pichia stipitis* ferments xylose at a higher rate and with better yields than any other known native microbe. A genetic system that employs the auxotrophic markers *URA3* and *LEU2* has distinct limitations on how many genetic manipulations can be performed. This yeast also will resist high levels of most common antibiotics used for selection in eukaryotic protests. Conspicuously, no drug selection marker has been shown to work reliably in this yeast. Xylose fermentation is essential for a productive conversion of biomass into ethanol. We discovered that *P. stipitis* uses an alternative genetic code that substitutes serine for leucine when CUG is coded in the mRNA. To get around this problem, we have modified the *Streptoalloteichus hindustanus ble* gene that confers resistance for Zeocin. In addition we cloned the homologous promoter of *Sc ETF1*, a strong constitutive promoter in *Saccharomyces cerevisiae* and used to drive expression of *Sh ble* gene. Finally, we adapted the Cre Recombinase - *LoxP* system and showed we can disrupt a gene with *PsURA3* and recover the marker for future use. These tools together with the recently completed *Pichia stipitis* genome will facilitate rapid genetic development of this versatile xylose fermenting yeast. These tools could also be used for the rapid genetic manipulation of *Candida albicans*, *Debaromyces hansenii*, and other yeasts that use the alternative nuclear genetic code.

POSTER PRESENTATION 5-19

Detailed Analysis of Modifications in Lignin after Treatment with Cultures Screened for Lignin Depolymerizing Agents

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Lignin, due to its intimate association with cellulose and hemicellulose, forms a barrier to enzymatic attack. Termites, beetles and other arthropods can digest decaying wood and other lignocellulosic plant litter. Lignin-degrading filamentous bacteria have been isolated from the termite gut. Bacterial lignin degradation has also been reported to be more specific than with fungal systems, an advantage, leading to many industrial applications like vanillin, adhesives, binder for laminated or composite wood products, etc.

Screening of different inoculum sources for lignin depolymerization was conducted. Sources selected were cultures obtained after dissection of guts of various insects like termites, beetles etc. that are known to digest wood. Other sources included cow rumen/dung, deer dung, extremophiles from Yellowstone National Park, and soils high in lignin content. Simultaneous fermentations with *Geotrichum Klebahnii* (Slavikova, 2001), a yeast-like strain, *Trametes cingulata* and *Phanerochaete chrysosporium* from ATCC were used as references for their documented ability to depolymerize lignin. Detailed analysis with Near Infrared Spectroscopy (NIR) and Atomic Force Microscopy (AFM) along with HPLC (UV detector) were conducted for the sources showing potential for lignin degradation. *Spread plate techniques, gram staining and Biolog were used for isolation and identification of the cultures showing potential.*

POSTER PRESENTATION 5-20

Optimization of L (+)-Lactic Acid Production Using Pellet-Form *Rhizopus oryzae* NRRL 395

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Lactic acid is used as a food additive for flavor and preservation and in the development of poly-lactic acid, a product used to make biodegradable plastics and textiles. *Rhizopus oryzae* NRRL395 is known to be a strain producing optically pure L (+)-lactic acid. The morphology of *Rhizopus* cultures is complex by forming filamentous, clumps and pellet mycelia. Different morphology growth has significant effects on the lactic acid production. In bioreactors, the filamentous or clump mycelia increase the viscosity of the medium, wrap around impellers, block the nutrient transportation, leading to a decrease in production efficiency and bioreactor performance. Growing fungi in pellet form can overcome these problems. The factors that affect the lactic acid production using *Rhizopus oryzae* NRRL395 with pellet form in flask cultures were investigated in detail in this work. CaCO3 addition should keep in certain level since too much extra CaCO3 would decrease the final lactic acid yield. A completely randomized design (CRD) with a three-way treatment structures was used to determine the influence of culture temperature, time and concentration of substrate. A second order (quadratic) polynomial model was used to characterize lactate concentration as a function of glucose concentration, fermentation temperature and time. Inoculum size was also studied at the optimal condition gotten from the model. Finally, lactic acid fermentation was performed in both 1L and 5L fomenters at the optimal values obtained from flask culture using both glucose and cull potato hydrolysate as raw material. The data presented in the paper can provide useful information on optimizing lactic acid production using alternative source materials.

POSTER PRESENTATION 5-21

The Influence of Oxygen Transfer on Production and Rheological Properties of Hyaluronic Acid Produced by *Streptococcus*

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Hyaluronic acid (HA) is a linear high molecular weight glycosaminoglucan polysaccharide composed by D-glucuronic acid e N-acetylglucosamine. HA holds a great potential of medical and cosmetic applications, due to its ability to retain large volumes of water and its rheological properties. The oxygen transfer plays an important role on production and rheological properties of hyaluronic acid when *Streptococcus zooepidemicus* are cultivated in submerged fermentation. This work investigated the mass and molecular weight of produced HA, as well as their viscous and viscoelastic properties, when 150, 250 and 400rpm agitation and 0.5 and 2vvm flow rates were used along fermentation. In addition, aeration was also cut off at the medium or at the end time of the exponential phase of growth and their effects investigated. The oxygen transfer was characterized by the mass transfer coefficient k_La determined by dynamic method. The experimental results showed a lower HA concentration 3.31g/L but a maximum molecular weight $3,2x10^6$Da and viscoelastic modulus 69,7Pa when aeration and agitation were 2vvm and 150rpm respectively. No viscoelastic HA was obtained at 400rpm, in spite of the higher produced concentration 12g/L. The results of aeration cut off on the production and rheological properties of HA, are also described.

POSTER PRESENTATION 5-22

Bioelectrochemical Denitrification Using Biocatalyst and Mediator

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Biological denitrification among various denitrification methods is very attractive because of economical and environmental advantages. However, carbon source feeding is required to maintain biological activity in biological denitrification. Permeabilized cells were used for the achievement of denitrification without feeding carbon source. Permeabilized *Ochrobactrum anthropi* SY509 containing denitrifying enzymes; nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase was used in this study. When using the permeabilized cell, cofactor is required for biocatalytic activity because the denitrification is accomplished by electron transfer. In this study, mediator was used for electron transfer to the enzyme and the denitrification was therefore performed by bioelectrochemical method. Carbon nanopowder was selected as a support material to immobilize the mediator, neutral red, because mediator molecule itself is difficult to immobilize on the electrode. Carbon nanopowder has advantages of large surface area and good conductivity. Neutral red was immobilized by two methods, with and without a linker. When both the permeabilized cells and the carbon nanopowder linked indirectly with the mediator were immobilized on the carbon felt electrode, high denitrification efficienty was obtained.

POSTER PRESENTATION 5-23

Homo-Ethanol Fermentation by a Novel Mutant of *Escherichia coli*

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We have previously described an ethanologenic *Escherichia coli*, strain KO11, that ferments all the sugars in biomass to ethanol, carrying the pyruvate decarboxylase and alcohol dehydrogenase genes from *Zymomonas mobilis*. As a continuation of these studies, a non-recombinant *E. coli*, strain SE2378, that also ferments both glucose and xylose to ethanol was constructed by introducing appropriate mutations in the various enzymes responsible for the metabolism of pyruvate. Strain SE2378 was derived from a double mutant, AH242, that lacks both lactate dehydrogenase and pyruvate formate lyase, the two enzymes that are responsible for production of lactate and acetyl-CoA (further metabolized to acetate and ethanol) during the mixed-acid fermentation of sugars by wild type *E. coli*. Strain AH242 (Δldh, Δpfl) is anaerobic minus due to its inability to reoxidize NADH generated during glycolysis. A mutant derivative of this double mutant that can grow anaerobically was obtained after mutagenesis and this mutant, strain SE2378, produced ethanol as the major fermentation product at a yield of 85% (mol/mol) from glucose and 79% (mol/mol) from xylose. Two mutations were mapped in pdhR, the gene that controls the expression of the genes encoding pyruvate dehydrogenase. Apparently, the mutant PdhR supported the production of active pyruvate dehydrogenase (PDH) complex in the anaerobic cell and the acetyl-CoA produced by the PDH was reduced by the native alcohol dehydrogenase to ethanol.

POSTER PRESENTATION 5-24

A Kinetic Model for Lipase Production by *Penicillium restrictum*

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In this work it is presented a simple mathematical model for the production of lipase as a function of the kinetics of growth and of the substrate consumption; taking into consideration the interaction of this enzyme with the protease, which affects directly the stability of the lipase. The death phase is not considered in this model.

Experimental data of growth, of production of lipase and protease are used, which were obtained at the Federal University of Rio de Janeiro. It is used *Penicillium restrictum* isolated from the wastes of a Brazilian babassu coconut oil industry.

The production of lipase was modeled by resolving the differential equations formulated for the microbial growth, for the substrate consumption, for the production of lipase and protease. The model is fitted to the experimental data through the least square method by using an optimization algorithm.

The fitting of the model to the experimental data, measured through the correlation coefficient, is very close to 1.00.

POSTER PRESENTATION 5-25

Butyric Acid and Hydrogen Production by Metabolically Engineered Mutants of *Clostridium tyrobutyricum*

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Clostridium tyrobutyricum is an anaerobe producing butyrate, acetate, H_2 and CO_2. In this study, metabolically engineered mutants were developed by inactivation of *ack* and *pta* genes, which impaired PTA-AK pathway and increased butyric acid production. Compared with the wild type, the mutants gave higher butyrate yield (>0.4 g/g vs. 0.34 g/g) and final concentration (43 g/L vs. 29 g/L), had higher tolerance to butyric acid inhibition, and produced more hydrogen (2.61 vs. 1.35 mol/mol glucose). Fed-batch fermentations with cells immobilized in a fibrous-bed bioreactors (FBB) further improved butyric acid and hydrogen production from sugars, including glucose, xylose, and fructose. Through adaptation in the FBB, a high butyric acid concentration of ~80 g/L was obtained at pH 6.3. This concentration is the highest ever attained in butyric acid fermentation. Metabolic flux analysis showed that the global metabolic flux distributions were altered in the mutant. These results suggested that enhancements in butyric acid and hydrogen production from sugars by *C. tyrobutyricum* can be achieved by metabolic engineering and cell adaptation in the fibrous-bed bioreactor. The increased productivity by *C. tyrobutyricum* mutants should lower the fermentation cost of biobased butyrate and allow it to compete more favorably in the chemical market.

POSTER PRESENTATION 5-26

Estimation of Oxygen Transfer in Solid-State Cultivation by *Drechslera (Helmninthosporium) monoceras* Using a Packed-Bed Bioreactor

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Solid-state cultivation (SSC) is generally defined as the growth of microorganisms on solid substrates with a continuous gas phase and no free-flowing water. In recent years, a resurgence of interest concerning SSC processes have been observed due to their numerous economic advantages over submerged cultivation. However, studies on the oxygen transfer and performance of bioreactors still are scarce. The aim of this research was to study the influence of bed height, air flow-rate and particle size on the oxygen transfer for the production of immunotherapics proteins from biomass of *Drechslera* (*Helminthosporium) monoceras.* The experiments were carried out in a packed bed bioreactor (180 mm height and 30mm diameter) at 25°C, pH 9,5, inoculum 0,4 mg/mL, initial moisture 45,8%, average particle size 0,59 and 0,35 mm, air flow-rates (0,2 – 1,0 L/min) and the bed heights (30, 80, 130 and 180 mm). The results show there are optimized levels of variables for which the initial moisture is approximately constant along fermentation and the k_La coefficient and biomass growth are maximized. In these experiments the higher k_La was 9,85 s^{-1} for 30 mm bed height, air flow-rate 0,4 L/min and particle size 0,35 mm. These results are important for the project and analysis of bioreactors for SSC.

POSTER PRESENTATION 5-27

Gibberellic Acid Production by Submerged Fermentation Using *Gibberella fujikuroi*

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Gibberellic acid represents one the most important plant growth regulators. Its production has been widely studied with different strains of *Gibberella fujikuroi* in different culture media. At present, the production of gibberellic acid by submerged fermentation with the filamentous fungi *Gibberella fujikuroi* can be a practical alternative to increase the production of vegetal biomass for the production of more and better foods, fuels and chemicals.

In this work, we present the preliminary results on optimization of the gibberellic acid production cells of two strains of *Gibberella fujikuroi* : NRRL-2278 and NRRL-2284. We use a complex culture medium in submerged batch fermentations. We try to optimize the culture conditions in batch reactors by using a method of surfaces response for the production of the plant regulator. Using this statistic method we try to select the most suitable culture medium and fermentation conditions for the production of gibberellic acid using these two strains of *Gibberella fujikuroi*.

POSTER PRESENTATION 5-28

Withdrawn

POSTER PRESENTATION 5-29

Proteome Analysis of the Xylose-Fermenting *Saccharomyces cerevisiae* Strain TMB 3400

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Saccharomyces cerevisiae is unable to naturally utilize the pentose sugar xylose, which is abundant in lignocellulose hydrolysates used for biofuel ethanol production. Heterologous expression of *Pichia stipitis* genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) enables xylose utilisation in *S. cerevisiae*, however at low rates. In a previous study, an industrial *S. cerevisiae* strain TMB3399 carrying the heterologous XR and XDH genes has been subjected to random mutagenesis in order to improve the xylose utilization rate (Wahlbom et al. 2003 FEMS Yeast Res 3: 319-326)**.** The resulting strain TMB 3400 grew and fermented xylose better than the parental strain TMB 3399. In order to find the key elements of the improved phenotype of TMB 3400, analyses at the transcript level have first been carried out (Wahlbom et al. 2003 Appl Env Microbiol 69: 740-746). In this study, results from a proteome analysis will be presented and compared with the previous transcriptome analysis.

POSTER PRESENTATION 5-30

Ethanol Fermentation by Overexpressing a Gram-Positive PDC Gene in *Lactobacillus plantarum* Strain TF103

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The objective of the current research is to convert the lactic acid fermentation capacities of select strains of lactic acid bacteria (LAB) into that of ethanol production. *Lactobacillus plantarum* ferments glucose to pyruvate through the Embden-Meyerhof-Parnas pathway, and pyruvate is then converted into lactate via lactate dehydrogenase (LDH). By substituting LDH with pyruvate decarboxylase (PDC) activity, pyruvate may be redirected away from lactic acid and toward ethanol. A pyruvate decarboxylase gene from the Gram-positive bacterium *Sarcina ventriculi* (*Spdc*) was introduced into a LDH deficient strain *L. plantarum* TF103 in which both *ldhL* and *ldhD* genes were inactivated. Four different fusion constructs between *Spdc* and either the *S. ventriculi* promoter or the *Lactococcus lactis* promoter in pTRKH$_2$ were introduced into TF103. PDC activity was detected in all four recombinant strains. The engineered strains were examined for ethanol and other metabolite products in flask fermentations. The recombinant strains grew slowly and produced 90-170 mM ethanol. An alternative approach to facilitate growth and increase ethanol production might be to insert *pdc* and inactivate *ldh* simultaneously by direct gene replacement.

POSTER PRESENTATION 5-31

Insoluble Exopolysaccharide Production of *Agrobacterium sp* (ATCC 31749 and IFO 13140)

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Determined bacteria of *Agrobacterium* genus, non-pathogenic and isolated from soil samples, are producers of two extracellular polysaccharides: acid and soluble succinoglucan, and the gum, curdlan, a neutral and insoluble polymer. The latter was approved by the Food and Drug Administration (FDA) in 1996, and has been used in the food industry, because of its capacity to form an excellent firm and resistant gel.

Corn glucose, cassava (or mandioca) glucose and corn maltose were used in fermentation medium for insoluble polysaccharide production as substitutes for the source of the carbon in the medium proposed in the literature. The two lineages of *Agrobacterium* sp used (ATCC 31749 and IFO 13140) in the production of insoluble exopolysaccharide presented equal or higher production compared to those cited in the literature (which were approximately 50.00%). The lineage ATCC 31749 presented higher production when used with corn maltose, with a production of 84.68%, while the lineage IFO 13140 showed higher production using corn glucose, with a production of 50.00% (in consequence of sugar reducers).

POSTER PRESENTATION 5-32

Co-Fermentation of Corn Stover Hydrolyzates for Bioethanol Production by Coimmobilized Cells of *Saccharomyces cerevisiae* and *Pichia stipitis* Adapted to Microbial Inhibitors

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Utilization of both biomass cellulose and hemicellulose for bio-ethanol production is an ambitious goal that would make the process more convenient. Besides the difficulty of converting xylose (the major hemicellulose component), the microbial inhibition of pretreatment by-products is another critical step that still needs effective solutions. Chemical detoxification has been widely used to remove toxic compounds. The main limit of this approach lies in the increase of the process costs and in the generation of wastes. *In situ* biological detoxification could turn useful only when the hydrolyzate is slightly inhibiting otherwise metabolism repression of yeast is likely to occur. Cells adaptation to toxic media before inoculation represents a valuable option although it has been poor practised because it is still unclear how it occurs.

The purpose of this work was the cofermentation of un-detoxified hydrolyzates of corn stover by using co-cultures of inhibitors-adapted *Saccharomyes cerevisiae* and *Pichia. stipitis.* Enzymatic hydrolyzates from steam-pretreated corn stover were fermented by using coimmobilized yeast cells in alginate beads.

Of known inhibitors in the enzymatic hydrolyzates from steam exploded corn stover, acetic acid, furfural, 5-hydroxymethyl furfural (5-HMF), and syringaldehyde have the highest concentrations (3g/L, 1.7 g/L, 0.3 g/L and 0.2 g/L respectively). The experimental procedure of adaptation provided the sequential transfer of yeast cells in medium prepared with increasing concentrations of the degradation by-products. The adaptation media were prepared by using the water-soluble fraction of steam exploded corn stover that includes degradation by-products generated by the thermal pretreatment.

The results indicated that both yeasts adapted to the toxic compounds being able to grow and ferment when inoculated in un-detoxified hydrolyzates. In particular *S. cerevisiae* revealed stronger adaptation capability than *P. stipitis*.

POSTER PRESENTATION 5-33

The Role of the Pentose Phosphate Pathway in Fermentation Inhibitor Tolerance

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Acid hydrolysis pretreatment of lignocellulose biomass releases sugars (glucose, xylose, etc.) for industrial ethanol fermentation. During fermentation, degradation products of xylose and glucose can form inhibitory products, furfural and 5-hydroxymethylfurfural (HMF), respectively. At high concentrations these inhibitors inhibit cell growth and reduce ethanol yield. Engineering yeast to be more tolerant of these inhibitors will lead to a more efficient lignocellulose to ethanol bioconversion. Recently, the pentose phosphate pathway (PPP) was implicated in furfural, HMF, and ethanol tolerance. The PPP contains nine genes, *ZWF1*, *GND1*, *GND2*, *RPE1*, *RKI1*, *TKL1*, *TKL2*, *TAL1*, and *YGR043C* (*TAL2*). Strains lacking *ZWF1*, *GND1*, *RPE1*, or *TKL1* have severe growth defects in the presence of furfural. In the presence of HMF or ethanol, these mutants have noticeable growth defects but less severe compared to furfural. These mutants are further characterized in regards to their effects on cellular physiology. In addition, the individual effect of overexpression of all nine PPP genes is also characterized in regards to growth, inhibitor tolerance, and ethanol yield using both standard lab medium and lignocellulosic derived medium.

POSTER PRESENTATION 5-34

Production of Lactic Acid from Glucose and Xylose by Genetically Modified *Saccharomyces cerevisiae*

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Cellulosic biomass is the largest renewable resource in the world and an ideal raw material for the production of chemicals by microbial processes. However, cellulosic biomass contains large amounts of xylose in addition to glucose. This limits the use of the naturally occurring *Saccharomyces* yeasts to convert cellulosic sugars to ethanol or other industrial products. In recent years, we have succeeded in using recombinant DNA techniques to modify the *Saccharomyces* yeasts to effectively metabolize xylose. This was accomplished by cloning and overexpressing three major xylose-metabolizing genes – xylose reductase, xylitol dehydrogenase, and xylulokinase genes – in yeast and by modifying the control mechanisms present in microbial cells. As a result, the metabolically engineered yeast can co-utilize glucose and xylose efficiently. We have further modified the recombinant *Saccharomyces* yeast to produce lactic acid and the resulting yeast produces lactic acid effectively from glucose, xylose, or a mixture of both. The results presented here serve as examples demonstrating that the safe, user-friendly *Saccharomyces* yeasts can be engineered to efficiently use cellulosic sugars to produce various important industrial products.

POSTER PRESENTATION 5-35

Thin, Multi-Layer Latex Coating Photobioreactors for Investigation of Optimal Light Adsorption and Hydrogen Evolution Using Non-Growing *Rhodopseudomonas palustris* Nitrogenase Mutants

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Rhodopseudomonas palustris CGA009 is an excellent model genetic system for optimization of the rate and efficiency of photosynthetic hydrogen production from organic substrates using nitrogenase. *R. palustris* contains three functional nitrogenase enzymes, a nonfunctional uptake hydrogenase, and can utilize waste carbohydrates, organic acids, and aromatic compounds which could be derived from lignin. Many of the obstacles to large-scale biohydrogen production may be overcome by using this organism under non-growth conditions in thin, porous, multilayer translucent latex coating photobioreactors. The multilayer approach could also be used to increase photosynthetic efficiency by overcoming light saturation by use of antenna pigment and light harvesting complex mutants in different layers. The H_2 evolution rate of *R. palustris* CGA009 from acetate in 40µm thick porous latex coatings (argon atm) is 3.0-3.4mmol H_2 m^{-2} h^{-1}. Hydrogen evolution from acetate is a function of light intensity, is continuous for over two weeks, and H_2 production activity is sustained after storage at -80°C. H_2 production rates have been measured for latex entrapped single nitrogenase expressing mutants (Nif+, Vnf+, Anf+) showing that some nitrogenases are more active in coatings than others. Altering the expression of Nif, Vnf, or Anf in order to increase coating specific activity, half-life, and the fate of substrate carbon (stored as PHAs or evolved as CO_2) are being investigated.

POSTER PRESENTATION 5-36

Development of a New Bioprocess for Organic Acid Production Using *Corynebacterium glutamicum*

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Under oxygen deprivation, aerobic *Corynebacterium glutamicum* produces organic acids from glucose at high yields in mineral medium even though cell proliferation is arrested. To develop a new, high productivity bioprocess based on these unique features, characteristics of organic acid production and the metabolic pathway of *C. glutamicum* under oxygen deprivation were investigated.

The main organic acids produced from glucose under these conditions were lactic and succinic acids. Addition of small amounts of pyruvic acid decreased intracellular NADH/NAD^+ ratio, leading to increased glucose consumption rate and organic acid production rate. The two rates were also increased in the presence of bicarbonate, which is a co-substrate for anaplerotic enzymes. With increasing bicarbonate concentration, the yield of succinic acid increased whereas that of lactic acid decreased.

To investigate the metabolic pathways of *C. glutamicum* under oxygen deprivation, select genes were inactivated by gene disruption and replacement. The uniqueness of the lactate dehydrogenase gene (*ldhA*) was demonstrated by the inability of *ldhA* deficient mutants to produce lactic acid. Although a pyruvate carboxylase (*pyc*) mutant exhibited similar behavior to that of the wild type, phospho*enol*pyruvate carboxylase (*ppc*) mutants were characterized by a dramatic decrease in succinic acid production, which was concomitant to decreased lactic acid production and glucose consumption rates.

POSTER PRESENTATION 5-37

Comparison of Genome Structure and Global Gene Expression Profiles between *Zymomonas mobilis* Type Strain ZM1 and Highly Ethanologenic ZM4 Strain

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Whole genome microarrays were used for genomic comparisons with the *Z. mobilis* type strain ZM1 (ATCC10988) and highly ethanologenic ZM4 (ATCC31821) revealing that the ZM4 strain had 54 predicted ORFs absent from ZM1, encoding transport and secretory proteins, transcriptional regulators and oxidoreductase. Furthermore, most of the additional ORFs were found to be transcribed actively during ethanol production.

Global expression profiles of *Z. moblilis* ZM4 were also analyzed with the DNA microarray during the cell growth. At the early stage of growth, especially starting point of exponential growth stage, ribosomal proteins and translation elongation factors were highly expressed. On the contrary, at the late stage of exponential growth, ATP synthetase, alcohol dehydrogenase and heat shock proteins were actively expressed. These results imply that ethanol production, ATP synthesis and cell growth were tightly related to each other. The higher expression of heat shock proteins was due to the accumulation of ethanol of which concentration was more than 40g/L.

POSTER PRESENTATION 5-38

Characterization of Xylose Transporters in Xylitol-Producing Recombinant *Saccharomyces cerevisiae*

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Xylitol is a five-carbon sugar alcohol which has been used as a natural sweetener. In order to produce xylitol by bioconversion processes, xylose and cosubstrate should be supplied into the cell where xylose is converted to xylitol. Sugar transport is known as one of the rate-limiting steps in bioconversion of xylose to xylitol by recombinant *Saccharomyces cerevisiae* containing the *Pichia stipitis* xylose reductase (XR) gene. Sugar transporters HXT 1~6, AGT1 and STL1 genes were analyzed by introducing those genes into a mutant strain, which cannot take up xylose at all. Batch fermentations with each of the recombinant *S. cerevisiae* strains were performed to investigate effects of coexpression of sugar transporter genes on xylitol formation. Xylitol was produced in the ranges from 0.05 to 0.45 g/L·hr, suggesting that HXT 1~6, AGT1 and STL1 genes are involved in xylose transport. The STL1 gene which was characterized as a new xylose transporter was fused with green fluorescent protein (GFP). The confocal microscopic analysis showed that the STL1 gene was localized to the outer membrane of recombinant *S. cerevisiae*.

POSTER PRESENTATION 5-39

Rational Approaches for Construction of *Candida magnoliae* Proteome Map

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Candida magnoliae is an industrially important organism because of its ability to produce erythritol from glucose. Erythritol is a noncariogenic, low calorie sweetener and safe for diabetics. Proteomic analysis of *C. magnoliae* was performed to construct the proteome map and to investigate physiological differences between the wild and its mutant strain. The response to osmotic stress at a protein level was also. As the genome of *C. magnoliae* has not been sequenced yet, limiting the available proteome database, proteomic analysis with rational approaches has been done based on two-dimensional electrophoresis, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), tandem mass spectrometry (MS/MS) and database interrogation. First, 51 protein spots were analyzed and then potentially identified in the response to osmotic stress at a protein level. The identified proteins were involved in glycolysis, stress response, other essential metabolisms and cell structures. Second, 6 proteins overexpressed in the mutant strain compared with its wild type seemed to play a role in the improved bioconversion of erythrose-4-phosphate in the mutant strain.

POSTER PRESENTATION 5-40

Homolactic Production of Lactic Acid from Pentose Sugars Using Moderately Thermophilic Bacillus Species

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Lactic acid can be used as a substrate for the production of poly-lactic acid (PLA) based plastics. These fully biodegradable plastics are derived from renewable resources, such as corn, and are an attractive alternative for petroleum-based materials, both from an ecological and political perspective. The commercial success of PLA is however dependent on the availability of low cost lactic acid.

The commercial production of lactic acid is currently based on fermentation of glucose, starch or sucrose. These relatively expensive substrates are an important contributor to the manufacturing cost price of lactic acid. Lignocellulosic biomass offers a cost attractive alternative feedstock because it is readily available, has no competing food value and is less expensive than the above mentioned substrates.

Conversion of lignocellulose into lactic acid requires microorganisms that are able to ferment both hexose and pentose sugars. While several microorganisms can efficiently ferment the glucose component in cellulose, efficient conversion of the pentose fraction has proven more difficult. Many heterolactic lactic acid bacteria are able to ferment pentoses via the phosphoketolase pathway, but co-produce significant amounts of acetic acid, which makes this economically unattractive.

We have found that some naturally occurring moderately thermophilic Bacillus species are capable of homolactic production of lactic acid from pentose sugars.

POSTER PRESENTATION 5-41

Respiration-Restrictive *Agrobacterium tumefaciens* ATCC4452 Has Improved Coenzyme Q_{10} Contents

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For the production of Coenzyme Q_{10} (CoQ_{10}), an electron carrier in the respiration chain with antioxidant activity, we investigated effects of aeration and agitation rates, dissolved oxygen levels, an electron flux inhibitor of azide, a proton gradient releaser of 2,4-dinitrophenol (DNP), and an oxidative stressor of hydrogen peroxide (H_2O_2) on the intracellular CoQ_{10} contents in *Agrobacterium tumefaciens* ATCC4452. With decrease of dissolved oxygen level from 20 to 5%, the intracellular CoQ_{10} content increased about 4-fold, yielding 2 mg per g-dry cell weight at 5% dissolved oxygen level. Azide significantly increased the intracellular CoQ_{10} content, with the highest value of 5.3 mg per g dry cell weight in the presence of 0.45 mM of sodium azide. However, DNP (up to 200 μM) and H_2O_2 (up to 10 μM) did not affect the intracellular CoQ_{10} content, indicating proton gradient release and oxidative stress do not affect the synthesis of CoQ_{10}. These results show that restricted electron flux by limited oxygen supply and the addition of azide increases the intracellular CoQ_{10} content, suggesting a feedback regulation of CoQ_{10} biosynthesis by its physiological function of electron carrier in the respiration chain.

POSTER PRESENTATION 5-42

Cloning and Characterization of S-Adenosylmethionine Synthetase Gene from *Sacchaopolyspora erythraea* ATCC11635 and *Streptomyces avermitilis* NRRL8165

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S-Adenosylmethionine has an important role for DNA methylation and cell signaling. S-Adenosylmethionine synthetase catalyzes the formation of S-Adenosylmethionine fom L-methionine and ATP. The antibiotic of *St. avermitilis,* avermectin, is a potent antiparasitic compound with a broad spectrum of activity against nematode and arthropod parasites. The antibiotic of *S. eythaea,* erythromycin, is of medical and commercial importance. It is considered the archetype of macrolide antibiotic, as evidence from extensive studies of its production. Recently, additional effects of S-Adenosylmethionine were reported such as positive regulator fort production of secondary metabolites. To determine the function of S-Adenosylmethionine synthetase, the key enzyme to synthesize S-Adenosylmethionine, we cloned S-Adenosylmethionine synthetase from *S. erythraea* and *St. avermitilis*. Both S-Adenosylmethionine synthetase nucleotides size were 1.2kb and molecular weight of deduced amino acid sequence was calculated about 44kDa. At the molecular level, an extremely high identity(>95%)and homology(>95%)was observed with S-Adenosylmethionine synthetase of other *Streptomycetes*. For characterization, it was purified through affinity chromatography using Ni-NTA resin. We corroborated S-Adenosylmethionine synthetase activity by TLC and HPLC. We confirmed S-Adenosylmethionine synthetase gene from *S. erythraea* and *St. avermitilis.*

POSTER PRESENTATION 5-43

Avermectin Production by Optimization of Medium Composition from *Streptomyces avermitilis* NRRL815

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The goal of this research was the production of avermectin that is widely used for the treatment of diseases caused by nematodes and arthropods in the veterinary and agricultural fields, respectively. The avermectin family is composed of four major components (i.e., A1a, A2a, B1a, and B2a) and four minor components (i.e., A1b, A2b, B1b, and B2b), which are structurally very similar. Among them, avermectin B1 complex is the most efficient component.

In this study, we tried to optimize the composition of medium and culture conditions for the total avermectin and avermectin B1 production from *Streptomyces avermitilis*, which is a natural producer of the avermectin family. Among various carbon and organic nitrogen sources tested, fructose and malt extract were most effective on avermectin production. Next, addition of polyethylene glycol and K_2HPO_4 in the medium significantly improved the intracellular contents of avermectin. Thus the optimized medium composition was 50 g/L fructose, 30 g/L malt extract, 5 g/L casamino acid, 2.5 g/L PEG 3,350, and 1 g/L K_2HPO_4, which increased the avermectin production from 10 to 478 mg/L. The contents of avermectin B1 complex was about 50% of the total amount of avermectin. These results would be very useful for enhancing productivity of B1 in up-scaled processes.

POSTER PRESENTATION 5-44

Effects of Inhibitors Formed During the Pretreatment of Lignocellulosic Biomass on the Ethanol Yield

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The choice of feedstock and yield of alcohol per ton of raw material are two of the most important factors affecting the economy of alcohol production. Lignocellulosic materials represent an abundant, up to this time unexploited, feedstock for fuel ethanol production. Recalcitrance of lignocellulosics to degradation is one of the main processing problems. Pretreatment of lignocellulosic raw material is necessary to open up the structure and to increase accessibility to enzymatic attack, thereby improving the overall conversion to ethanol. During this process a range of sugar and lignin degradation compounds toxic to yeast are formed, which reduce the ethanol yield in the fermentation process. The spectrum of inhibitors and their amounts are determined by the raw material used and the pretreatment technique applied.

The aim of the present study was to investigate the conversion of spruce, willow and corn-stover to ethanol under similar conditions. Raw materials were first steam pretreated applying previously optimized methods for each lignocellulosic biomass. The slurry obtained after pretreatment was subjected to complete analysis. Thereafter, the liquid fraction, comprising the solubilized hemicellulose fraction and inhibitory compounds were separated from the solid, cellulose containing, fibrous fraction. Batch-wise alcoholic fermentation of this liquid supplemented with glucose equivalent to the amount of cellulose present in the slurry obtained in the pretreatment was carried out in stirred, temperature controlled flasks at low pH using a yeast strain resistant to typical inhibitors found in hemicellulose hyrolysates. During anaerobic fermentation, CO_2 evolution was continuously monitored, while the ethanol content was only measured at the end of the fermentation. Detailed results will be presented on the conference.

POSTER PRESENTATION 5-45

"From Innovation to Industrial Production" a Review on the Strategies and Factors that Led to the Successful Development of the Recombinant Yeast for Cellulosic Ethanol Production

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Since 1980, there has been a concerted effort to genetically engineer the *Saccharomyces* yeast or other microorganisms to ferment sugars from cellulosic biomass (agricultural residues and related plant-derived material) to ethanol for use as transportation fuel. It is known that more than 70% of cellulosic biomass can be converted to sugar molecules, which theoretically can be fermented to ethanol by microorganisms. However, the major sugars derived from cellulosic biomass include not only glucose but also xylose. The main problem is that the *Saccharomyces* yeast, traditionally used for the industrial production of ethanol, cannot ferment xylose. Furthermore, no other naturally occurring microbes can effectively ferment xylose either. The culmination of this work was the successful genetic engineering of the *Saccharomyces* yeast to effectively convert both glucose and xylose from cellulosic biomass to ethanol in a single process. This groundbreaking work is being done by a small group of researchers from Purdue University (West Lafayette Indiana, USA). Their yeast, known as the "Purdue yeast", can effectively convert both glucose and xylose from cellulosic biomass to ethanol. One of the Purdue yeasts, 424A(LNH-ST), is currently used by logen (www.iogen.ca), to produce cellulosic ethanol (also referred to as cellulose ethanol) from wheat straw in the world's first commercial cellulosic ethanol production facility. logen has acknowledged that the Purdue yeast is currently the most effective microorganism for the production of industrial scale cellulosic ethanol. In this presentation, we will examine the strategies we used to engineer the yeast and other factors that have made our Purdue recombinant yeast exceptional for the industrial production of ethanol. These might be useful for engineering yeast or other industrial microorganisms to enhance their performance in the production of other industrial products.

POSTER PRESENTATION 5-46

Improvement of Ethanol Production by *Pichia stipitis* in High-Xylose-Content Liquor Obtained for Sugarcane Bagasse Hydrolyses

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The sugarcane bagasse is a material produced in great amount in Brazil, mainly as a residue of sugar and ethanol production, and represents an important source of sugars such as xylose from hemicellulose and glucose from cellulose. A high-xylose-content liquor (50 gr/L) was obtained by chemical hydrolysis with diluted sulfuric acid solution. This work aims at defining a strategy of cell acclimatization for improving ethanol productivity in the fermentation with *Pichia stipitis*, as well as the investigation of the effect of medium components in the process performance.

Experiments were carried out in 500mL shaken flasks and in a 2L-bioreactor (BIOSTAT B- B. Braun Biotech International). The process variables such as glucose, xylose, xylitol and ethanol were measured by HPLC, and cell by optical density in 570nm.

The previous cell exposure to non-detoxificated hydrolisate, twice before fermentation, resulted in an increase of ethanol concentration from 7.4 to 20.0 g/L and a reduction in fermentation times from 57 to 38 hours. Additionally, the influence of medium components was investigated, applying a factorial experimental design. The results showed that nitrogen sources such as yeast extract and urea play an important role on fermentation performance and KH_2PO_4 displayed a negative effect.

POSTER PRESENTATION 5-47

Evidence for Enzyme-Microbe Synergy in Cellulose Utilization by *Clostridium thermocellum*

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The phenomenon of synergy between components of cellulase enzyme systems, whereby the rate realized by two or more components in combination is greater than the sum of the rates observed when the components act separately, has frequently been observed and evaluated in the literature. A different type of synergy involves enhancement of the effectiveness of cellulase by virtue of expression on the surface of a microbial cell as compared to when the cellulase is not cell bound. In this presentation, such "enzyme-microbe" synergy is quantitatively evaluated for the first time.

Microbially-mediated cellulose hydrolysis by *Clostridium thermocellum*was systematically compared to enzymatically-mediated hydrolysis carried out by purified *C. thermocellum* cellulosomes in the presence and absence of *Thermoanaerobacterium thermosaccharolyticum,* a non-cellulolytic thermophile capable of utilizing soluble products of cellulose hydrolysis. It may be noted that the ternary cellulose-enzyme-microbe complexes are present in the case of microbially-mediated hydrolysis whereas cellulose hydrolysis occurs exclusively because of the action of binary cellulose-enzyme complexes in the case of enzymatically-mediated hydrolysis.

In batch culture under controlled conditions, it was found that Avicel was completely consumed within 16 hours by the *C. thermocellum* culture (microbial hydrolysis) as compared to about 32 hours for purified cellulosome in the presence or absence of *T. thermosaccharolyticum* (enzymatic hydrolysis). The amount of cellulase present throughout enzymatic hydrolysis was equal to the final amount present, and twice the initial amount present, for microbial hydrolysis. Thus, batch results support a degree of enzyme-microbe synergy, equal to ratio of the cellulosome-normalized hydrolysis rates observed for the microbial system divided by that for the enzymatic system, of between 2 and 4. Data for comparison of microbial and enzymatic hydrolysis rates in steady-state continuous cultures will also be presented, and the potential significance of enzyme-microbe synergy will be considered in fundamental and applied contexts.

POSTER PRESENTATION 5-48

Whole Genome Transcription Analysis of Yeast Stress Response During Very High Gravity Ethanol Fermentation

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Very high gravity (VHG; >30% dry substance) ethanol fermentations using whole ground corn as a raw material are carried out in the fuel ethanol industry using Simultaneous Saccharification and Fermentation (SSF). During VHG SSF the added enzymes rapidly produce glucose and maltose resulting in accumulated concentrations imposing osmotic stress to the yeast. Later the yeast biomass has increased to a concentration where it utilizes the sugars faster than the enzymes produce them, thus reducing the sugar concentrations. At this point the ethanol concentration has risen to stressing levels. During the first phase biomass grows exponentially, later it enters a long lasting stationary phase, during which half of the total ethanol is produced. Two strains of *Saccharomyces cerevisiae* have been employed in this study: A laboratory strain and an industrial strain. The strains were grown anaerobically in a standard laboratory medium containing 20 g/L glucose and in a VHG medium mimicking the industrial corn substrate containing 280 g/L maltodextrin and operated as SSF. For both strains whole genome transcription analysis using Affymetrix DNA array chips were performed on samples taken at both early and late phase VHG SSF and in standard mineral medium.

POSTER PRESENTATION 5-49

Biosurfactant Production by *Rhodococcus erythropolis* Growing on Glycerol as Carbon Source

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Biosurfactants are surface-active compounds of microbial origin with detergency, emulsification, de-emulsification, foaming and wetting properties. Biosurfactants have special advantages over their chemically manufactured counterparts because of their lower toxicity, their biodegradable nature and effectiveness at extreme temperature, pH and salinity. Therefore, they can be produced from renewable substrates. Microbial surfactants have potential applications in petrochemical industry to enhance oil recovery and for hydrocarbon bioremediation.

In the present work, the production of biosurfactant synthesised by *Rhodococcus erythropolis* was studied, during growth on hydrophilic substrates. The process was carried out at 28°C in a 1,5L bioreactor using glycerol as carbon source and $NaNO_3$ and $(NH_4)_2SO_4$ as nitrogen sources. The aeration was adjusted to 30% of dissolved oxygen and the pH was maintained at 7.0 by automatic addition of acid and base solutions. The bioprocess was monitored through measurements of biosurfactant concentration, glycerol and nitrate consumption. After 72 hours of cultivation, 1,5 g/L of biosurfactant, surface and interfacial tensions values (with hexadecane) of 33 and 5.5 mN/m, respectively, and 67% of emulsifying index (E_{24}) for a hexadecane-water binary system were obtained. The use of glycerol, rather than what happens with hydrophobic carbon source, allowed spontaneous release of biosurfactant, originally associated to cell wall.

POSTER PRESENTATION 5-50

Developing Viable Cereal-Based Biorefineries

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Creating cost-competitive cereal-based biorefineries as substitutes for petroleum refineries would require significant improvements in current cereal processing practices. More efficient processing of cereal kernels for the production of a spectrum of value-added products is necessary. In addition, current cereal-based biorefineries do not exploit the full potential of cereal crops for the production of highly efficient bioconversion feedstocks. This paper will present on-going research incentives in the Satake Centre for Grain Process Engineering aiming at the improvement of cereal processing and microbial feedstock production. Cereal kernels are initially processed by an advanced separation technology, pearling, that separates sequentially the outer layers of cereal grains. These layers can be used in various applications depending on the cereal grain. Enhancement of bioconversion yields is achieved by producing nutrient supplements, rich in amino acids, longer peptides, sources of phosphorus, minerals, vitamins and glucose, by exploiting the natural process of fungal autolysis. Any future biorefinery would be dependent on fungal bioconversions to produce a range of hydrolytic enzymes (e.g. amylase, protease, cellulase) that are required to hydrolyse natural macromolecules contained in cereals into directly assimilable micronutrients. On-site production of these enzymes would result in the production of a high amount of fungal biomass. Fungal autolysis can be initiated under oxygen-limited conditions and the resulting fungal extract could provide an inexpensive substitute for yeast extracts. The importance of fungal extracts in microbial bioconversions will be demonstrated in the production of the biodegradable bioplastic, poly-hydroxybutyrate (PHB), by *Ralstonia eutropha*.

POSTER PRESENTATION 5-51

Metabolic Engineering to Improve Ethanol Production in the Xylose Utilizing Thermophile *Thermoanaerobacterium saccharolyticum* JW/SL-YS485

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Thermoanaerobacterium saccharolyticum JW/SL-YS485 is a gram positive, thermophilic, obligate anaerobe that utilizes xylose and xylan but not cellulose, grows at temperatures from 30°C to 66°C, and produces ethanol, acetate, and lactate as fermentation products. This organism is of potential interest as a biocatalyst because its high growth temperature and ability to grow at low pH (3.9 to 6.3) may allow reduced cellulase loadings in processing featuring a simultaneous saccharification and fermentation configuration. In addition, this thermophilic, pentose-utilizing microorganism could also be used in a consolidated bioprocessing configuration, with no added saccharolytic enzymes, in conjunction with *Clostridium thermocellum*.

Using recently-established gene transfer techniques (*Tyurin* et al., Appl. Environ. Microbiol., 2004 and unpublished), we report development of a strain of *T. saccharolyticum* in which the lactic acid dehydrogenase (*ldh*) gene has been knocked out, and the further creation of a second strain in which the acetate kinase/phosphotransacetylase (*ack/pta*) has been knocked out. For both strains, gene knockout resulted in undetectable production of the targeted organic acids. The ldh^- strain exhibited a growth rate and cell yield comparable to the wild-type. The ack^-/pta^- strain initially did not grow well and did not completely utilized the substrate present. However, complete substrate utilization and growth at rates comparable to the parent strain was realized after sequential batch transfer, apparently indicative of selection for strains with compensatory metabolic changes that alleviated imbalances present in the first-generation engineered strains. Efforts to develop strains in which both *ldh* and *ack/pta* are deleted will be described.

POSTER PRESENTATION 5-52

Induction of Pleiotropic Drug Resistance (PDR) Gene Expression Indicates Important Roles of PDR to Cope with Furfural and 5-Hydroxymethylfurfural Stress in Ethanologenic Yeast

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Furfural and 5-hydroxymethylfurfural (HMF) derived from dehydration of pentose and hexoses by dilute acid hydrolysis of lignocellulosic biomass are major inhibitive compounds for enzymatic saccharification and microbial fermentation in ethanol conversion. Mechanism of tolerance to furfural and HMF stress is unknown. Our interest lies on identification of relevant target genes and understanding of molecular mechanisms involved in the inhibitive stress tolerance in ethanologenic yeast. By means of functional analysis and comparative gene expression using microarray and quantitative real-time RT-PCR, we identified genes significantly enhanced in expression during the initial exposure to furfural and HMF stress. These genes belong to the pleiotropic drug resistance (PDR) gene family. Many PDR genes function as transporter of ATP-binding cassette proteins and are encoded for plasma membrane proteins. These genes mediate membrane translocation of ions and a wide range of substrates. This presentation will cover methods of our well-established quality-controlled microarray and qRT-PCR technologies and convincing evidence of yeast gene expression in the earlier stage to cope with the furfural and HMF stress.

POSTER PRESENTATION 5-53

Effect of Mixing in Anaerobic Digestor on Conversion of Farm Waste to Methane in 100L Upflow Bioreactor

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Conversion of manure waste from dairy farms has been used for methane production for decades, however problems such as digestor failure are routine. This has been investigated in small scale digestors previously, however a larger scale detailed study has not been reported yet. We report production of methane in 100L digestor and the results of investigation of the effect of partial mixing induced by gas upflow/recirculation in the digestor. The digestor was run for over 45 days (three times the hydraulic retention time) with and without the mixing induced by gas upflow. The results show a dramatic effect of mixing on digestor operation. Without mixing, the digestor fails within 30 days, while with mixing continuous production of methane is observed. This study demonstrates the importance of mixing and its critical role in design of anaerobic digestors.

POSTER PRESENTATION 5-54

Removal of Mercury from Coal via Microbial Bioleaching

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A biological process for removal of mercury from coal is under investigation. Iron and sulfur oxidizing bacteria have previously been used for desulfurization of coal. We have shown that removal of mercury is also possible via the same principles. Two pure cultures, *Leptospirillium ferrooxidans* and *Acidothiobacillus ferrooxidans* and four environmental consortium samples obtained from acid mine drainage were studied for mercury removal from coal. Four different coal samples were examined. The results have shown that up to 20% of the mercury can be removed in batch cultures compared to control. Additional parameters such as media composition, pH and inoculum size were also studied. Preliminary results from a continuous system for mercury removal will also be presented.

POSTER PRESENTATION 5-55

Optimization of Pentose Fermentation in *Zymomonas mobilis* Through Kinetic Modeling and Experimental Analysis

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Zymomonas mobilis has been engineered with four new enzymes to ferment xylose along with glucose. A network of pentose pathway enzymatic reactions interacting with the native glycolytic Entner Doudoroff pathway has been hypothesized. We have analyzed the complex interactions between the pentose phosphate and glycolytic pathways in this network by developing a large-scale kinetic model for all the enzymatic reactions. Based on the experimental literature on *in vitro* characterization of each of the 20 enzymatic reactions, the large-scale kinetic model is numerically simulated to predict the dynamics of all the intracellular metabolites along the network of interacting metabolic pathways. This kinetic model takes into account all the feedback and allosteric regulations on the enzymatic reaction rates and is better suited to the systems level analysis of interacting metabolic pathways compared to the standard linearized methods of metabolic flux analysis and metabolic control theory.

This nonlinear kinetic model is simulated to perform numerous in silico experiments by varying different enzyme concentrations and predicting their effects on all the intercellular metabolic concentrations and the ethanol production rates in continuous fermentors. Among the five enzymes whose concentrations were varied and given as input to the model, the ethanol concentration in the continuous fermentor was optimized with xylose isomerase was needed at the highest level, followed by the transaldolase. Predictions of the model, that interconnecting enzyme phosphoglucose isomerase, does not need to be overexpressed, were recently confirmed through experimental investigations. Through this kinetic modeling approach, we can develop efficient ways of maximizing the fermentation of both glucose and xylose, while minimizing the expression of the heterologous enzymes.

Poster Abstracts for Session 6

Bioprocess R&D

POSTER PRESENTATION 6-08

Grape Pomace as a Source of Fuels and Chemicals

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In producing wine or juice from grapes in commercial wineries, approximately 10 percent of the grapes end up as pomace, a solid waste which is most typically land applied as a method of disposal. There are a number of potential opportunities for obtaining value from this waste pomace including composting for sale, the production of steam or electricity, its use as a cattle feed, fermentation to methane or ethanol, and extraction to obtain anthocyanins and procyanidins. This paper presents detailed analyses of pomace from a number of grape varieties and examines value added opportunities based on these analyses. Experimental results from some of the more promising opportunities are presented and discussed.

POSTER PRESENTATION 6-09

Concentrating Egg Albumin Solution to Solid Using Foam Fractionation

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To concentrate a protein solution into a solid form often requires a costly process. Foam fractionation is a low cost adsorptive separation process which can be used to concentrate protein solutions. Earlier studies (ca. 1940) may have indicated that enzymes like cholinesterase could be collected in crystalline form. Our previous studies with ovalbumin showed that when this enzyme is foamed from solution it can yield a white solid precipitate. In this study, foam fractionation is used to concentrate a solution of egg albumin (made from a yellow solid powder) into a white solid precipitate. A mass recovery of around 50% is achieved directly after foaming. There are three control variables that need to be adjusted in order to produce a solid protein egg albumin product. One, the initial concentration needs to be less than 250 parts per million. Two, the air superficial velocity needs to be between 1 and 5 cm/min. Three, a large physical space for the internal holdup needs to be in place in order to allow the produced foam to drain well.

POSTER PRESENTATION 6-10

Simultaneous Saccharification and Fermentation of Steam-Pretreated Spruce Using Yeast Cultivated on the Pretreatment Liquid

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Raising the dry matter content, as well as utilizing the substrate efficiently in simultaneous saccharification and fermentation of spruce is important when attempting to reduce the ethanol production price. In the current study, different *S. cerevisiae* strains, including a xylose-fermenting strain, were used in SSF-experiments aiming at as high fibrous content as possible with the minimum yeast concentration. The SSF cultivations were analyzed on-line by the carbon dioxide evolution rate (CER) and off-line by measurements of ethanol and sugar concentrations. Furthermore, the cell viability was studied by measuring colony forming units (CFU). The strains were adapted by cultivation on the liquid from the pretreatment. Results comparing the different strains will be presented.

POSTER PRESENTATION 6-11

Supercritical Extraction Process for Pro-Vitamin A Recovery: Simulation and Optimization

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Supercritical extraction process represents an alternative to conventional separation methods, because of the favorable properties of supercritical fluids, such as solvent recovery; simple separation, favorable thermal conditions and mass transfer properties and solvent free products.

CO_2 is the most commonly employed solvent, because it is relatively inert, cheap, non-toxic, recyclable and non-flammable and its critical properties (critical pressure=7370.00 kPa and critical temperature=30.95°C) are easily reachable. The use of co-solvent is also possible, in order to improve the separation. However, the solvent choice depends on the system.

In this work, a simulation procedure of this process was developed through the use of the commercial simulator HYSYS™, adapting the existing units to the operating conditions typical of the supercritical extraction process. As case study, the Palm Oil system with carbon dioxide/ethanol as supercritical solvent was used. This example characterizes the problem for recovering pro-vitamin A (beta-carotene) from natural sources, as the palm oil. This is very interesting because the supercritical extraction is a clean technology and the carotenes are obtained from a natural source, which is desired by the market. The optimization procedure was carried out analyzing the variables involved in the process.

POSTER PRESENTATION 6-12

An Evolutionary Training Method for Hybrid Models of Fermentation Processes

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This work brings a hybrid modeling technique that facilitates the effective development for an extractive alcoholic fermentation process. In the hybrid model, differential equations based on mass and energy balances are combined with multilayer perceptron neural networks which represent the kinetics relations of the biological system. Initially, it was required to estimate the output of the neural network sub-model which is the reaction rate. Then the neural network sub-model parameters are directly identified from input/output data obtained using a kinetic model, whose kinetic parameters were experimentally determined. These parameters are then used as an appropriate initial parameter set for the following optimization where the parameters of the neural network sub-model are directly optimized within the complete model. The performance criterion used to update the parameters is calculated using evolutionary algorithms in the form of real-coded and binary-coded genetic algorithm. These algorithms have been found to address the problem with success in terms of the quality of the solution. Typical nonlinearities present in these kind of biotechnological process are adequately represent through this kind of approach.

POSTER PRESENTATION 6-13

Simultaneous Saccharification and Fermentation of Steam Pretreated *Salix* at High Consistency in Order to Increase the Ethanol Concentration

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A high substrate concentration is necessary in simultaneous saccharification and fermentation (SSF) in order to lower the overall energy utilization and water consumption, as well as improving the process economy. However, the use of higher dry matter contents leads to increased concentrations of inhibitory compounds, which, at a certain level, result in reduced fermentability. Therefore, a robust and tolerant yeast is required. This can be accomplished by cultivating the yeast on the pretreatment hydrolysate, as the yeast then becomes better adapted to the environment.

The main objective of this study was to increase the final ethanol concentration by performing SSF at higher consistencies, maintaining a high ethanol yield. Experiments were carried out on the slurries from steam pretreatment of non-, SO_2-, and H_2SO_4-impregnated *Salix* chips. At first, the ethanol concentration that could be obtained with commercial baker's yeast (5 g DM/L) was investigated, by performing experiments at initial water-insoluble solids (WIS) contents of 5, 7, and 9 %. The study was continued using yeast adapted to the medium, in an attempt of increasing the WIS concentration even further. The results from this on-going study will be presented.

POSTER PRESENTATION 6-14

Optimization of Lactic Acid Production from Cheese Whey Using *Lactobacillus helveticus* Under Batch Conditions

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Cheese whey is a liquid effluent from cheese-making processes. There is an increased interest in the economic utilization of whey produced by the dairy industries, because the whey is a pollutant, due to its lactose content. The aim of this work was to find the most suitable values of some fermentation parameters for lactic acid production from whey by an lactic acid bacterium, Lactobacillus helveticus (ATCC 15009). The effects of lactose content, temperature, pH, and the supplementation of whey with yeast extract were investigated. In order to accomplish this, a composite central design was used with three repetitions in the center, making a total of 27 operational conditions. The experiments were conducted in a batch reactor and the time of each experiment was 32 hours. The results show that the smallest concentration of product was obtained in experiments with pH smaller than 6. The same happens when the yeast extract concentration was less than 12 g/L. The highest lactic acid concentration, 57 g/L, was obtained with a temperature of 36°C, pH = 7 and lactose and yeast extract concentrations of 85g/L and 20.37, respectively.

POSTER PRESENTATION 6-15

Operation of a Percolation Column Apparatus for Oxidative Lime Pretreatment and Solid State Conversion of Chipped Yard Waste to Carboxylic Acids

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The MixAlco process is a recently-developed method for converting biomass wastes such as solid wastes, cow manure and other waste organic materials into commercially marketable chemicals such as ethanol, acetone, and acetic acid. Processing steps are as follows: (1) oxidative lime pretreatment, using air and lime at ambient pressures, (2) non-sterile fermentation of lime-treated biomass to carboxylate salts using a mixed culture of acid-forming microorganisms, (3)dewatering, (4) thermal conversion of carboxylate salts to ketones, and (5) hydrogenation of ketones to alcohols. To date, research on the MixAlco process has been applied primarily to slurried substrates.

Chipped yard waste contributes a considerable waste stream in municipal landfills. Attempts to recycle yard waste as compost or mulch has met with some success, but typically the quantities available quickly saturate local markets. This project seeks to investigate the application of the MixAlco process to the conversion of chipped yard waste using a percolation system for in-situ solid state conversion of particulate biomass. This study presents results from oxidative lime pretreatment and acidogenic fermentation carried out in a custom-built percolation reactor column apparatus. The reactor is capable of performing both the pretreatment and fermentation steps. Results on mixing effectiveness, percolation behavior and conversion to mixed acids are presented.

POSTER PRESENTATION 6-16

On-Site Lime Pretreatment and Conversion of Dairy Manure to Mixed Acids for the Production of Chemical Feedstocks Via the MixAlco Process

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The MixAlco process is an anaerobic fermentation system that converts biomass into organic chemicals and mix-alcohol fuels via alkaline pretreatment, non-sterile, acidogenic digestion, product concentration, thermal conversion and hydrogenation. Because they have low capital costs and relatively simple operation, it is proposed that the pretreatment and fermentation steps of the MixAlco process may be suitable to be carried out on location at confined animal feeding operations. This project focuses on converting lime-pretreated dairy manure into carboxylic acids using a four-stage counter-current fermentation system installed on-site at a dairy farm.

The pretreatment and fermentation system was constructed on a dairy farm in Central Texas and operated for several months to determine the effectiveness of on-site conversion. The system consists of five plastic barrels that serve as both pretreatment and fermentation reactors. The setup was configured with a series of pumps to transport liquids from barrel to barrel enabling the counter-current fermentation system. A temperature controlled water circulation system was designed to maintain a uniform temperature of 40˚C throughout the digesters. The temperature and pH of the fermentation system was logged automatically to ensure steady state was achieved. Initial conversion results and comparisons with laboratory studies are presented.

POSTER PRESENTATION 6-17

Closure of the Phosphate Balance in the Conversion of Lime-Pretreated Dairy Manure to Mixed Acids

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Acidogenic fermentation is proposed to address dairy-derived phosphate water pollution in Central Texas. Biomass such as cattle manure can be converted to mixed-alcohol fuels or chemicals via the MixAlco Process, which includes: alkaline pretreatment, acidogenic digestion, product concentration, thermal conversion and hydrogenation. Because it is non-sterile, mixed acid fermentation can process problem waste streams such as manure and biosludges and utilizes low-cost materials for construction. The flexibility of the down-stream processing, which enables the production of a wide variety of value-added products, is also a unique and advantageous characteristic. It is hypothesized in this study that dairy farm-derived phosphate contamination of surface waters could be diminished by the application of the MixAlco conversion process to dairy waste streams.

Soluble and total phosphate concentrations were measured in samples taken from dairy manure before, during, and after lime pretreatment and fermentation steps. It was found that phosphate is present in each step, but at different concentrations. Lime moderately reduced the amount of phosphorus in dairy cattle manure, and the fermentation step further precipitated out calcium phosphate salts with the addition of calcium carbonate. A comparison was made with methanogenic fermentation of manure, in which less neutralization of acids is required and thus less calcium becomes available for precipitation of phosphate. Thus, it was found that the MixAlco process effectively sequesters phosphate into the solid phase, removing it from problematic waste streams. Further downstream processing may enable recycling of the phosphate compounds as fertilizer components.

POSTER PRESENTATION 6-18

Direct Capture Immobilized Separator: A Novel Approach to Separations

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A team comprised of a national laboratory and a small company is developing a novel Direct Capture Immobilized Separator. In this separator, using actual biological cells as the feed, ANL/Prime Separations, Inc. has redesigned the fundamental approach to separation of fermentation products. Product cells can produce acids as a part of normal metabolism. With this novel patented separator, the anion of the organic acid is directly captured onto an immobilized surface that is later released with appropriate buffers. The technology will substantially enhance separation efficiency in bio-reactors. This is accomplished because it overcomes three basic barriers commonly found in bio processing systems utilizing microorganisms. First, the desired products are trapped in complex mixtures containing hard-to-separate components. Second, the bio reactions tend to be inhibited by the presence of many products that need to be separated. Consequently, by increasing separation capability, the fundamental bio reactions can proceed at a faster rate resulting higher overall throughputs. Third, the complexity of the bio reactor broth also needs a complex pretreatment prior to product separation. This often includes several stages of particulate filters with complex regeneration and cleaning-in-place accessories, which are not required with the novel separator.

The novel separator under development has a three-fold benefit. First, is a simultaneous high separation capability due to the direct capture mechanism with associated boost of production rate. The second benefit is due to the reduction of product concentration within the bio reactor which pushes the concentration-driven reactions forward at a higher rate. The third advantage of the immobilized separator is that it can operate in complex bio-broths without the substantial pretreatment required by conventional separation schemes. Because the proposed separator is fundamentally different in the way it can deal with sludge/solids compared with conventional systems (which are often plagued by the suspended solids found in bio reactors) this third feature is expected to be the most significant benefit of this novel separator.

Preliminary process economics have been explored using the NREL's bioprocess simulation program**. Actual lab data will be reported in this presentation.

**Courtesy of Michael Pacheco and John Jechura.

POSTER PRESENTATION 6-19

Lactic Acid Recovery from Cheese Whey Fermentation Broth Using Membrane System

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Lactic acid was produced by free and immobilized cells on spiral-sheet bioreactor from cheese whey. The lactic acid was recovered from the fermentation broth of cheese whey by a combination of membrane separation. The separation steps include ultrafiltration/ microfiltration to concentrate whey protein and bacterial cells, nanofiltration to separate lactic acid from

sugars and reverse osmosis to concentrate lactic acid. This presentation will introduce the lactic acid production and recovery system.

Lactic acid was produced from cheese whey with free cell and immobilized *Bifidobacterium longum* (*B. longum*). *B. longum* produces high yield of L (+) lactic acid compared with D (-) lactic acid. The immobilized fermentation was conducted in a bioreactor containing immobilized cells coupled with a 2.0-liter bench top fermentor to control pH and temperature. The free cell fermentation was conducted in a 2.0-liter bench top stirred fermentor. The lactic acid product was separated and purified by a three-stage membrane separation system named ultrafiltration, nanofiltration and reverse osmosis. The recovery ratio of protein, lactose and lactic acid on each step was measured and analyzed.

POSTER PRESENTATION 6-20

Inulin Containing Biomass for Bioethanol Production: Fructose Extraction Methods and Fermentation Assays

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Jerusalem artichocke (*Helianthus tuberosus*) is a perennial herbaceous plant that develops underground stolons forming shaped tubers which are similar to potatoes. The tubers consist of 75-79% water, 2-3% proteins and 15-16% carbohydrates, of which the D-fructose polymer inulin can constitute 8% or more. This D-fructose polymer can be used to produce ethanol or to obtain chemicals for pharmacy, fine chemistry or materials industry.

The carbohydrates, which are initially in high levels in the stems, have been transferred to tubers by the end of the growing cycle. The utilization of tubers to obtain inulin presents some drawacks as it is the high percentage of water which may promote the risk of contamination under storage conditions and the need for deep tillage to obtain the tubers. On the other hand, the possibility to harvest the above-ground biomass before tubers development and use the inulin containing stalks as feedstock for ethanol production is now envisaged as an interesting option to this species mostly used for animal feeding.

In this work the extraction conditions of inulin from Jerusalem artichocke stalks have been studied and optimized in order to attain a high fructose content fermentable extract. Furthermore, fermentation of those extracts using *Kluyveromyces marxianus* CECT 10875 and baker´s yeast has been performed and the potential to use this feedstock for bioethanol production assessed. Reults from these experiments will be presented.

POSTER PRESENTATION 6-21

Butanol Extraction from Fermentation Broth: Mathematical Equations

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Butanol, a major product of acetone butanol ethanol (ABE) fermentation, is toxic to the cells of *Clostridium acetobutylicum/C. beijerinckii* that produce it. The maximum concentration of butanol in a batch reactor does not exceed 20 g/L thus hampering recovery of this solvent due to a higher boiling point than water. In order to make butanol recovery energy efficient, extraction of butanol using an organic solvent was sought. In order to avoid cell growth inhibition and achieve an efficient recovery, four different processes were considered and mathematical equations were developed. These four processes include: 1) Staged extraction from cell free broth [extraction without concurrent production, EW/OCP]; 2) Staged extraction from whole broth [extraction with concurrent production, EWCP]; 3) Differential extraction from cell free broth [EW/OCP] and; 4) Differential extraction from whole broth [EWCP]. In all the four cases flow directions were counter current. For these four processes, the effect of various parameters on butanol extraction will be presented.

POSTER PRESENTATION 6-22

Development of Functional Link Hybrid Neural Model for an Alcoholic Fermentation Process

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There is great interest in optimizing all the steps of the ethanol production and the development of reliable mathematical models is essential for the effective design and high performance operation of bioprocesses. Phenomenological models can be developed considering industrial conditions, but changes happen normally in an industrial unit invalidating the prediction of the model in different conditions. Taking this into consideration the objective of this work is to develop a model for an alcoholic fermentation process with multiples stages in a simple and rapid way. A hybrid neural model was developed, combining mass and energy balances with neural networks, the functional link networks (FLN), which describe the process kinetics. The obtained structure has a good non-linear approximation capability and the estimation of the network weights is linear. As the proposed model is able to adjust to kinetic and environmental changes, it is suitable for use in the development of optimization strategies for the bioreactors. Thus, the training is fast, require low computational effort and convergence is guaranteed. The simple structure of the FLNs allows the easy and rapid estimation of network weights and, consequently, the use of the hybrid model in an adaptive form to be used for posterior optimization and control applications.

POSTER PRESENTATION 6-23

Kinetic Analysis of Growth and Xanthan Production with *Xanthomonas campestris* TISTR 1100 in Coconut Water

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Xanthan gum is an important biopolymer that is produced efficiently by a gram negative bacterium, *Xanthomonas campestris*, through the fermentation process. It has been used in a wide variety of foods for a number of reasons, including emulsion stabilization, temperature stability and its pseudoplastic rheological properties and in other industrial applications such as pharmaceuticals, agriculture, textile and petroleum production. The aim of this research is to study the influence of dissolved oxygen on xanthan production from synthetic medium and coconut water by *Xanthomonas campestris* TISTR 1100. The experiment was carried out in a 3.7 litre fermenter with a 2.5 litre of working volume. The ranging of dissolved oxygen from 10 to 30 % were tested by using synthetic medium at 33 ^{0}C. At 10, 15, and 30% of dissolved oxygen, the maximum viscosity of fermentation broth were 582.5, 690 and 950 centipoint. The fermentation kinetics showed that the maximum biomass concentrations were 1.95, 2.14 and 2.60 g/l, the maximum specific growth rate were 0.236, 0.266 and 0.247 h^{-1}, the maximum xanthan production were 9.25, 13.75 and 15.65 g/l , the maximum specific rate of xanthan production were 0.214, 0.352 and 0.318 g/g/h, the maximum substrate utilization were 19.37, 19.68 and 21.99 g/l and the maximum specific rate of substrate utilization were 1.740, 0.800 and 1.141 g/g/h, respectively. The results indicated that the 15% of dissolved oxygen was the most suitable for xanthan fermentation. The coconut water was then tested under the 15 % dissolved oxygen using the same condition as previously investigated. Results revealed that the maximum viscosity was 630 centripoint. The fermentation kinetics showed that the maximum biomass concentration was 2.60 g/l, the maximum specific growth rate was 0.321 h^{-1}, the maximum xanthan production was 16.25 g/l, the maximum specific rate of xanthan production was 0.478 g/g/h, the maximum substrate utilization was 23.28 g/l and the maximum specific rate of substrate utilization was 5.826g/g/h, respectively.

POSTER PRESENTATION 6-24

Influence of the Concentration of Sucrose and Temperature in Alcoholic Fermentation

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The use of ethanol as a fuel is an alternative which economical and technical viability have already been demonstrated throughout history. Due to the economic and ecological matters in the fuel sector, the interest on ethanol in the last years have increased in a great amplitude in the whole world, stimulating scientific research in the area of the alcoholic fermentation. The applications of etanol in energy obtainment go beyond the direct burning of this compound , being in phase important researches on biodiesel and hydrogen production.

In this work are presented the results of research which involves the optimization of alcoholic fermentation of solutions formulated of commercial sucrose, enriched with some nutrients. The fermentations are being carried in a concentration of sucrose of 100 to 300 g/L, and from 25 to 35 ºC of temperature. The results obtained until the moment allows to conclude that the incomes of the fermentations are very next to the stoichiometric value, due to the high cellular concentration used, and due to the fact that the cellular growth was very low. The biggest rates synthesis of product are in 30 and 35ºC with complete consumption of sucrose in all the fermentations.

POSTER PRESENTATION 6-25

Integrated Process for Microbial Formation of Poly(β-hydroxyalkanoates) from Activated Sludges with Enhancement of Wastewater Treatment

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By investigating the capability of the different microbial community of activated sludge from various industries in their accumulation of poly-β-hydroxyalkanoates (PHAs) and the feasibility in enhancing the efficiency of wastewater treatment, enhanced sludge mineralization and the optimization in the PHAs production might be achievable. Under specific conditions of biological reactor, the accumulation of PHAs associated with glycolysis of intracellular carbohydrates occurred. Under specific treatment conditions, lactate was converted to 3-hydorxyvalerate rich polyhydroxyalkanoates via acetyl-CoA and propionyl-CoA. This study included biomass screening comparison, the performance of PHAs production under different carbon sources and PHAs accumulation associated with wastewater treatment efficiency by different types of sludge (such as the domestic sludge, piggery sludge, oily sludge, purified bio-filter sludge, food waste sludge and also mixed sludges).

There were totally 22 types of biomasses of activated sludge from different industries isolated and confirmed as PHA biomasses. Among the isolated biomasses, the highest dry cell yielding (4,900mg/L) was found by the biomasses of purified bio-filter sludge. Cellular content of PHA more than 30% were achieved by biomasses of purified bio-filter sludge, food sludge and mixed sludge. There were about 60% stimulation of PHAs cellular content and 900% concentration level enhanced by the induction effect of cross-mixing and co-culture effect of the mixed sludge. The performance of microbial PHAs production by different carbon sources was different. Methanol and sodium octanoate showed toxicity to most of the isolated biomasses. Lactic acid seemed perform a stimulation effect (1,300% enhancement) in PHAs production in comparing with glucose as carbon sources. In the co-culture induction conditions, more than 32% PHB and 4% PHV of cellular weight were achieved. For wastewater treatment enhancement, certain parameters were monitored, such as BOD, COD, TOC, suspended solids and sludge content. Preliminary results showed that during the PHAs production process by using a bioreactor, the COD level of the wastewater can be removed from about 10,000ppm to about 260ppm with 97% COD removal efficiency achieved. Besides, suspended solids could be removed from about 3,000ppm to about 10ppm (more than 99% removal efficiency). In addition, oil & grease removal was from about 60ppm to less than 10ppm. Thus, the goal of gaining double benefits (PHAs production and enhance wastewater treatment) was achieved.

POSTER PRESENTATION 6-26

Cloning and Expressioning the PHA Synthase Gene phaC1 and phaC1AB into *Bacillus subtilis*

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Polyhydroxyalkanoates (PHAs) are polyesters of hydroxyalkanoates synthesized by numerous bacteria as intracellular carbon and energy storage compounds and they accumulate as granules in the cytoplasm of cells. In this work, we constructed two recombinant plasmids pBE2C1 and pBE2C1AB. Then we transformed the two plasmids into *Bacillus subtilis* DB104, respectively and generated *Bacillus subtilis*/ pBE2C1 and *Bacillus subtilis*/pBE2C1AB. The two recombinants strains were subjected to fermentation and exhibited PHA accumulation, which was the first report to demonstrate mcl-PHA production in *Bacillus subtilis*. The result of GC analysis suggested that the product produced by *Bacillus subtilis*/ pBE2C1 was identified to be a hydroxydecanoate-co-hydroxydodecanoate (HD-co-HDD) polymer while the product produced by *Bacillus subtilis*/pBE2C1AB was identified to be a hydroxybutyrate-co-hydroxydecanoate-co-hydroxydodecanoate (HB-HD-HDD) polymer.

POSTER PRESENTATION 6-27

Effect of Ca^{2+}, Mg^{2+} and Trace Heavy Metal Ions on PHB-Producing Bacteria

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The effect of Ca^{2+}, Mg^{2+} and several trace heavy metals (Cu^{2+}, Mn^{2+}, Cr^{2+}, Zn^{2+}) ions on three types of PHB accumulation bacterial strains was studied. Two of the bacterial strains, *Sphaerotilus natans* (*S. natans*) and *0B17* (*Pseudomonas sp.*) are wild type strains isolated from activated sludge. The remaining one is the recombinant *E. Coli DH5α / pUC19 / CAB.*

Under the effect of Ca^{2+} and Mg^{2+}, the PHB accumulation due to the three bacterial strains will be reduced. It indicates that Ca^{2+} and Mg^{2+} are not essential elements for the PHB production. As Recombinant *E. Coli* has an ineffective depolymerase system, the suppression in PHB accumulation may be due to the inhibition of PHB synthesis system.

Under the effect of heavy metals, Cu^{2+} inhibits the growth of the three bacterial strains. For *S. natans*, Mn^{2+} is found to enhance the PHB accumulation. For *0B17*, Cr^{3+} enhances the PHB accumulation. However, for recombinant *E. Coli*, slightly enhancement in PHB accumulation is observed under Cr^{3+}.

POSTER PRESENTATION 6-28

Enhanced Production of mcl-Polyhydroxyalkanoates by *Pseudomonas aeruginosa*

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Polyhydroxyalkanoates are biodegradable, and biocompatible thermoplastics that are produced by bacteria. In this study, we aimed at producing medium chain length polyhydroxyalkanoates (mcl-PHA) by growing *Pseudomonas aeruginosa* in fatty acids-containing medium. The carbon sources used were glucose, and also fatty acids such as citrate acid, nonanoic acid, decanoic acid and lauric acid. Our experiments demonstrated that *Pseudomonas aeruginosa* could produce the 10 carbons and 12 carbons mcl-PHA in the glucose, citrate acid, nonanoic acid, lauric acid medium and the 8C,10C and 12C mcl-PHA in decanoic acid medium. Higher yield of mcl-PHA could be produced when using decanoic acid as the carbon source. The cells dry weight and PHA accumulation percentage were 3.632g/L and 27.1%, respectively. The bacterium also accumulated 1.9%, 1.48%, 2.3% and 7.9% in glucose, citrate acid, nonanoic acid and lauric acid medium, respectively. Our study illustrated that the decanoic acid is suitable for using in the production of mcl-PHA with a high productivity.

POSTER PRESENTATION 6-29

Evaluation of Experimental Systems for Xylitol Separation

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Xylitol production from lignocellulosic residues is a well known subject, however there are few works dealing with its separation from fermentation broth. In order to evaluate this process, three experimental systems for xylitol separation employing zeolites were studied. The xylitol was obtained by fermentation in a bench-scale reactor using an ion-exchanged detoxified bagasse hydrolysate medium and the yeast *Candida guilliermondii*. The downstream experiments were performed by varying the number of columns and the pulse volume, the best results being attained with 2 and 3 columns of zeolite Baylith WE894 exchanged with barium and fed with a pulse volume equal to 8% of bed volume. Although the separation efficiencies were enhanced by using 3 columns, it was observed that the amount of xylitol in the enriched fractions and its recovered amount were only sligthly higher than those obtained using 2 columns. In addition, the 2-column system contributed to a more rapid elution of the compounds and required a lower eluent volume. Therefore, among the three experimental systems evaluated, the one composed by 2 columns of zeolite led to a better xylitol separation.

POSTER PRESENTATION 6-30

Scale-Up of Two-Stage Biofilter System for Removal of Odorous Compounds

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The latest waste treatment has risen to a big problem in Korea. Unlike offensive odors that are produced by chemical processes, waste landfill leachate showed that complex compounds with various concentrations were closely related to process characteristics. Thus, this study investigates the removal efficiencies of complex odorous compounds using a two-stage biofilter at the GwangGyang waste landfill area, and seeks desirable ways to treat the offensive odors. The reactor(2000 W × 2500 L× 2000 H) used in the experiment was composed of scrubber, 1st-stage biofilter, 2nd stage biofilter, and `media packed 2.5m^3 into the reactor by cutting out a 20 W × 20 L × 20 H section of polyurethane foam. Strain was isolated from compost of composting facilities of Seo San in Chungnam, Korea. Tests in a reactor with offensive odors including methyl mercaptan, hydrogen sulfide, dimethylsulfide, trimethylamine, ammonia, and mist etc at 20˚- 30˚ temperature and 15 sec of retention time for 35 days. Overall, 8 kinds of offensive odor were shown removal efficiencies more than 95% at two-stage biofilter system.

POSTER PRESENTATION 6-31

Simultaneous Removal of SO_2 and NO_x Using Aqueous Homogeneous Catalyst

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SO_x and NO_x, which are formed when fossil fuel burns, are known major precursors of acid rain and thus abatement of their emission is a major target in air pollution control. Although the wet FGD system has experienced high SO_2 removal efficiencies, that is not SO_x for NO_x. The reason for this process failure is that NO_x, which accounts for more than 90% of NO_x in the flue gas, is quite insoluble in water. Since the wet scrubbing system currently dominates the FGD market, a minor adjustment of the system may work for the combined SO_x/NO_x removal system should be cost-effective. In order to simplify and thus reduce the cost associated with deSO_x and deNO_x of flue gas, processes for simultaneous removal of SO_x and NO_x have been developed. To obtain the basic data on the process of simultaneous SO_2/NO removal, the objectives of this study were to determine the optimal reaction conditions and aqueous solution compositions for simultaneous removal of SO_2/NO using a bubbling column reactor. Nitric oxide and sulfur dioxide can be removed from flue gas by a bubbling column reactor containing ferrous EDTA. Tests in a 45 mm the inside diameter column and 68 mm outside diameter and 1.2 L volume made of pyrex glass with several commercial packings gave 99 to 99.9 percent SO_2 removal and 50 to 80 percent NO removal. The experimental result of molar ratio of ferrous EDTA for simultaneous SO_2/NO removal shows that SO_2 and NO removal efficiencies were increased in proportion to ferrous EDTA concentration. When considering cost-effectiveness, the optimal ferrous EDTA concentration was 0.03 M. Also, the effect of O_2 concentration on the simultaneous SO_2/NO removal was investigated. At 4% O_2 concentration, removal efficiencies of SO_2 and NO were more efficient than those of 20% O_2. Based on the optimal operating conditions, the effects of addition of selected 6 additives on the combined SO_2 and NO removal using a bubbling column reactor were carried out. Maximum SO_2/NO removal efficiencies were achieved under ferrous EDTA 0.03 M, ascorbic acid 0.024 M, adipic acid 0.024 M, and sodium sulfite 0.09 M. The removal efficiencies of combined SO_2 and NO under optimal aqueous solution were obtained 99% of SO_2 during reaction period and above 85% of NO for 2 hours, while removal efficiency of NO was decreased after 2 hours. Overall, addition of various additives to ferrous EDTA enhanced removal of NO by 85-100% in the initial step, but this effect is decreased as a result of oxidation of ferrous EDTA. To meet the requirement of large scale combined SO_2 and NO removal, while our results are promising, further studies are needed, including configuration of suitable reactor, cost-effective process, and optimal operating conditions etc.

POSTER PRESENTATION 6-32

Macroscopic Mass and Energy Balance of a Pilot Plant Anaerobic Bioreactor Operated Under Thermophilic Conditions

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In West Virginia intensive poultry generates annually over 100,000 tons of manure. Nationally, this residual represents over 9 million tons. An alternative to face the problem is the use of thermophilic anaerobic digestion. Nowadays the technological alternatives available based on this process are very diverse. A modification of the typical continuous stirred tank reactor is particularly a promising process due to its capability to manage considerable amounts of residuals at low operational cost, and to the relative process stability. Experiments were carried out in a 40-m^3 pilot plant digester, using poultry manure. Data acquisition systems allowed monitoring of power consumption of all the devices used for feeding, transport and mixing. Chemical composition of feed and effluent were monitored during the experiments as well as methane production. Results suggested that some changes are necessary to the pilot plant configuration in order to reduce power consumption but without detriment of the biodigestor performance.

POSTER PRESENTATION 6-33

Use of Granular Starch Hydrolyzing Enzymes for Low Energy Grain Ethanol Production

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Production of fuel ethanol from grain continues to expand at double-digit rates. Use of energy in the process is a major cost with cooking of the grain to aid in enzymatic digestion a significant expense. Recently, novel enzyme systems (granular starch hydrolyzing enzymes, or GSHE) have been developed that are capable of converting granular (i.e., uncooked) starch to fermentable sugars in a simultaneous saccharification fermentation. The successful demonstration of enzymatic drilling of granular starch using GSHE for replacing an expensive energy intensive cooking process and its benefits in the carbon conversion efficiency, osmolarity and overall process operation and economics will be presented.

POSTER PRESENTATION 6-34

Ethyl Alcohol Production Optimization by Coupling Genetic Algorithm and Multilayer Perceptron Neural Network

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In this work, genetic algorithms (GA) and multilayer perceptron neural network (MLPNN) were used in combination with modeling and simulation to optimize an extractive alcoholic fermentation process. The objective is to maximize productivity and conversion in the fermentor. A comparison is made between the performances of two models when the process is optimized using a real-coded and binary-coded genetic algorithms. This first one is a deterministic model, whose kinetics parameters were experimentally determined as functions of the temperature, the second is a non-linear model represented for a MLPNN. The optimization results are compared and the efficiency of the genetic algorithm is demonstrated. Besides, the result demonstrated the prediction accuracy of MLPNN. Thus, MLPNN showed to be a very powerful and flexible tool well suited for modeling the fermentation process due to an implicit corrective action arising from the training methodology and the associated estimation procedure. In addition to establish optimal conditions for operation, the present methodology also makes possible to predict both conversion and productivity when the system is disturbed in some way. This is useful no only for the additional knowledge supplied about the process, but also for fermentation monitoring and control.

POSTER PRESENTATION 6-35

Studies on Glucose and Fructose Adsorption by Activated Carbon

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Fructose is an important natural sugar, sweeter than glucose and sucrose. One of the most important sources of fructose is sucrose, that can be converted into glucose and fructose. To produce a high fructose syrup from sucrose, it is necessary to separate the glucose formed. Generally, the separation of sugar, like glucose and fructose, is difficult, because these sugars are isomers. Adsorption is the process utilized in these cases.

In this work batch runs in a stirred tank were carried out to study the adsorption of fructose and glucose by activated carbon and ion exchange resin, separately. The activated carbon was used in three different sizes. The glucose and fructose concentrations were determined by Somogyi & Nelson method and Glucose-Oxidase method. Batch runs were carried out with pretreated and non-pretreated carbon to investigated the influence of the treatment in glucose and fructose adsorption. It was observed that the carbon size did not influence the adsorption process and the treatment of the carbon either. Analyzing the adsorption kinetic curves, it was observed that activated carbon adsorption capacity is bigger with glucose than with fructose. Although, with ion exchange resin the inverse occurred. Preliminary batch tests were carried out with a mixture of glucose and fructose to analyze the selectively of the adsorbents.

POSTER PRESENTATION 6-36

Application of RAPD Technique for Microorganism Screening in the Bioconversion of Limonene

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Limonene is a low priced monoterpene and a precursor of many flavors and fragrances. Terpenes and their oxygenated derivatives are extensively used by aroma industries. The use of microorganisms for these transformations has stimulated the biotechnology market. However, the microorganisms responsible for this bioconversion demand a long fermentation time and high medium costs. Aiming at the highest efficiency in the process of strain screening, the application of molecular biology techniques, such as RAPD, have been proposed. The goal of this work is to correlate the results of RAPD with previous tests of oxidation for the detection of bands, which denote the distinguished expression of responsible genes for the product of interest. Assays with 120 random primers have been carried out. From these primers, 12 were chosen for RAPD, since they have better amplified the DNA of the 18 studied microorganisms. Simultaneously, fermentations have been performed to oxidize limonene to carvone, and for the purpose of comparison with the results of molecular biology. The results demonstrated that it is possible to use the RAPD technique for screening some strains with oxidative ability.

POSTER PRESENTATION 6-37

Assessment of the Influence of Process Variables on the Biotransformation of (+)-Limonene by *Penicillium digitatum* ATCC 26821

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Limonene is the major compound in essential oils of citrus fruits, generally with a concentration above 90%. It is the precursor of many flavors and fragrances and is used as a starting material for manufacture of terpene alcohols and ketones. Biotransformation can be used to convert monoterpene precursors or intermediates into more valuable "natural" flavors and fragrances. In this context, the main objective of this work was to investigate the biotransformation of (+)-limonene by *Penicillium digitatum* ATCC 26821.The effect of the following variables were evaluated by means of a semi-factorial experimental design with 8 variables and 2 levels, keeping constant the agitation and the culture medium: temperature (20-35ºC), concentration (1-5v/v%) and type of co-solvent (EtOH and acetone), concentration (1-5v/v%) of substrate and sequential addition of substrate. The effect of the adaptation of the pre-cultures with small amounts of substrate, the time of growth of inoculum and addition of vitamin solution into the medium was also investigated. The experiments were performed in conical flasks with liquid cultures. The strain of *P. digitatum* was able to convert, in some experimental conditions, limonene to carvone and limonene oxide as majority products.

POSTER PRESENTATION 6-38

Production and Rheological Caracterization of Biopolymer of *Sphingomonas capsulata* ATCC 14666, Using Conventional and Industrial Media

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Glucose and sucrose are generally used as preferential carbon sources for the production of biopolymers. Nevertheless, alternative sources such as molasses, soybean pomace, cheese whey and others, have been suggested aiming at the reduction of production costs. The main objective of this work was the production and rheological characterization of biopolymer by *Sphingomonas capsulata* ATCC 14666, using conventional and industrial media in rotary shaker at 28ºC and 72h. The production of biopolymer in conventional medium was carried out using a full 2^2 factorial design with 3 replicates at the center point to establish the optimum conditions of each independent variable (concentration of sacarose and agitation). The productivity reached the maximum of about 0.038 g/Lh, at 208 rpm and 4% (wt/v) of sucrose. For this optimum condition, different concentrations of industrial medium was tested (2.66, 4, 6 and 8%). Tukey's test was used to check significant differences among conditions studied. The best productivities was obtained using pretreated molasses 8% (wt/v) (0.296 g/Lh), residue of PTS 6% (wt/v) (0.244 g/Lh) and crude molasses 8% (wt/v) (0.192 g/Lh), respectively. The rheology (apparent viscosity) presented similar values compared to those of the literature for other biopolymers.

POSTER PRESENTATION 6-39

Biosurfactant Production by a Strain of *Pseudomonas aeruginosa* in a Bioreactor Coupled to a Membrane Oxygenator

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Biosurfactants are gaining importance in various industries like agriculture, food, textiles, and petrochemicals. The rhamnolipids have been pointed out as the most promising biosurfactants because emulsification, demulsification, detergency and wetting, amongst others, are important properties ascribed to such molecules. The most studied microorganisms for the production of rhamnolipids are the bacteria of the genus *Pseudomonas* and factors like carbon and nitrogen, trace elements, temperature, and oxygenation affect the production. The major problem found in these aerobic fermentations is the excessive foam formation, due to the air injection in the bioreactor. In order to overcome this difficulty, a membrane contactor can be used to promote the oxygen transfer from the gaseous to the liquid phase, without phase dispersion. The aim of this work was to produce rhamnolipid type biosurfactant by one strain of *Pseudomonas aeruginosa* (PA1) isolated from oil environments. This production was carried through a bioreactor (3L) coupled to a membrane oxygenator in order to maintain dissolved oxygen concentration between 3 and 5 mg/l. The productivity of 21 mg/l.h (expressed in rhamnose units) was obtained when the nitrogen source was depleted and a glycerol consumption of 94 mg/l.h was observed. This proposed process seems to be a good alternative for the rhamnolipid production, since it favors the process control and makes the raise of the production scale possible.

POSTER PRESENTATION 6-40

Influence of the Concentration of Sacarosis and Temperature in Alcoholic Fermentation

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The use of ethanol as a fuel is an alternative which economical and technical viability have already demonstrated throughout history. Due to the economic and ecological matters in the fuel sector, the interest on ethanol in the last years have increased in a great amplitude in the whole world, stimulating scientific research in the area of the alcoholic fermentation. The applications of ethanol in obtaining energy go beyond the direct burning of this compound, being in phase important researches on biodiesel, using ethanol and hydrogen production.

In this work are presented the results of a research which involves the optimization of alcoholic fermentation of solutions formulated of commercial sacarosis, enriched with some nutrients. The fermentations are being carried in a concentration of sacarosis of 100 to 300 g/L, and from 25 to 35 ºC of temperature. The results obtained until the moment allow us to conclude that the incomes of the fermentations are very next to the stoichiometric value, due to the high cellular concentration used, and due to the fact that the cellular growth was very low. The biggest speeds of synthesis of product are in 30 and 35ºC, and having total consumption of sacarose in all the fermentations.

POSTER PRESENTATION 6-41

Anaerobic Degradation of Horticultural Wastes in Batch Process

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Nowadays, in Mexico several thousands of tons of organic wastes are produced daily in the horticultural products distribution centers of México (Centrales de Abasto). Anaerobic digestion could be used successfully to treat more than 300,000 tons/year of wastes (only in Mexico City). In this work, the methanogenic process is characterized by using organic solid wastes from these big markets.

Results show a very good biodegradability of wastes in a relatively short period of time. This is because the hydrolysis step is shorter due to the biochemical composition of the organic matter fed in the reactor, since lignin and cellulose rates are low. Thus, the hydrolysis is not slow and rate-limiting in comparison with such steps when lignocellulosic materials like grasses or agricultural crop residues are used. COD was reduced 92 % in ten days. An advantage of using this kind of wastes, which are entire fruits and vegetables, is that sulfide production is not strong because most of horticultural products have low sulfates.

POSTER PRESENTATION 6-42

Affinity Foam Fractionation for Selective Separation of *Trichoderma* Cellulase

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Foam fractionation, which involves bubbling air or an inert gas into liquid solutions, is a simple, low-cost and environmentally friendly separation/purification process. Surface-active substances partition onto bubble surfaces and become enriched in the collected foamate. When applying foam fractionation to cellulase separation, we found that cellulase is surface-active but not the most hydrophobic in the fermentation broth. To enhance their selectivity and enrichment, we have developed a new technology, affinity foam fractionation. Cellulose hydrolysates and analogs, carboxymethyl celluloses (CMC) with different molecular weights (MW) and degrees of substitution (DS), are added to bind the cellulase selectively and form hydrophobic complexes that readily partition onto bubble surfaces. The effects of cellulase concentration (FPU), hydrolysate/analog-to-FPU ratio, type of hydrolysate/analog, and presence of cells are evaluated. The foaming properties measured included foaming speed, foam stability and dryness, foamate volume and FPU, and enrichments of FPU and individual cellulase components. Foamate FPU could be as high as 4 folds of that in the broth. Among cellulase components, exoglucanase was enriched the most (3 folds), endoglucanase the next (2.3 folds), and β-glucosidase the least (1.4 folds). With CMC, those having low DS and high MW performed better. More detailed results and correlations will be presented.

POSTER PRESENTATION 6-43

Coupling Foam Fractionation with Fermentation for *Trichoderma* Cellulase Production

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Foam fractionation is a promising tool for protein concentration and separation. It is simple, inexpensive, and environmentally friendly, and can be readily scaled-up from laboratory to commercial-size equipment. In this work, we coupled the foam fractionation with fermentation of *Trichoderma reesei* Rut C-30 for cellulase production. Cellulase is a secondary metabolite. To maximize its productivity, a continuous process with cell retention is desirable. The fermentation is therefore made with continuous addition of fresh medium containing concentrated cellulase-inducing C substrate(s), and continuous removal of (almost) cell-free medium through foam fractionation columns. Lactose and hardwood hydrolysate are used as the cellulase-inducing C substrates. The fresh medium addition is computer-controlled using an algorithm designed to maintain the rate of pH decrease, after each base addition for pH control, to a value predetermined for the culture at early stationary phase (prior to sporulation). Cell retention is achieved by immobilizing cells on Celite particles, which are retained during medium removal by foaming (with the help of backwash). The foamate removal rate is continuously measured with an electronic balance and used to adjust, via computer control, the aeration rate so that the foamate removal rate matches the combined rate of medium addition and backwash. Experience and results obtained will be presented.

POSTER PRESENTATION 6-44

Syngas Fermentation as a Route to Hydrogen and PHA from Biomass

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We are investigating the use of microbes to catalyze the conversion of syngas to high-value chemicals (syngas fermentation) as an alternative to chemical synthesis from petroleum-based precursors. There are two main advantages of syngas fermentation compared to conventional chemical synthesis: 1) Microbial catalysts are relatively insensitive to syngas composition, which reduces gas processing and cleaning costs; and 2) microbes can be genetically engineered to produce a variety of value-added chemicals not readily obtainable by chemical synthesis, including chirally pure chemicals of the type described in this proposal. This paper will focus on the use of *Rhodospirillum rubrum* to produce hydrogen and polyhydroxyalkanoates (PHA) as co-products from fibrous feedstocks, including low-vlaue waste streams.

We have assembled a team of researchers at ISU to investigate the major technical issues related to syngas fermentation: thermal gasification, gas-liquid mass transfer, and metabolic engineering of suitable microbes. Gasification studies are aimed at identifying gasifier operating conditions that produce syngas suitable for anaerobic fermentations of CO, CO_2, and H_2 and investigating minimal gas-cleaning strategies that provide non-toxic gas streams to bioreactors. The mass transfer effort is investigating ways to increase the rate at which syngas can be dissolved into aqueous media to enhance the growth rate of the microbes that ferment it. Metabolic engineering activities focus on designing microbes to produce diverse PHA in high yields for use as biodegradable plastics. This paper reports on recent progress in each of these elements of syngas fermentation.

POSTER PRESENTATION 6-45

Industrial Biomaterials and Fuels from Lignocellulosic Biomass: Development Initiatives in Australia and New Zealand

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As a result of increasing consumer awareness of the environmental footprint left by either the manufacture or degradation of everyday products, sustainability has become a marketable product attribute. Many large-scale industries are seeking to incorporate more and more sustainable raw materials into their manufacturing processes. AgriGenesis (New Zealand) and the Sugar Research Institute (Australia) have partnered to apply their joint expertise in process engineering, biochemical engineering, polymer chemistry, and biotechnology for the production of fuels and industrial raw materials from lignocellulosic biomass resources available in each of these geographies.

The proponents have identified low-cost bio-refining systems to separate the lignin and carbohydrate components of renewable biomass from farmed crops. Proprietary technologies have been identified for the production of lignin-based functional replacements for non-renewable raw materials and transport fuels. An early stage milestone for this project is the construction of an integrated pilot process for the production of test products for industrial partners. A description of the proposed process plus progress made in optimizing the unit operations for the different target biomass sources and product concepts will be presented for discussion.

POSTER PRESENTATION 6-46

Acetate Removal from the Hydrolyzate of Lignocelluloses by Methanogens: The Feasibility Study

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Acetate is one of the major toxic byproducts generated during the pretreatent of lignocelluloses, because acetate can decrease the yield and productivity of ethanol for almost all ethanol producing microorganisms including *Z. mobilis* and *S. cerevisiae*. Overliming and ion exchange are widely accepted conditioning methods for the removal of these toxic byproducts in hydrolyzate. However, the formation of gypsum in overliming and the high cost of ion exchange operation are still barriers for such applications. In this work, the feasibility of using biological means to selectively remove acetate from hydrolyzate is evaluated. In particular, methanogens were applied as detoxic agents for acetate removal, as some methanogens, such as *Methanoplanus* species, only use acetate as carbon source and convert it into CO_2 and methane. The existence of methanogens does not cause contamination problems to ethanol fermentation, as methanogens do not compete with ethanolgens for substrate, and methanogens do not produce inhibition molecules.

POSTER PRESENTATION 6-47

Inulinase Production by *Kluyveromyces marxianus* NRRL Y-7571 Using Solid State Fermentation

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Inulinase is an enzyme relevant to fructose production by enzymatic hydrolysis of inulin. This enzyme is also used in the production of fructooligosaccharides that may be used as a new food functional ingredient. Commercial inulinase is currently obtained using inulin as substrate, which is a relatively expensive raw material. In Brazil, the production of this enzyme using residues of sugarcane and corn industry (sugarcane bagasse, molasses and corn steep liquor) is economically attractive, due to the high amount and low cost of such residues. In this context, the aim of this work was the assessment of inulinase production by solid state fermentation using by *Kluyveromyces marxianus* NRRL Y-7571. The solid medium consisted of sugar cane bagasse supplemented with molasses and corn steep liquor. The production of inulinase was carried out using a full 2^4 factorial design with 3 replicates at the center point to establish the optimum conditions of each independent variable (temperature, moisture, concentration of corn steep liquor and molasses). The enzymatic activity reached a maximum of 189 units of inulinase per gram of dry substrate.

POSTER PRESENTATION 6-48

In situ Batch Extractive Fermentation Using *Clostridium beijerinckii* BA101: Scale-Up and Mixing

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Microbial inhibition by butanol is an important obstacle to the commercialization of this fermentation when using the solventogenic *Clostridia*. In order to overcome this inhibition, several methods have been used to selectively remove butanol during the course of the fermentation, including pervaporation, gas-stripping, perstraction, and liquid-liquid extraction.

Liquid-liquid extraction using oleyl alcohol was examined as a means for butanol recovery because of its low energy requirement and high selectivity for butanol. 200mL and 1.5L *Clostridium beijerinckii* BA101-based batch fermentations were carried out in combination with in situ extraction. When examined at 200mL, improved butanol production and glucose consumption was observed over the traditional batch fermentation. Subsequently, the system was scaled up to 1.5L. Extractive fermentation at 1.5L required modifying the fermentor and the extraction technique as mass transfer was insufficient in the absence of mixing. The effects of mixing in model solutions and in 1.5L volume fermentations and issues concerning emulsion formation and fouling associated with the mixing process were examined.

POSTER PRESENTATION 6-49

Experimental Development, Instrumentation and Control of an Extractive Fermentation Process to Ethanol Production

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The objective of this work was to develop studies in lab scale of a continuous fermentative process coupled to a vacuum flash evaporator. The phases of this work consisted in assembly and instrumentation of the prototype and elaboration of a supervisory system coded in LabVIEW 6.1, which allows the acquisition of data and the control of the process through computers.

The experiments in continuous fermentation, with the coupling of the vacuum flash evaporator, used *Saccharomyces cerevisiae* and cane molasses as substrate. The analytical follow up was done through analysis of total reducing sugars, ethanol, glycerol, dry mass and viable cells.

The flash system has worked correctly, producing an alcoholic solution at the condenser with 50 °GL. The fermentation operated with concentrations of ethanol at 5 °GL, which is a weakly inhibitory value for the yeast of the process, even when fed with concentrated cane molasses, containing up to 330 g/l of sugar. The result meets the initial goal, which was to operate the system with low level of ethanol and to guarantee high productivity, even in high concentrations of sugar in the feeding. The results showed that system productivity was superior to that of the conventional continuous process.

POSTER PRESENTATION 6-50

The Effect of Varying Structured Catalyst Packing on Conversion in Microchannel-Based Bioreactors

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Continuous flow enzymatic micro scale bioreactors have been fabricated and operated successfully in proof of concept experiments. Subsequently, one of these reactors has been simulated using CFD-ACE+ software. The design of the original reactor was arbitrarily chosen. It contains rectangular channels with cross dimensions of 100+ micrometers and regularly spaced obstructions (packing particles) within the channels to provide additional surface area. Simulation efforts are now under way with the ultimate goal of design optimization. The packing particle size, shape, location, and density will be designed precisely for any application in order to maximize the use of expensive immobilized enzymes. The reactors are fabricated by a micro molding process where any arrangement of packing particles recommended by simulations is physically realizable.

The scope of the present work is to study the effects of selected design changes on conversion by computer simulation. The reactive surface area provided by enzyme-coated packing particles is varied and found to have a non-linear effect on conversion. The fluid mechanics responsible for this complex relationship is analyzed. It is also found that some choices for the locations of packing particles can cause channeling with a corresponding drop in conversion. For very dense packing configurations, the high sources pressures required to produce the desired flow rates can threaten the structural integrity of the reactors.

POSTER PRESENTATION 6-51

Reactions Involved in Raw Sewage Sludge Combustion

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Thermal conversion technologies for sewage sludge include several processes such as pyrolysis, gasification, and combustion/incineration, which have the potential to produce low cost activated carbon, bio-oil, syn-gas as a gaseous fuel, or construction materials. The process used depends on the targeted product and energy recovery desired.

When raw sludge is subjected to high temperatures, a very complex system of reactions is initiated, most of which may occur simultaneously, such as dehydration, devolatilization, gasification, volatile matter burning, combustion, ash melting and so on. Volume shrinkage is also an important physical/chemical phenomena, which generally takes place in biomass with high contents of water and volatile matter.

In this paper, two specific areas will be carefully examined based on the characteristics of sludge--one is the reactions and routes involved in raw sludge combustion; the other is the solid char or ash properties, which include its morphology, melting behavior and the fate of some trace elements.

POSTER PRESENTATION 6-52

Process Improvements for Corn Ethanol Production

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Bio-Process Innovation has been developing a 'High Speed/ Low Effluent' (HS/LE) fermentation process as well as a Low Energy Distillation Process. The HS/LE fermentation process allows near complete fermentation of 18 to 28% glucose to ethanol in 4 to 10 hours, in either a Continuous Cascade or Consecutive Batch (CB) mode over extended periods of several to many months. In the Consecutive Batch (CB) mode of operation, the fermenter is available for immediate re-set after completion of fermentation and a settling period during which completed beer is decanted. This allows 3 or even 4 batches of 10 to 14% (v/v) ethanol to be produced per reactor per day. The HS/LE process is characterized by

1. Increasing productivity of fermenters by a factor of 3 to 8 times
2. Decreasing effluent stillage by allowing a high degree of backset
3. Decreasing nutrient needs/costs
4. Producing a clean, nearly sparkling clear, non-fouling 'beer' to take to the distillation column- perhaps a low energy column of BPI design.
5. Producing a clean high density yeast paste by-product with no need for centrifuges
6. Reducing waste water/ cleaning chemicals by eliminating need for CIP of fermenter(s) between batches.

In this paper/presentation, design and performance of a pilot scale 6000 liter HS/LE fermenter run in a small ethanol plant in Iowa using wet mill corn syrup will be described. Concepts and process flows for 'next generation' corn ethanol facilities, with applications of the HS/LE process to dry mill ethanol facilities, will also be described.

POSTER PRESENTATION 6-53

Biomass Gasification and Progress Towards Commercial Application

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The Energy & Environmental Research Center (EERC) located at the University of North Dakota has been actively engaged in verification, development, and testing of biomass gasifiers. A multitude of projects have been completed covering co-firing, gasification, hydrogen production, gas testing, and cleaning. This paper will describe data produced from the operation of a downdraft gasifier located at the EERC. The specific focus concerns the reliability for operation, and the ability of the gasifier to provide consistent clean gas for conversion to fuels and chemicals, or production of power (turbines, and piston engines). Data will be presented providing gas quality, contaminant levels, and the relationship to the operation of microturbines and piston engines. The EERC is operating systems in the range of 15 to 200 kWe. The gasification system is modular and portable. The gasifier consumes dry bulky materials such as wood chips, and utilizes a downdraft reactor configuration. Gas cleaning includes wet scrubbing, and filtration. The results from various fuels will be reported including sawdust, wood chips, and municipal debris. Capital costs are low and systems represent a significant opportunity to utilize biomass residues, and provide generation at attractive economics. Commercial biomass gasification processes are desired for biorefinery concepts.

POSTER PRESENTATION 6-54

Hybrid Neural Modeling of Batch Alcoholic Fermentation

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One of the main barriers to a more widespread use of advanced optimization and control techniques in the biochemical industry is the cost for model development and validation. Usually modeling costs account for over 75% of the expenditures of an advanced control project. Thus, it is of great advantage to find some simple and rapid way of modeling these processes, accurately enough for optimization and control. One method that has been proposed in recent years to achieve this goal is the use of hybrid neural modeling.

In this work two hybrid neural network models were developed for a batch alcoholic fermentation using data obtained from an experimental study. The hybrid models combine mass balance equations with neural networks to describe the kinetics. The first model uses the state variables (biomass, substrate and product concentrations) as inputs to the neural networks while the second one uses secondary variables (pH, brix and turbidity). The two hybrid models were shown to describe the dynamic behavior of the process accurately. Their performance is compared to that of a first principles model whose kinetic parameters were determined as functions of the temperature from the same experimental data set used to develop the hybrid models.

POSTER PRESENTATION 6-55

Spreadsheet Tools for Determination of Derivatives and Microbial Kinetics

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Determination of enzyme kinetics, microbial growth rates, substrate utilization rates, and product accumulation rates requires derivatives to be calculated with a method that can be duplicated and yield consistent results. Here I discuss two methods that have been suggested in the literature and provide spreadsheet formulas for the needed equations. A cubic spline method proved more versatile in its use even though the spreadsheet programming was elaborate. The methods discussed were tested with published literature data for cell growth and a preferred technique was developed based on smoothing the data and using this smooth data for rate calculations.

POSTER PRESENTATION 6-56

Biotechnological Production of Xylitol from Sugar Cane Bagasse: Effect of Glucose on Xylose Reductase and Xylitol Dehydrogenase Activities

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The influence of the glucose in the xylose-to-xylitol metabolism was evaluated from sugarcane bagasse hydrolysate fermentations with different glucose:xylose ratios (1:25, 1:12, 1:5 and 1:2.5) employing inoculum of *C. guilliermondii* obtained from the cultivation in medium containing glucose, a mixture of glucose and xylose or only xylose as carbon sources. According to the results, the glucose:xylose ratio interfered positively in this bioconversion and it was not observed a correlation between the favorable condition of xylitol production and the XR and XDH enzymes activities. The maximum activity of XR (0.582U/mg_{prot}) occurred in the fermentation carried out un medium with 1:25 glucose:xylose ratio, using the inoculum grown in medium containing glucose. The maximum activity of XDH (0.360U/mg_{prot}) was verified in the fermentation of medium with 1:2.5 glucose:xylose ratio and inoculum grown in a mixture of glucose and xylose, whereas the maximum values of xylitol yield (0.59g/g) and volumetric productivity (0.53g/L.h) were reached when the glucose:xylose ratio was 1:5 and the inoculum was grown in medium containing only xylose. This indicates that these results were influenced not only by the glucose:xylose ratio in the fermentation medium, but also by the presence or lack of glucose in the inoculum growth medium.

POSTER PRESENTATION 6-57

Butanol Extraction from Fermentation Broth: Mathematical Equations

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Butanol, a major product of acetone butanol ethanol (ABE) fermentation, is toxic to the cells of *Clostridium acetobutylicum/C. beijerinckii* that produce it. The maximum concentration of butanol in a batch reactor does not exceed 20 g/L thus hampering recovery of this solvent due to a higher boiling point than water. In order to make butanol recovery energy efficient, extraction of butanol using an organic solvent was sought. In order to avoid cell growth inhibition and achieve an efficient recovery, four different processes were considered and mathematical equations were developed. These four processes include: 1) Staged extraction from cell free broth [extraction without concurrent production]; 2) Staged extraction from whole broth [extraction with concurrent production]; 3) Differential extraction from cell free broth [extraction without concurrent production] and; 4) Differential extraction from whole broth [extraction with concurrent production]. In all the four cases flow directions were counter current. For these four processes, the effect of various parameters on butanol extraction will be presented.

POSTER PRESENTATION 6-58

Production of Lactate Ester by Extractive Fermentation and Enzymatic Esterification

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Lactic acid is an important specialty chemical that can be used to synthesize biodegradable polymers and green solvents, such as polylactic acid (PLA) and lactate esters. Lactate esters in many situations can replace toxic solvents. Conventional fermentation processes for organic acids production usually suffer from low product concentration, low productivity and low yield due to end product inhibition. With *Rhizopus oryzae* immobilized in a rotating fibrous bed bioreactor, on-line extractive removal of lactic acid from the fermentation broth was demonstrated by using alamine in 1-octanol contained in a hollow fiber membrane extractor. The extractant containing lactic acid was further reacted with octanol to produce octyl lactate catalyzed by lipase. Ethyl lactate can be further made from octyl lactate. The extractive fermentation coupled with esterification with immobilized lipase reactor provides an energy-efficient green process for production of lactate esters from biomass.

POSTER PRESENTATION 6-59

Mathematical Modeling of Cell-Immobilized Bioreactors for Alcohol Production

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New bioprocesses based on the immobilization of enzymes and cells have significantly called the attention of both industry and academia in the last decade. The continuous production of ethanol based upon bioreactors with immobilized cells enables the economic reuse of biomass, improves stability, and diminishes product contamination. This work presents the experimental design of pellets investigating the effects of alginate concentration, calcium chloride concentration and pellet diameter in the overall fermentation performance of bioreactors. The paper also presents a novel mathematical model of a cell immobilized bioreactor. The model is investigated by computational simulation and experimentally validated for a system with a support prepared using the calcium alginate gel-entrapping method. Besides the selection of the support, the substrate adsorption and product diffusion, which are controlling steps in the system, were addressed. The results indicate that the bioreactor investigated in this work is very promising and suggest modes for cells immobilized based bioreactors operation.

POSTER PRESENTATION 6-60

Polysaccharide Production by *Haemophilus influenzae* Type B

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This work deals with the study of capsular polysaccharide biosynthesis by *Haemophilus influenzae* in order to develop a Brazilian technology for further vaccine production. Several experiments were conducted to select the best strain, investigate the proper size inoculum and the influence of nutritional and environmental conditions for the polysaccharide production.

Kinetic studies showed that the highest level of the macromolecule(at about 105 mg/L) was obtained with initial cell concentration of around 30 mg/L. Increased concentrations of carbon source negatively affected the fermentation process whereas increasing the yeast extract concentration improved the polysaccharide synthesis. Low levels of NAD and hemin were suitable for metabolic activity. The cyclic feed batch was a promising system allowing higher product concentration than the batch process. The polysaccharide recovered and partially purified presented a high molecular weight.

POSTER PRESENTATION 6-61

Combined Effects of Acetic Acid, Formic Acid and Hydroquinone on *Debaryomyces hansenii* Physiology

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Xylitol is a high value product that can be effectively produced by some yeasts from pentose rich hemicellulosic hydrolysates and its production is recognized as an advantageous alternative for upgrading such hydrolysates. Xylitol yield and productivity are affected by several factors e.g. oxygen availability, initial biomass and the concentrations of hexoses, pentoses and inhibitors formed during the hydrolysis. Among those factors, the influence of inhibitors is still the less understood. Although the assumption that the toxicity depends on combined effects of the inhibitors present, as it has been established for ethanol production by yeasts, this has not yet been clearly shown for the xylitol bioproduction.

In this work, the yeast *Debaryomyces hansenii* was cultivated in shake flasks, at the initial pH of 5.5, in a chemically defined medium containing glucose, xylose and arabinose in similar concentrations to the concentrated hydrolysates. Based on previous work and considering the impact on yeast physiology and the concentration in the hemicellulosic hydrolysates, acetic acid (0-6 g/l), formic acid (0-4.6 g/l) and hydroquinone (0-3 g/l) were used as model inhibitor compounds. A modified central composite design was used to investigate the main effects and interactions on the biomass and product formation kinetic and stoichiometric parameters. The results clearly indicate that the interaction effects play an important role on the xylitol bioprocess.

POSTER PRESENTATION 6-62

Dynamic Modeling of a Fermentation Process for Ethanol Production by Deterministic and Hybrid Models

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The main goal of this research project is the development of hybrid models for description of the dynamic behavior of biological processes. These models are developed by coupling Artificial Neural Networks (ANN) and deterministic models. ANNs are computer techniques that present a mathematical model inspired by the neural structure of intelligent organisms that acquire knowledge through the experience. The hybrid models will be used as mathematical representations of the process, for applications in real time control and optimization and mainly as soft-sensors.

The biological process studied is related to fermentative ethanol production using the microorganism *Saccharomyces cerevisae*. The deterministic model was validated properly in industrial scale and is used as a data source for studies of the dynamics of the process, development of the models with ANN as well as of the hybrid models.

For the construction of the hybrid model it was accomplished through the training of an ANN that predicts the specific speed of growth of the microorganism when supplied the values of the product (P), cells (X) and substrate concentrations (S). After adjustment of the ANN, it is coupled to the deterministic model supplying the dynamic profiles of the variables: S, X and P, efficiency and productivity.

POSTER PRESENTATION 6-63

Use of Different Adsorbents for Sorption and *Bacillus polymxa* Protease Immobilization

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Proteases constitute one of the most important groups of industrial enzymes, accounting for at least 25% of the total enzyme sales, with two-thirds of the proteases produced commercially being of microbial origin [1]. Proteases produced by *Bacillus* species are by far the most important group of enzymes produced commercially, being used in detergent, protein, brewing, photographic, leather, and dairy industries. Bacteria of genus *Bacillus polymxa* are active producers of extra cellular proteases and characteristics of enzyme production by *Bacillus* species have been well studied [2].

The application of cell immobilization techniques to production of metabolites by culture of micro-organisms appears as a very interesting alternative to the conventional process carried out with free cells. Proteases have been immobilized on natural and synthetic supports [3]. Inorganic supports are widely used for immobilization of enzymes mainly due to their good flow through properties, mechanical strength and regeneration. The commonly used inorganic supports are controlled porous glass, ceramics and alumina [4]. Furthermore, it was reported that enzymes could be activated by complexation with polysaccharides such as chitin or chitosan.

In the present work, *Bacillus polymxa* protease was studied using different adsorbents (chitin, chitosan, alginate, synthetic zeolite and raw zeolite) and evaluated the storage stability and re-usability of the immobilized protease. It was estimated that protease activity was shielded the ratio of 64% and 62% by using alginate and synthetic zeolite respectively under 24 hours operation condition. Additionally, the yield of the immobilization was 17% for alginate for 16 hours and it was determined that protease enzyme was not adsorbed on the chitin, chitosan, synthetic zeolite, raw zeolite.

These results indicate that the interactions of enzymes with some materials strongly affect the activity and stability of the enzymes in hydro-organic reaction systems.

POSTER PRESENTATION 6-64

Effect of Different Parameters on the Enrichment of DHA by Enzymatic Hydrolysis from Cod Liver Oil

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Docosahexaenoic acid (22:6, DHA) is n-3 polyunsaturated fatty acid and it is mostly found in fish. Because of its beneficial effects on the human body, usage of DHA in the food and pharmaceutical industries increases day by day. DHA plays an important role in the prevention of a number of diseases in humans [1-3].

The aim of this project is to observe the effect of the amount of enzyme, reaction time, pH, temperature and stirring velocity on the enrichment of DHA by enzymatic hydrolysis of fish oil and to be helpful for food and pharmaceutical industry by this study.

Purification of DHA was attempted by enzymatic hydrolysis of cod liver oil. Cod liver oil (CLO) was hydrolyzed with *Candida rugosa* lipase.

The optimal conditions of hydrolysis of CLO by *Candida rugosa* lipase were established as pH 5, 35°C and 12 h with 2000 U/g_{oil} enzyme concentration. The DHA amount of original CLO was increased from 10.72% to 17.6% in TG fraction of hydrolyzed CLO which means 64% increase in DHA content.

Also, at the end of this study it was seen that performing hydrolysis reaction of CLO with 1000U/g_{oil} *Candida rugosa* lipase at the pH 5.0, 35°C for 24h and fish oil: buffer solution was 12g:18ml resulted in 83.12% hydrolysis and 1.45 fold increase in the amount of DHA according to original CLO.

The comparison of the results was in a good agreement with the examples given in the literature. As a conclusion, it can be said that *Candida rugosa* lipase is effective on CLO and this method is efficient to obtain the DHA rich glyceride mixture from the fish oil.

POSTER PRESENTATION 6-65

Hydrogen Generation from Sugars via Aqueous-Phase Reforming

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Virent Energy Systems, Inc. is commercializing the Aqueous Phase Reforming (APR) process that allows the generation of hydrogen from biomass-derived compounds such as glycerol, sugars, and sugar alcohols. The APR process is a unique method that generates hydrogen from aqueous solutions of these oxygenated compounds in a single step reactor process compared to the three or more reaction steps required for hydrogen generation via conventional processes that utilize non-renewable fossil fuels. The key breakthrough of the APR process is that the reforming is done in the liquid phase. The APR process occurs at temperatures (180 °C to 270 °C) where the water-gas shift reaction is favorable, making it possible to generate hydrogen with low amounts of CO in a single chemical reactor. Furthermore, the APR process occurs at pressures (typically 15 to 50 bar) where the hydrogen-rich effluent can be effectively purified. Virent is currently developing the APR system for the purpose of generating hydrogen-rich fuel gas from biomass-derived sorbitol and integrating this reformer with power generating devices such as an internal combustion engine driven generator, a gas-fired turbine, or a solid-oxide fuel cell. The utilization of biomass-based compounds allows the APR process to be a carbon neutral method for on-demand production of hydrogen. This presentation will discuss the current development of catalysts, reactor configurations, and process equipment of the APR process for the hydrogen generation from biomass-derived oxygenated compounds such as glycerol and sorbitol.

POSTER PRESENTATION 6-66

Countercurrent Fermentation and Continuum Particle Distribution Modeling of Rice Straw in the MixAlco Process

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Currently, rice straw is either burned in open fields or incorporated into the soil. Increasing environmental concerns and government legislation call for a decrease in the quantity of rice straw burned (California Rice Commission's Library on Rice Straw Utilization). Incorporating rice straw into soil increases foliar disease, reduces crop yield, degrades soil conditions, and produces methane, a greenhouse gas. Therefore, a low-cost technology to convert these wastes into useful fuels and chemicals is valuable. Significant potential benefits result from fuel and chemicals derived from cellulosic biomass such as rice straw, a renewable nonfood feedstock.

The MixAlco process involves anaerobic fermentation of biomass using a mixed culture of microorganisms to produce carboxylic acids. Countercurrent fermentation of rice straw and chicken manure to carboxylic acids was performed using a mixed culture of marine mesophilic microorganisms. Fermentations were performed at various liquid residence time (LRT) and volatile solid loading rates (VSLR). The Continuum Particle Distribution Model (CPDM) was used to validate the experimental results and predict acid concentrations at other VSLR and LRT.

POSTER PRESENTATION 6-67

Molecular Distillation: A Powerful Technology for Obtaining Tocopherols from Soya Sludge

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Nowadays, great importance is being given to products that come from natural sources, such as products of high added value and obtained with high technology. This is the case of tocopherols obtained from soya sludge. Quite often, most of these products are molecules of high molecular weight or thermally sensitive, hindering the separation or purification through traditional methods, because they are subjected to high temperatures, which decompose these materials. An alternative to succeed in the separation/purification of such products is the use of molecular distillation, which is a peculiar case of evaporation: it operates under low pressure and, therefore, at relatively low temperatures, minimizing the thermal decomposition. Furthermore, this process can take advantages in relation to other techniques that use solvents as the separating agent avoiding problems with toxicity.

Taking all of these in consideration, in this work, the molecular distillation process for recovering tocopherols from Soya Sludge was studied. The simulation is very important to establish the availability of this process or not and its operating conditions, to improve the yield and purity of the final product and to establish the flexibility index of the process. Finally, it was made a comparison of simulated and experimental data, in order to validate the simulation results.

POSTER PRESENTATION 6-68

Evaluation of the Chromatographic Column Method for Xylitol Recovery Obtained by Fermentative Pathway

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Xylitol is a five carbon sugar alcohol with important applications in food and pharmaceutical industries. This compound is produced in a large scale by chemical process, but it also can be obtained by a fermentative pathway using hemicellulosic hydrolysates as substrates. However, for the fermentation process to be economically competitive to the traditional process, the procedure for recovering and purifying xylitol needs to be easy and efficient.

In the present work, xylitol was produced by *Candida guilliermondii* FTI 20037 yeast from sugarcane bagasse hemicellulosic hydrolysate. Subsequently the use of a chromatographic column employing silica gel as the stationary phase to recover and purify the xylitol obtained was evaluated. The results showed that with the use of this technique it was possible to eliminate the colored substances from the medium and to recover 60% of xylitol in the crystalline and pure form. The chromatographic column using silica gel thus appears as an interesting and potential technique for further studies on xylitol purification.

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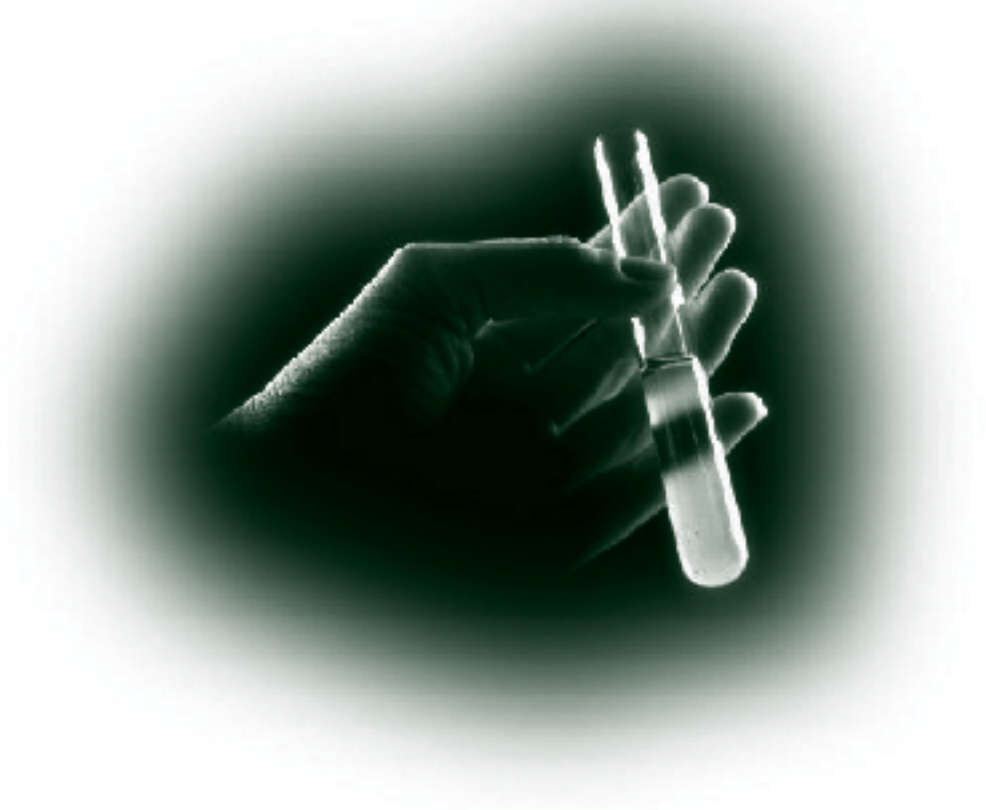

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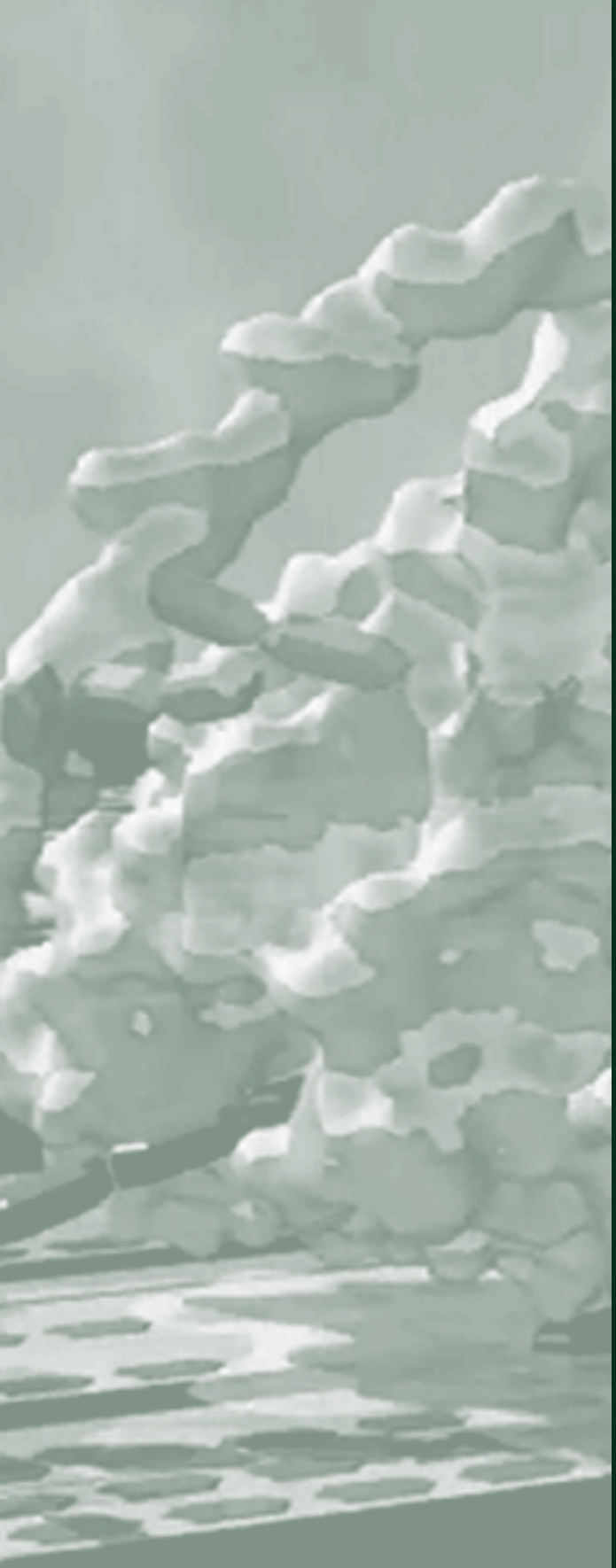

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